plant. This was evident from changes in infrared absorption of residues isolated from both leaves and roots. The greatest change was the disappearance of the 1-hydroxyl group which suggests glycoside formation at this position. This is similar to the reported metabolism of abscisic acid to a water-soluble complex with glucose in apple (Powell and Seeley, 1974).

For these reasons, it is considered more likely that the disappearance of the plant regulator dimethylheptyl-(1-hydroxy-*p*-menth-2-yl)ammonium bromide from grapefruit leaves is also due to its metabolism and deactivation by the tree. The biological half-life determined (10.8 days) explains the short term (14 day) growth retardant effect invariably observed when this regulator is applied to grapefruit trees.

LITERATURE CITED

Mooney, R. P., Pasarela, N. R., *J. Agric. Food Chem.* **15**, 989 (1967).
Newhall, W. F. (to Amchem Products, Inc.), United States Patent 3 564 046 (1971).
Newhall, W. F., Pieringer, A. P., *J. Agric. Food Chem.* **15**, 488 (1967).
Newhall, W. F., Pieringer, A. P., *HortScience* **7**, 254 (1972).
Pieringer, A. P., Newhall, W. F., *J. Agric. Food Chem.* **16**, 523 (1968).
Pieringer, A. P., Newhall, W. F., *J. Am. Soc. Hortic. Sci.* **95**, 53 (1970).
Powell, L. E., Seeley, S. D., *J. Am. Soc. Hortic. Sci.* **99**, 439 (1974).
Schill, G., *Anal. Chim. Acta* **21**, 341 (1959).
Schill, G., Danielsson, B., *Anal. Chim. Acta* **21**, 248 (1959).

William F. Newhall*
Béla S. Buslig
Institute of Food and Agricultural Sciences
Agricultural Research and Education Center
and Florida Department of Citrus
University of Florida
Lake Alfred, Florida 33850

Received for review February 10, 1976. Accepted August 19, 1976. Florida Agricultural Experiment Stations Journal Series No. 8086.

Nutritive Value of Rye and Wheat Breads Assessed with *Aspergillus flavus*

The relative nutritive value of crust and crumb portions of rye and wheat breads has been assessed using the fungus *Aspergillus flavus*. Rye bread crumb was rated superior to wheat bread crumb. Crusts of both the breads showed a reduced nutritive value. The fungus graded these samples in the same order as has been done by the protozoan *Tetrahymena pyriformis* and by rat bioassays using the same material.

While looking for simple, rapid, and inexpensive biological tests for evaluating relative nutritive value (RNV) in plant breeder's material, we found that *Aspergillus flavus* produced a biomass response which was negatively correlated with protein content and positively correlated with lysine in protein. The material was tested at equal nitrogen level and included barley (Mohyuddin et al., 1976a) and rye, wheat, and triticale cultivars (Mohyuddin et al., 1976b). Encouraged by these results, we tested this fungus on a set of wheat and rye bread samples comprised of crust and crumb portions. Data on protein efficiency ratio (PER) using rat and RNV determined by *Tetrahymena pyriformis* were available with the same material (Kaestner et al., 1976).

MATERIALS AND METHODS

A non-toxin-producing strain of *Aspergillus flavus* Link ex. Fr. was initially isolated from spoiling bread crumbs and maintained on 2% malt extract agar. Spores from a 10-day old culture were harvested according to Mohyuddin and Skoropad (1972). The rye and wheat breads were prepared in the Bundesforschungsanstalt für Getreideverarbeitung, Detmold, West Germany. The preparation and further fractionation into crust and crumb portions have been reported by Brümmer and Seibel (1975). The data on the PER values using rat and RNV determined with *T. pyriformis* have been taken from Menden et al. (1975) and Kaestner et al. (1976), respectively.

The samples were weighed at 3 mg nitrogen equivalent in duplicate in 100-ml Erlenmeyer flasks. Soluble potato starch was added in a quantity that brought the total weight to 500 mg. Thus, the samples were tested at isonitrogenous and isoweight levels. Twenty milliliters of distilled water was added to each flask, allowed to stand for 1 h, and then autoclaved at 1 kg/cm^2 pressure (121 °C) for 10 min. A vitamin solution (Stott and Smith, 1966) was similarly autoclaved. One milliliter of spore suspension ($\sim 10^6$) and 1 ml of vitamin solution were added to each flask.

The contents were shaken on a flat flask shaker for 72 h at 26–28 °C. The mycelium produced was filtered through a nylon sieve, washed with water, transferred to a preweighed filter paper, and dried at 100 °C for 1 h, and the dry weight was calculated. This dry weight in milligrams was used as an index of biomass. Available lysine data were kindly supplied by Dr. Kaestner and were determined by the method of Booth (1971).

Table I. Biomass Development of *Aspergillus flavus* on Crust and Crumb Portions of Rye and Wheat Breads, in Comparison with RNV Determined by *Tetrahymena pyriformis* and PER Values Obtained with Rat

Sample	Nitrogen, %	Avail Lys, g/16 g of N	*A. flavus* biomass,[a] mg	*T. pyriformis*[b] RNV	Rat[c] PER
Rye bread crumb	1.57	2.6	101.5	33.3	1.7
Wheat bread crumb	2.00	2.1	86.9	22.1	1.2
Rye bread crust	1.50	1.2	77.2	10.3	−0.3
Wheat bread crust	1.96	1.0	72.2	10.3	−0.6

[a] Each value is the mean of two replicates. [b] RNV = relative nutritive value, expressed as direct microscopic counts of cells/milliliter in relation to casein; casein counts taken as 100. [c] PER = protein efficiency ratio.

RESULTS AND DISCUSSION

Table I presents the data on the biomass produced by *Aspergillus flavus* on the crust and crumb components of rye and wheat breads when administered at an equal

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.

LITERATURE CITED

Booth, V. H. J., *J. Sci. Food Agric.* **22**, 658 (1971).
Brümmer, J. M., Seibel, W., *Ernaehr. Umsch.* **22**, 107 (1975).
Kaestner, H. W., Kaul, A. K., Niemann, E.-G., *Qual. Plant.–Plant Foods Hum. Nutr.*, **25** (3–4), 361 (1976).
Kidder, G. W., Dewey, V. C., in "Biochemistry and Physiology of Protozoa", Vol. 1, Academic Press, New York, N.Y., 1951, p 323.
Menden, E., Elmadfa, I., Horchler, V., *Getreide Mehl Brot* **29**, 253 (1975).
Mohyuddin, M., Kaul, A. K., Sharma, T. R., Niemann, E.-G., *Qual. Plant.–Plant Foods Hum. Nutr.* **25** (3–4), 317 (1976a).
Mohyuddin, M., Sharma, T. R., Kaul, A. K., Niemann, E. G., *J. Sci. Food Agric.* **27**, in press (1976b).
Mohyuddin, M., Skoropad, W. P., *Can. J. Bot.* **50**, 1431 (1972).
Stott, J. A., Smith, H., *Br. J. Nutr.* **20**, 663 (1966).

Mirza Mohyuddin*
Trilok Raj Sharma
Ernst-Georg Niemann

Institut für Strahlenbotanik
Gesellschaft fur Strahlen-und Umweltforschung
3000 Hannover, West Germany

Received for review May 24, 1976. Accepted October 4, 1976. One of us (M.M.) acknowledges the financial assistance of Alexander von Humboldt Foundation, Bonn, West Germany.

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