MYCOTOXINS IN FOODSTUFFS. VII. INACTIVATION OF PATULIN IN WHOLE WHEAT BREAD BY SULFHYDRYL COMPOUNDS

J. REISS, Mikrobiologisches Laboratorium, Grahamhaus Studt K. G., 655 Bad Kreuznach (Germany)

ABSTRACT Cereal Chemistry 53(2): 150-152

Patulin is inactivated by an aqueous extract of whole wheat bread at pH 5. The detoxification of patulin by glutathione and cysteine suggests that sulfhydryl compounds are responsible for the failure to detect this toxin in bread after prolonged incubation time.

The carcinogenic mycotoxin patulin is inactivated by a variety of compounds containing sulfhydryl (SH), *e.g.*, glutathione (GSH) and cysteine (CSH) (1,2,3). On the other hand, this reaction between patulin and enzymes with SH groups is considered the cause for the extreme toxicity of patulin toward bacteria (4,5,6).

It was demonstrated that patulin is inactivated by SH compounds in various foodstuffs, *e.g.*, in meat (7,8) and in fruit juices and wheat flour (9). Bread contains SH compounds (10,11) and may be contaminated with patulin (12,13), mainly excreted by *Penicillium expansum,* which is a common spoilage fungus on bread (14). Earlier observations (13) showed that the amount of the toxin which is detectable by thin-layer chromatography decreases with increasing incubation time of the inoculated bread. In view of the widespread occurrence of *P. expansum* on bread, the possible inactivation of patulin by SH compounds in bread was investigated.

MATERIALS AND METHODS

Preparation and Treatment of the Bread Extract

Whole wheat bread "Graham" (2 g) was ground in 10 ml distilled water (no buffer was used) and the solution was filtered. To 1 ml of the filtrate, 1 ml $10^{-3}M$ aqueous solution of patulin (F. A. Norstadt, Agricultural Research Service, Fort Collins, Colo.) was added. The final pH was 5. Samples were stored at room temperature (20°–22°C) in the dark and were analyzed immediately and every 2 days up to a period of 8 days.

Reaction of Patulin with SH Compounds

The $10^{-3}M$ solution of patulin (1 ml) was mixed with 1 ml of 1 μM solution of CSH or GSH (Serva, Heidelberg, Germany). The pH value of all test solutions was 5.

Determination of Patulin

Individual test solutions (2 μl) were spotted on a precoated silica gel sheet (Polygram SIL N-HR; Macherey-Nagel and Co., Düren, Germany). The standard applied to the sheet was a solution of 0.1 mg patulin in 2 ml chloroform. The spots were made visible by spraying with a saturated solution of *o*-dianisidine in glacial acetic acid (15) and the concentration of patulin present in the spots was calculated from a standard curve based on the fact that the square

TABLE I
Inactivation of Patulin by Glutathione, Cysteine, and Extract of Whole Wheat Bread "Graham"

Incubation Time days	Patulin Recovered (in %) When Added to:		
	Glutathione	Cysteine	Bread Extract
0	100	100	100
2	100	...[a]	80
4	28	80	40
6	35	20	30
8	1	4	14

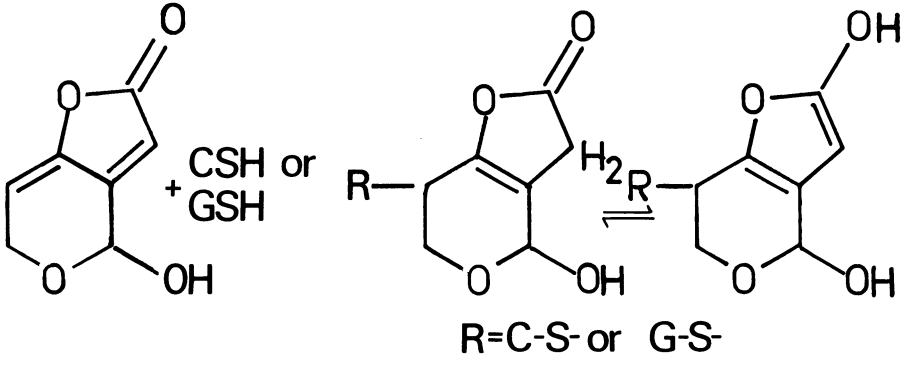
[a]Not analyzed.

root of the area of the spot is a linear function of the logarithm of the weight of the toxin (16). All tests were made in duplicate.

