Determination of zearalenone (ZEA) in wheat samples collected from Jeddah market, Saudi Arabia

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The incidence of fungal contamination in wheat grains in Saudi Arabia market was frequently studied; however, the potential presence of Zearalenone was not examined thoroughly. Therefore, the objective of the current study was to explore the occurrence of Zearalenone and the fungal flora in wheat samples and to correlate between the detected Zearalenone level and the level of fungal contamination as well as the wheat production source. 30 samples were collected from food stores and mills located at Jeddah. Fungal flora was determined using PDA media and Zearalenone concentrations were determined using HPLC method. Out of the 30 collected samples only 18 samples (60%) showed fungal contamination that belongs to six genera (Aspergillus, Penicillium, Alternaria, Emericella, Eurotiun and Acremonium). Aspergillus species were the most dominant species in the infected samples where they represented 70.33% of the total infected species. Zearalenone was detected in 40% of the total samples. Only 25% of the local samples were positive to Zearalenone with a maximum level that reached 4,000 µg.kg⁻¹. The percentage of positive samples were higher in the imported wheat samples (50%), while Zearalenone maximum level reached 10,000 µg.kg⁻¹ and the overall mean was 1663 µg.kg⁻¹ compared to the local samples.

Key words: Zearalenone, ZEA, wheat grains, fungal contamination, Jeddah, Saudi Arabia.

INTRODUCTION

Plant parasites moulds are frequently found in livestock foodstuffs and alter the quality of grain by producing secondary metabolites (mycotoxins), having several types of toxic effects. Mycotoxins of major concern for human and animal health are produced by three main genera of fungi (Aspergillus, Fusarium and Penicillium) during crop growth, harvesting or storage. Fusarium mycotoxins are worldwide occurring in small-grain cereals and frequently reported in fresh or stored grains (Manka et al., 1985; D’Mello, 1997; Bottalico, 1998; