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Natural Occurrence of Moniliformin and Fusarin C in Corn Screenings Known To Be Hepatocarcinogenic in Rats[1]

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A sample of corn screenings associated with a field outbreak of leukoencephalomalacia in horses in Pennsylvania and shown to be hepatocarcinogenic in rats was analyzed chemically for the presence of the *Fusarium* metabolites moniliformin, fusarin C, T-2 toxin, and diacetoxyscirpenol. The corn screenings were found to be naturally contaminated with moniliformin and fusarin C at levels of 2.82 and 0.39 mg/kg, respectively, while no T-2 toxin or diacetoxyscirpenol could be detected. This is the first report of the natural occurrence of both moniliformin and fusarin C in corn in the U.S. It is however unlikely that moniliformin is involved in either equine leukoencephalomalacia or hepatocarcinogenesis while it is unknown whether fusarin C plays any role in the etiology of these two syndromes.

INTRODUCTION

During 1983–1984, 9 of 15 horses on a farm in southeastern Pennsylvania fed corn screenings naturally infested with *Fusarium moniliforme* Sheldon died of leukoencephalomalacia (LEM) (Wilson et al., 1985). This material was fed unsupplemented to rats for 176 days and proved to be hepatotoxic as well as hepatocarcinogenic. Liver lesions in the rats fed these corn screenings were characterized by neoplastic nodules, adenofibrosis, and cholangiocarcinoma (Wilson et al., 1985). This was the first report of the hepatocarcinogenicity of corn associated with field cases of LEM and naturally infested with *F. moniliforme*. The chemical nature of the compound(s) that causes LEM in horses and liver cancer in rats is unknown.

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A sample of the corn screenings from a field outbreak of LEM and found to be hepatocarcinogenic in rats by Wilson et al. (1985) was analyzed chemically to determine whether fusarin C, moniliformin, or the *trichothecenes*, T-2 toxin and diacetoxyscirpenol occurred naturally in this material.

Fusarin C is mutagenic to *Salmonella typhimurium* (Loeffler) Castellani & Chalmers in the Ames test and is known to be produced by *F. moniliforme* and *Fusarium graminearum* Schwabe (Wiebe and Bjeldanes, 1981; Gelderblom et al., 1983, 1984a,b; Cheng et al., 1985; Gaddamidi et al., 1985). Moniliformin is a highly toxic compound that causes rapid death and pathological lesions including mycocardial degeneration and necrosis in experimental animals and is produced by several *Fusarium* species (Cole et al., 1973; Kriek et al., 1977; Marasas et al., 1979a,b, 1984b, 1986; Rabie et al., 1982; Thiel et al., 1982). Both of these compounds have been found to occur naturally in a sample of moldy home-grown corn from Butterworth, Transkei, an area with a high human esophageal cancer rate in southern Africa (Thiel et al., 1982; Gelderblom et al., 1984a). T-2 toxin and diacetoxyscirpenol are potent dermotoxic and hemorrhagic agents and are two of the most toxic trichothecenes produced by several *Fusarium* species (Marasas et al., 1984b).

EXPERIMENTAL SECTION

Corn Sample. A sample (1 kg) of corn screenings, consisting of broken kernels, pieces of cobs and stalks, and

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other debris, was obtained from a farm in southeastern Pennsylvania, where it had been fed to horses during an outbreak of LEM during 1983–1984 (Wilson et al., 1985). The corn was grown locally and had no history of fungicide treatment. The corn screenings were finely ground in a laboratory mill and used for chemical analyses.

Chemical Analyses. Moniliformin was extracted by vigorously shaking 1 g of the finely ground sample with 10 mL of distilled water for 30 min and centrifugation. Quantitative determination of moniliformin was done by HPLC on the clear extract without any further purification using an ion-exchange procedure as well as a paired ion-chromatography technique as described by Thiel et al. (1982). In both procedures the peak heights of the moniliformin peaks were compared to that of a moniliformin standard after separation of 20 μL of the extracts and detection of the peaks at 227 nm. The ion-exchange separations were done on a Partisil 10 SAX column (4.6 mm i.d. × 25 cm) using 0.01 M sodium dihydrogen phosphate (pH 5.0) as mobile phase at a flow rate of 1.0 mL/min. Paired ion chromatography was done on a μBondapak C_{18} column (Waters, 3.9 mm i.d. × 30 cm) using 0.1 M sodium phosphate buffer (pH 7.0, 0.005 M tetrabutylammonium hydrogen sulfate, 8% methanol) as mobile phase at a flow rate of 1.0 mL/min.

