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Fluorimetric Detection and Quantification of Deoxynivalenol with Zirconyl Nitrate-Ethylenediamine

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Deoxynivalenol (3,7,15-trihydroxy-12,13-epoxytrichothec-9-ene-8-one, vomitoxin) is a cytotoxic trichothecene mycotoxin recently discovered worldwide in grain in association with infestations of Fusarium species, especially in cool, temperate regions (Ueno 1983). Ingestion of this mycotoxin results in feed refusal, emesis, and growth depression in swine and other livestock (Vesonder and Hesseltine 1981), but the specific long-term effects on humans remain unknown.

Quantification of this mycotoxin is relatively difficult because trichothecenes neither fluoresce nor appreciably absorb ultraviolet light (Pathre and Mirocha 1977). Currently, the generally recommended procedure for analysis of deoxynivalenol requires gas-liquid chromatography with electron-capture detection or gas chromatography - mass spectrometry (Scott 1982). Several colorimetric procedures have been investigated in the search for more practical methods for detection and quantitation and include 4-(p-nitrobenzyl)pyridine, a chromogenic reagent for epoxides (Takitani et al. 1979) and chromotropic acid which reacts with formaldehyde released from trichothecenes upon treatment with sulfuric acid (Kato et al. 1979, Baxter et al. 1983). Type-B trichothecenes such as deoxynivalenol also react with aluminum chloride to produce fluorescent reaction products (Ueno 1973), and this reaction has recently been confirmed for deoxynivalenol (Kamimure et al. 1981). Several type-B trichothecenes, however, produced significantly greater fluorescence upon reaction with zirconyl salts in comparison with aluminum salts and based on this, Kato et al. (1976) developed a fluorimetric assay for fusarenon-X, a toxin related to deoxynivalenol. Through modification of this procedure, we have developed a rapid, simple, sensitive method for the detection and quantitation of deoxynivalenol by fluorimetry.

MATERIALS AND METHODS

Deoxynivalenol (Research Foods Ltd., Downsview, Ontario) standard solution was prepared in methanol at a concentration of 0.04 ug/uL. Fifty microliters (2 ug deoxynivalenol) or less of this standard was added to 2 mL of methanol in 5-mL test tubes. To

this, 25 uL of 5% (v/v) ethylenediamine in methanol and 50 uL of 6% (w/v) zirconyl nitrate in methanol were added. The test tubes were sealed with plastic film, briefly shaken on a vortex mixer, and heated at 40°C in a water bath for 40 min. After the test tubes were cooled to room temperature, the fluorescence of the reaction product was determined in relation to a reagent blank by using a Turner Model 430 spectrofluorimeter with excitation at 360 nm and emission at 454 nm. The half-power band width of the monochrometer was set at 15 nm, and fluorescence intensities were recorded uncorrected.

RESULTS AND DISCUSSION

The fluorescence spectra of the deoxynivalenol reaction product showed excitation maximum at 360 nm and emission maximum at 454 nm. Similar, but not identical values, were reported for related trichothecenes including fusarenon-X, nivalenol, and dehydronivalenol (Kato et al. 1976). The reagent concentrations, however, are critical for production of optimum levels of the fluorescent deoxynivalenol reaction product (Table 1 and 2). Values reported for fusarenon-X (Kato et al. 1976) would produce only 40% of maximum fluorescence for deoxynivalenol.

Table 1. Effect of concentration of ethylenediamine on fluorescence of deoxynivalenol reaction product[a].

% Ethylenediamine (v/v)	Relative Fluorescence
3.0	39.80
3.5	64.68
4.0	88.06
4.5	97.01
5.0	100.00
5.5	99.50
6.0	92.04
6.5	89.85

[a]25 uL deoxynivalenol (1 ug); 25 uL ethylenediamine; 50 uL 6% (w/v) zirconyl nitrate; 2 mL methanol; 40°C for 40 min. Values are means of three determinations.

Table 2. Effect of concentration of zirconyl nitrate on fluorescence intensity of deoxynivalenol reaction product[a].

% Zirconyl Nitrate (w/v)	Relative Fluorescence
3.0	57.46
3.5	65.61
4.0	69.40
4.5	75.11
5.0	80.90
5.5	88.51
6.0	100.00
6.5	99.57
7.0	95.48
8.0	81.90
9.0	65.15

[a]25 uL deoxynivalenol (1 ug); 25 uL of 5% (v/v) ethylenediamine; 50 uL zirconyl nitrate; 2 mL methanol; 40°C for 40 min. Values are means of three determinations.

We chose a 40 min reaction time at 40°C. Shorter durations are required at higher temperatures but the risk of thermal decomposition of the reaction product is also apparent(Fig. 1). Reactions at higher temperatures might be of interest for possible application to post-column derivitization to improve minimum detectability of deoxynivalenol following high performance liquid chromatography (HPLC).

The fluorescent reaction product was stable at room temperature for at least 4 h. Calibration curves showed excellent linearity from 25-2000 ng/2 mL methanol (r = 0.996). Individual determinations were highly reproducible with a coefficient of variation of 1.33% and 2.71% for 1000 and 100 ng, respectively (calculated for 10 determinations).

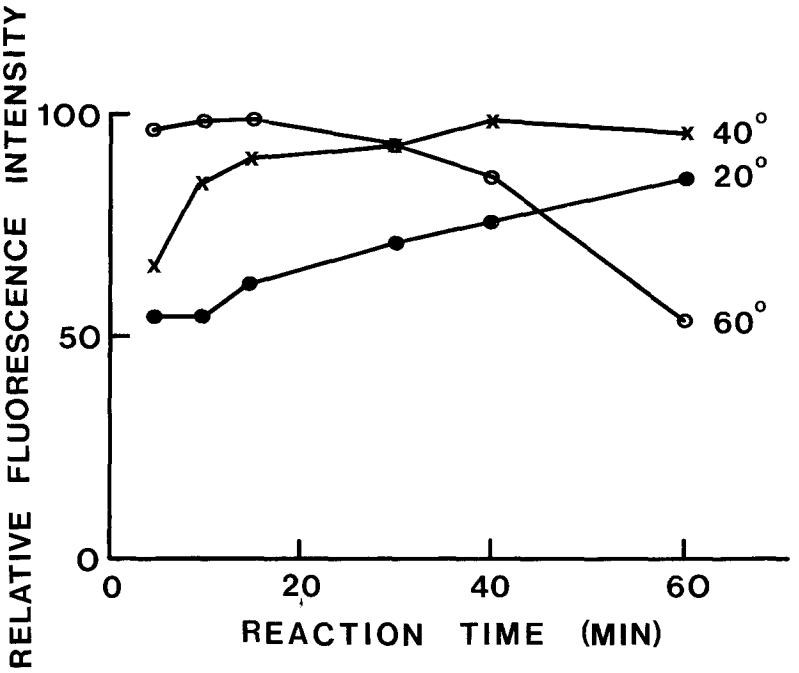

Figure. 1 Effect of incubation temperature and duration of heating on fluorescence intensity of deoxynivalenol reaction product.

This determination method for deoxynivalenol is rapid, sensitive, accurate and economical to perform. In view of its efficiency and practicality, further work is warranted on its possible adaptation as a determinative procedure in analytical methods to screen agricultural commodities.

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