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Determination of optimal conditions for hydrolysis of conjugated deoxynivalenol in corn and wheat with trifluoromethanesulfonic acid

S.T. Tran, T.K. Smith*

Department of Animal and Poultry Science, University of Guelph, 50 Stone Rd. E., Guelph, ON N1G 2W1, Canada

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ABSTRACT

Deoxynivalenol (DON) is a common mycotoxin contaminating corn and wheat and conjugated forms are also present. Recent studies have suggested that current analytical methods for DON analysis in feedstuffs do not detect conjugated forms in the absence of hydrolysis. The aim of the current study, therefore, was to determine the optimal conditions in which conjugated DON in corn and wheat can be hydrolyzed by trifluoromethanesulfonic acid (TFMSA). The optimal hydrolysis procedure was determined based on reaction duration, reaction temperature and TFMSA concentration. Total DON concentrations were determined using ELISA with free DON concentrations determined by ELISA and GC–MS. The optimal hydrolysis conditions for determination of conjugated DON in corn were found to be 0.5 M TFMSA incubated for 20 min at 22 °C. Optimal conditions for wheat samples were 0.5 M TFMSA incubated for 40 min at 40 °C. Using these optimal hydrolysis conditions, 10 corn samples and 10 wheat samples were analyzed to determine the presence of conjugated DON. All samples contained conjugated DON with an increase of 8–70% for DON in corn following hydrolysis and an increase of 7–75% for DON in wheat. This hydrolysis procedure will aid in the accurate determination of total DON and conjugated DON in feedstuffs.

1. Introduction

Deoxynivalenol (DON) is one of the most important mycotoxins produced by many *Fusarium* species (Ueno, 1983) and is found as a common contaminant of cereal grains worldwide (Abramson et al., 1997; Tanaka et al., 1988; Stratton et al., 1993). DON was first isolated by Vesonder et al. (1973) from *Fusarium*-infected corn in the United States and named "vomitoxin" because of its capacity to induce emesis in swine. DON has been reported to cause a variety of toxicoses in animals and to potentially affect on humans (Pestka, 2007; Pestka and Smolinski, 2005; Rotter et al., 1996). There are marked species differences with respect to relative DON toxicity. The pig is most sensitive to DON, followed by rodents, dogs, cats, poultry and ruminants (Pestka, 2007; Pestka and Smolinski, 2005; Rotter et al., 1996). Feed-born DON can reduce production efficiency and cause serious economic losses to livestock and poultry producers (Charmley et al., 1995). Acetylated forms of DON (3-acetyl and 15-acetyl) have been reported to be minor contaminants with equivalent or lower toxicity than DON based on LD50 values in mice and are thus unlikely to pose any additional risk (Pestka, 2007).

Abbreviations: ANOVA, analysis of variance; DON, deoxynivalenol; ELISA, enzyme-linked immunosorbent assay; GC–ECD, gas chromatography with electron capture detection; GC–MS, gas chromatography–mass spectrometry; LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry; RCF, relative centrifugal force; SEM, standard error of the mean; TCA, trichloroacetic acid; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid.

* Corresponding author. Tel.: +1 519 824 4120x53746; fax: +1 519 822 7897.
E-mail addresses: tsmith@uoguelph.ca, trsmith@Alltech.com (T.K. Smith).

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Young et al. (1984) reported that the DON content of yeast fermented food products was higher than that of the contaminated wheat flour used for their production. The most plausible reason for this apparent increase was that the toxin had been metabolized by the wheat to a compound other than DON, which, under certain conditions, could be transformed back to DON. In another study, Trenholm et al. (1994) found that the decrease in feed intake by pigs is higher when a naturally contaminated wheat based diet is fed compared to the feeding of the same quantity of purified DON. It has been shown that a DON conjugate arising from plant metabolism exists (Berthiller et al., 2005). Conjugated DON (also referred to as masked or non-extractable DON) is, therefore, defined as a structurally modified DON bound to different compounds including glucose (Berthiller et al., 2005, 2007; Sasanya et al., 2008; Savard, 1991), fatty acids (Chakrabarti and Ghosal, 1986; Savard, 1991), glutathione and hemicellulose (Lamoureux and Runess, 1986). Such conjugated forms of DON may be hydrolyzed during digestion thereby generating free DON. Recent studies have suggested that common analytical methods for DON analysis in feedstuffs do not detect conjugated DON (Berthiller et al., 2005). This may be because these substances are more polar than the precursor compounds and are, therefore, difficult to extract with conventional solvents. They might also be discarded in the cleanup process because of their unknown physical or chemical properties.