Fusarin C analyses were done as previously described by Gelderblom et al. (1984a). The sample (20 g) to which water (50 mL) was added was successively extracted with 150 and 100 mL of dichloromethane/2-propanol (1:1). The extracts were combined, dried, and successively extracted with petroleum ether and chloroform. The petroleum ether extract was reextracted with acetonitrile and the latter combined with the chloroform extract. The extracted material was fractionated on a silica gel 60 (Merck, 0.063–0.200 mm) column (2.5 × 16 cm) using methanol/dichloromethane (1:19) as eluent. Fusarin C was quantified in the column eluate by HPLC on an Ultrasphere column (Beckman, 5 μm, 4.6 mm i.d. × 25 cm) using methanol/chloroform (1:19) as mobile phase and detection at 360 nm.

T-2 toxin and diacetoxyscirpenol were extracted from the sample (20 g) with 100 mL of methanol/water (1:1). A 20-mL aliquot of this extract was transferred to a prepacked Extrelut 20 column (Merck Chemicals) from which the toxins were eluted with dichloromethane. The eluate was dried, redissolved in chloroform/hexane (1:2), and applied to a silica gel column equilibrated with chloroform. The column was washed with benzene followed by benzene/acetone (95:5), whereafter the toxins were eluted with ether. The dried fraction containing the toxins was dissolved in toluene/acetonitrile and derivatized with *N*-(heptafluorobutyryl)imidazole (Pierce Chemical Co.). The derivatized fraction was mixed with 0.1 M phosphate buffer (pH 6.0), and the two phases were allowed to separate. An aliquot of the organic phase was removed and the solvent evaporated under nitrogen. The residue was dissolved in benzene and analyzed by gas chromatography. Chromatographic analysis was performed on a Calor Erba Model 5300 gas chromatograph fitted with a split injector, a fused silica capillary column (SE-30, 25 m × 0.32 mm) and a ^{63}Ni electron capture detector. Quantification was done by comparison of peak heights against calibration curves obtained for derivatized standards of T-2 toxin and diacetoxyscirpenol.

RESULTS AND DISCUSSION

The corn screenings contained fusarin C and moniliformin at concentrations of 0.39 and 2.82 mg/kg, respectively, while no T-2 toxin or diacetoxyscirpenol could be detected (Table I). The presence of moniliformin was confirmed by the positive detection of a chromatographic peak at the retention time for moniliformin as well as by cochromatography with authentic moniliformin using both HPLC procedures (Thiel et al., 1982). The presence of fusarin C in the corn screenings was confirmed by cochromatography with fusarin C standard as well as by the demonstration that the suspected fusarin C peak breaks down to three additional chromatographic peaks upon exposure to long-wave UV irradiation in exactly the same way as authentic fusarin C (Gelderblom et al., 1984a). This is the first report of the natural occurrence of these two *Fusarium* metabolites in corn in the U.S.

Table I. Concentration of *Fusarium* Toxins in Corn Screenings

	concn, mg/kg		concn, mg/kg
fusarin C[a]	0.39	T-2 toxin	nd[c]
moniliformin[b]	2.82	diacetoxyscirpenol	nd

[a] Average of two determinations (0.34, 0.44 mg/kg). [b] Average of four determinations (2.71, 3.13, 2.26, 3.19 mg/kg). [c] nd = Not detected (detection limit 200 μg/kg).

Fusarin C and moniliformin have previously been reported to occur naturally in a sample of moldy, homegrown corn in Transkei. The levels of fusarin C detected in apparently healthy kernels from this sample and in visibly *Fusarium*-infected kernels were 0.02 and 0.28 mg/kg, respectively (Gelderblom et al., 1984a). The levels of moniliformin in the original sample and in the visibly *Fusarium*-infected kernels were 16 and 25 mg/kg, respectively (Thiel et al., 1982).

The major fungal contaminant of the sample of corn screenings was *F. moniliforme* (Wilson et al., 1985). Culture material of *F. moniliforme*, strain MRC 826, previously has been shown to cause LEM in horses (Kriek et al., 1981) and to be hepatocarcinogenic in rats (Marasas et al., 1984a). Although *F. moniliforme* has been reported to produce moniliformin and fusarin C, the significance of the presence of these two metabolites in the sample of corn screenings with respect to its relationship with LEM and hepatocarcinogenicity is not clear. It is highly unlikely that moniliformin is involved in the etiology of either of these two syndromes as it is not produced by any of the *F. moniliforme* isolates with which LEM has been reproduced experimentally in horses (Marasas et al., 1984b, 1986), nor is it a product of *F. moniliforme* strain MRC 826, the only isolate of *F. moniliforme* that has been shown to be hepatocarcinogenic in rats (Marasas et al., 1984a). Although fusarin C is highly mutagenic (Gelderblom et al., 1984a; Wiebe and Bjeldanes, 1981) and is produced by *F. moniliforme* strain MRC 826 (Gelderblom et al., 1983), it has not been shown to be carcinogenic nor is there any reason to suspect any relationship between fusarin C ingestion and LEM.

