nitrogen level. For the sake of comparison, the RNV data using *T. pyriformis* and rat PER values are also given. *Tetrahymena* data are expressed as percent microscopic counts, taking casein counts as 100. It appears from this table that *A. flavus* graded the bread samples in the same order as had been done by *T. pyriformis* and rat. Rye bread crumb was rated superior when compared to wheat bread crumb. Crusts of both the breads showed a reduced nutritive value, as is reflected in the biomass produced. This response corresponded with the available lysine in protein. The magnitude of differences in nutritive value within the samples as assessed by *A. flavus* and *T. pyriformis* probably reflects the sensitivity of the two organisms. It appears that *A. flavus* may not be as sensitive in picking up small differences in protein quality as is *T. pyriformis* or rat, in processed cereals. *Tetrahymena pyriformis* is known to have similar amino acid requirements as rat and, therefore, is expected to give a more sensitive protein quality response (Kidder and Dewey, 1951). *Aspergillus flavus* does not have specific amino acid requirements, but it appears that the fungus can differentiate between various bread samples on the basis of protein quality.

Our intention is not to make a direct comparison between *A. flavus* and the other two organisms, because of their diversities and dissimilarities. However, since most of the parameters of nutritional quality are related to available lysine in protein, an indirect comparison can be made. We only wish to emphasize that the fungal system graded the various bread samples in the same order (not to the same magnitude) as has been done by *T. pyriformis* and rat. Because of the various advantages this system offers (easy to culture, less susceptible to contamination, less amount of material required, reliable methods for biomass estimation), these data are of potential interest. *Aspergillus flavus* may prove to be a useful primary stage protein quality screening tool in processed cereals and in plant cultivars, where rapidity, simplicity, inexpensiveness, small use of material, and relative rather than absolute precision are desired.

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Residue Analysis of Chlorflurenol in Cucumbers

A convenient method for the residue analysis of chlorflurenol, methyl 2-chloro-9-hydroxyfluorene-9-carboxylate, involving electron-capture gas–liquid chromatographic quantitation is presented. The method is sensitive to 0.005 ppm.

The growth regulator, chlorflurenol (methyl 2-chloro-9-hydroxyfluorene-9-carboxylate), is a white solid, mp 152 °C. It has a relatively low mammalian LD_{50} of 310 mg/kg. Chlorflurenol is supplied as a 5 and 12.5% emulsifiable concentrate (EC) and has found use as a growth suppressant (Thomson, 1974). Recently, parthenocarpy induced by chlorflurenol in cucumber has been reported (Cantliffe, 1972; Cantliffe et al., 1972). Since chemically induced fruit set could be economically important, a convenient method for monitoring chlorflurenol residues in cucumber was needed. We now wish to report a method involving extraction of the chlorflurenol, Florisil column cleanup, and quantitation by electron capture GLC.

EXPERIMENTAL SECTION

Materials. Solvents were Fisher Reagent Grade redistilled in all-glass stills. Granular anhydrous sodium sulfate was Mallinckrodt Analytical Reagent Grade. Florisil was Fisher 60–100 mesh and was kept at 130 °C until used. Chlorflurenol analytical standard was supplied by EM Laboratories (Elmsford, N.Y.).

Preparation. Cucumbers were macerated completely in a food chopper. Fifty grams of subsample was blended for 2 min with 200 ml of acetone and filtered through a 350-ml coarse sintered glass filter with suction. The blender was rinsed with 50 ml of acetone and this acetone was used to rinse the filter pad. The filtrate was placed in a 1-l. separatory funnel with 500 ml of distilled water and 30 to 40 ml of a saturated sodium sulfate solution. To this was added 100 ml of benzene and the mixture was shaken 1 min. The layers were separated and the benzene layer was filtered through a 1.5 in. pad of anhydrous sodium sulfate. The benzene extraction was repeated with a second 100 ml of benzene. The benzene was filtered through the same drying pad. The combined extracts were reduced to 5–10 ml on a rotavap at 45 °C.