RESULTS AND DISCUSSION

Table I summarizes the data for the reaction of patulin with the bread extract as well as with CSH and GSH. It is evident that the SH compounds in the bread extract—probably CSH and GSH themselves—inactivate patulin greatly. The detoxification, however, is not complete and this might be partially due to the low pH value. Several authors (7,9) found a stronger reaction of patulin with thiol containing amino acids at higher pH values than at lower values. To preserve the natural properties of the bread, the pH value was kept constant in this study. Nevertheless, the data of Table I show a noticeable inactivation of patulin.

The addition reaction of patulin with SH compounds takes place at the double bond of the characteristic –CH=CH–CO– group (6). Hofmann *et al.* (7) have proposed the following scheme:



The addition products of patulin with SH compounds are apparently nontoxic (7).

The present study shows that SH compounds in bread inactivate patulin to a considerable extent. This may explain the observation that this mycotoxin cannot be detected in inoculated bread after a prolonged incubation time (13).

Acknowledgment

I thank F. A. Norstadt for a sample of patulin.

Literature Cited

1. CAVALLITO, C. J., and BAILEY, J. H. Preliminary note on the inactivation of antibiotics. Science 100: 390 (1944).
2. MIESCHER, G. Veber die Wirkungsweise von Patulin auf höhere Pflanzen, insbesondere auf Solanum lycopersicum L. Phytopathol. Z. 16: 369 (1950).
3. DICKENS, F., and COOKE, J. Rates of hydrolysis and interaction with cysteine of some carcinogenic lactones and related substances. Brit. J. Cancer 19: 404 (1965).
4. KISER, J. S., and ZELLAT, J. S. Antibiotics, other than penicillin, produced by Penicillia. Trans. N.Y. Acad. Sci. 7: 210 (1945).
5. RINDERKNECHT, H., WARD, J. L., BERGEL, F., and MORRISON, A. L. Studies on antibiotics. 2. Bacteriological activity and possible mode of action of certain non-nitrogenous natural and synthetic antibiotics. Biochem. J. 41: 463 (1947).
6. GEIGER, W. B., and CONN, J. E. The mechanism of the antibiotic action of clavacin and penicillic acid. J. Amer. Chem. Soc. 67: 112 (1945).
7. HOFMANN, K., MINTZLAFF, H.-J., ALPERDEN, I., and LEISTNER, L. Untersuchung über die Inaktivierung des Mykotoxins Patulin durch Sulfhydrylgruppen. Fleischwirtschaft 51: 1534 (1971).
8. ALPERDEN, I., MINTZLAFF, H.-J., TAUCHMANN, F., and LEISTNER, L. Untersuchungen über die Bildung des Mykotoxins Patulin in Rohwurst. Fleischwirtschaft 53: 566 (1973).
9. SCOTT, P. M., and SOMERS, E. Stability of patulin and penicillic acid in fruit juices and flour. J. Agr. Food Chem. 16: 483 (1968).
10. COLLINS, T. H. The uses of L-cysteine and azodicarbonamide in bread making. Bakery Management 1972: 5.
11. BLOKSMA, A. H. The relation between the thiol and disulfide contents of dough and its rheological properties. Cereal Chem. 49: 106 (1972).
12. REISS, J. Nachweis von Patulin in spontan verschimmeltem Brot und Gebäck. Mycotoxine in Nahrungsmitteln. II. Naturwissenschaften 59: 37 (1972).
13. REISS, J. Mycotoxine in Nahrungsmitteln. III. Mitteilung. Bildung von Patulin auf verschiedenen Schnittbrotarten durch Penicillium expansum. Chem. Mikrobiol. Technol. Lebensmittel 2: 171 (1973).
14. REISS, J. Die Schimmelpilze des Brotes. Zbl. Bakt. II. Abt. 128: 685 (1973).
15. REISS, J. Dünnschicht-chromatographischer Nachweis von Patulin. Chromatographia 4: 576 (1971).
16. REISS, J. Halbquantitative Bestimmung des Mycotoxins Patulin auf Dünnschichtchromatogrammen. Mikrochim. Acta 1975:473 (1975).

[Received March 25, 1975. Accepted June 10, 1975]