The *Fusarium* species isolated from corn screenings associated with the field outbreak of LEM included 64 cultures of *F. moniliforme* Sheldon, one culture of *Fusarium subglutinans* (Wollenw. & Reinking) Nelson, Toussoun, and Marasas, one culture of *Fusarium sporotrichioides* Sherb., and three cultures of *Fusarium equiseti* (Corda) Sacc. (Wilson et al., 1985). At present five cultures of *F. moniliforme* and one each of *F. sporotrichioides*, *F. equiseti*, and *F. subglutinans* are being tested for their ability to produce either moniliformin, fusarin C, or other unidentified toxic metabolites. It has now become even more important to identify the compound(s) responsible for the hepatocarcinogenic and LEM-producing activities of cultures of *F. moniliforme* strain MRC 826 (Marasas

et al., 1984a), especially since both these activities were detected in a field sample of corn in the U.S.

Registry No. Moniliformin, 52591-22-7; fusarin C, 79748-81-5.

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Functional Properties of Rapeseed Protein Products with Varying Phytic Acid Contents

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Rapeseed meal and three rapeseed protein isolates containing different levels of phytic acid (0.9–4.6%) were compared with soybean meal and a commercial soybean protein isolate in terms of functional properties. Rapeseed products exhibit lower nitrogen solubility, higher water absorption, lower moisture adsorption, and, in some cases, better oil absorption than the corresponding soybean products. In general, rapeseed products show similar or higher emulsifying activity and emulsion stability compared with soybean products. The low-phytate products have better emulsifying properties than their high-phytate counterparts. Rapeseed products are characterized by overall favorable foaming capacity and foam stability compared with soybean products. The foaming properties are concentration dependent and are influenced by NaCl and sucrose. The level of phytic acid barely affects the foaming properties of rapeseed products.

Several groups have developed processes for the production of rapeseed protein concentrates and isolates; the different approaches have been summarized in some reviews (Rutkowski, 1975; Olson and Anjou, 1979; Sosulski, 1983; Mieth et al., 1983).

A procedure was reported from this laboratory for preparing two rapeseed protein isolates of acceptable light colors in high yield, employing a countercurrent alkaline extraction of the meal protein and its subsequent two-step precipitation from the extract at pH 6.0 and 3.6 (El Nockrashy et al., 1977). In a later study, it was observed that conditions favoring a high yield of protein isolates also result in enrichment of phytic acid in the isolates (Blaicher et al., 1983). A procedure involving acidic extraction of phytates from rapeseed meal at pH 4.0 prior to countercurrent extraction of the protein at pH 11.0 and subsequent isoelectric precipitation of protein at pH 4.7 could eliminate most of the phytic acid from the isolate although the protein yield was thereby considerably reduced (Blaicher et al., 1983).

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The functional properties of rapeseed protein products have not been studied extensively (Sosulski, 1983). It is well-known that the processing steps employed as well as the associated nonprotein components can significantly influence the functional properties of protein products. In the present study, therefore, the functional properties of low- and high-phytate rapeseed isolates prepared by countercurrent extraction–isoelectric precipitation procedure (El Nockrashy et al., 1977; Blaicher et al., 1983) have been determined and compared with those of a commercial soybean isolate. A major objective of this study was to find if the phytic acid contents of the products had an influence on their functional properties.

EXPERIMENTAL SECTION

Materials. Rapeseed, *Brassica napus*, cv. Erglu, was defatted into rapeseed meal from which two rapeseed protein isolates, RPI-I and RPI-II, were prepared according to El Nockrashy et al. (1977) by countercurrent extraction of the meal protein followed by consecutive precipitations at pH 6.0 and 3.6, respectively. In addition, a low-phytate rapeseed protein isolate (RPI-III) was prepared from rapeseed meal by extraction of phytate at pH 4.0 prior to countercurrent extraction of the protein at pH 11.0 and its precipitation at pH 4.7 (Blaicher et al., 1983). Edible grade soybean, *Glycine max*, was ground, defatted by ex-

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