Berthiller et al. (2005) recently described the use of LC–MS/MS, Liu et al. (2005) used GC–MS while Zhou et al. (2007, 2008) used GC–ECD for the determination of conjugated DON. The hydrolysis procedures used for determination of conjugated DON, moreover, included very high temperatures and high acid concentrations including trichloroacetic acid (TCA) (1 M) at 140 °C for 40 min (Liu et al., 2005) and trifluoroacetic acid (TFA) (1.25 M) at 133 °C for 53 min (Zhou et al., 2007, 2008). These acids, however, were only tested for their capability of catalyzing the reaction at relatively high temperatures. In addition to TCA (pKa = 0.08) and TFA (pKa = 0.3), TFMSA is amongst the strongest known monoprotic Brönsted acids (pKa = −13) (Howells and McCown, 1977). TFMSA and its conjugate base possess several important properties such as extreme thermal stability and a high resistance towards both reductive and oxidative cleavage. TFMSA does not provide a source of fluoride ions even in the presence of strong nucleophiles (Howells and McCown, 1977). TFMSA has also been reported to deglycosylate glycoproteins regardless of linkage and composition (Edge, 2003; Edge et al., 1981). Although these properties suggest that TFMSA should be an efficient catalyst, it does not yet appear to have been investigated for hydrolysis procedures in the determination of conjugated DON in cereals.

The objective of the current study was, therefore, to determine the optimal conditions in which conjugated DON in corn and wheat can be hydrolyzed using TFMSA.

2. Materials and methods

2.1. Chemicals and reagents

TFMSA (980 ml/L), TFA (990 ml/L) (Sigma–Aldrich, St. Louis, MO, USA) and TCA (980 ml/L) (Fluka–Chemie, Buchs, Switzerland) were used as catalysts for hydrolysis. Deionized water (Millipore, MA, USA) and sodium carbonate (Fisher scientific, ON, Canada) were used as extraction solvents or as a neutralization agent.

2.2. Samples

Ten naturally contaminated grain samples of corn and ten naturally contaminated grain samples of wheat from the 2006 and 2008 crops in Ontario, Canada were collected (500 g/sample) and stored in polyethylene bags at 4 °C until analyzed. Before analyzing, all samples were ground (Wiley mill, Arthur H. Thomas Co., Philadelphia, PA, USA) to a particle size of less than 1 mm.

2.3. GC–MS analysis

Corn and wheat samples were analyzed for free DON, 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol at the Laboratory Services Division of University of Guelph using a gas chromatograph coupled with a mass spectrometry as described by the Canadian Food Inspection Agency (2007). The limit of detection for these mycotoxins was 0.060 μg/g, 0.050 μg/g and 0.050 μg/g, respectively.

2.4. ELISA analysis

AgraQuant® DON test kits with a detection range of 0.012–0.250 μg/g (Romer Labs, Inc., Union, MO, USA) were used. These direct competitive ELISA test kits quantified free DON and total DON in corn and wheat using horse-radish peroxidase as the competing, measurable entity and measured using a microplate reader (Bio-Rad 550, Hercules, CA, USA). The cross-reactivities of the AgraQuant® DON kit are as follows: DON (100%), 3-ADON (>100%), 15-ADON (103%), T2-toxin (0%), nivalenol (0%), fusarinon-X (0%) and DON-3-glucoside (4.8%).

Naturally contaminated corn and wheat samples were analyzed for free DON according to Abouzied et al. (1991) using the extraction procedure recommended by manufacturer.