Cleanup. A column 12 cm high with an o.d. of 2.54 cm containing 20 g of Florisil and 2-cm pads of sodium sulfate at the top and bottom was prepared and prewet with 60 ml of benzene. The benzene was drained to the top of the

Table I. Recovery of Chlorflurenol from Fortified Cucumber Samples

Chlorflurenol added, μg	Chlorflurenol recovered,[a] μg	Recovery,[a] %	Range, %
1	0.90	90	
2	1.57	79	75-91
4	3.51	88	85-101
5	4.45	89	86-93
8	7.15	89	80-105

[a] Average of several determinations.

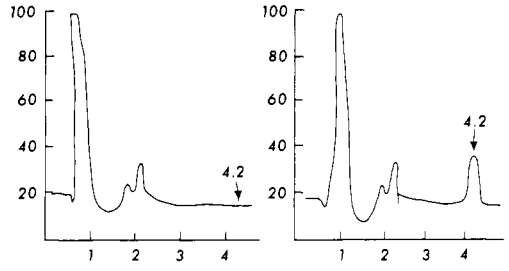

Figure 1. Chromatogram of: (A, left) unfortified cucumber sample; (B, right) cucumber sample fortified with 2 μg of chlorflurenol. The retention time of chlorflurenol is marked at 4.2 min.

column and the concentrate transferred to the column with several small benzene rinses. The column was eluted with 200 ml of 5% acetone in benzene and this fraction was discarded. The column was then eluted with 200 ml of acetone and this fraction was collected. The sample was reduced to 10 ml on a rotavap at 30 °C for GLC analysis.

Gas-Liquid Chromatography. Determinations were performed on a Micro-Tek 220 gas chromatograph equipped with a ^{63}Ni electron capture detector. The column was a 1.83 m × 4 mm i.d. glass column containing 6% DC 200 on 80–100 mesh Gas-Chrom Q. Operating parameters were nitrogen carrier at the rate of 80 ml/min; injector 225 °C; column 200 °C; detector 275 °C. Under these conditions chlorflurenol had a retention time of 4.2 min.

Standard Curve. One to four microliters of a 0.1 μg/ml solution of chlorflurenol was injected into the chromatograph. Peak area was plotted vs. concentration or nanograms injected. Normally 4 μl of sample was injected for quantitation.

RESULTS AND DISCUSSION

Blank and samples fortified in the blender with 1, 2, 4, 5, and 8 μg of chlorflurenol were taken through the extraction and cleanup procedures described above. These fortifications correspond to 0.02, 0.04, 0.08, 0.10 and 0.16 ppm residue samples. The average recovery for this series was 87%. The actual recoveries are reported in Table I.

Chlorflurenol was extracted quantitatively from aqueous acetone by benzene in control experiments. In separate control experiments, recovery of chlorflurenol from the Florisil column ranged from 85 to 100%.

Response is linear with both concentration and amount injected. The sensitivity was determined by assuming quantitation of a peak at two times the noise level, and was found to be 0.005 ppm. A typical chromatogram is shown in Figure 1.

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Antioxidant Activity of Browning Products Prepared from Low Molecular Carbonyl Compounds and Amino Acids

The antioxidant effect of browning products on safflower oil was investigated. The browning products were obtained by the reaction of low molecular carbonyl compounds with amino acids. Carbonyl compounds tested were methylglyoxal, glyoxal, glyoxylic acid, and dihydroxyacetone. The antioxidant effectiveness changed with the combination of carbonyl compounds and amino acids. Of four carbonyl compounds, methylglyoxal and dihydroxyacetone gave the most potent antioxidative products, followed by glyoxal and then glyoxylic acid. On the other hand, branched chain amino acids such as leucine and valine were most effective as amino acids.

It is well known that a nonenzymatic browning reaction between carbonyl compounds and amino acids functions in the formation of antioxidative substances (Kato, 1973; Maleki, 1973; Yamaguchi and Fujimaki, 1974). Most work was done on the products obtained by the reaction of hexose or pentose with amino acid. In our previous report (Itoh et al., 1975), the antioxidant activity of browning products made from a triose sugar (dihydroxyacetone) and amino acids was compared with the activity of those obtained from glucose or xylose and amino acids. The results indicated that dihydroxyacetone gave the most effective products. El-Zeany et al. (1973) reported the brown pigments produced by condensation of glyoxal with glycine showed excellent antioxidant effect. These facts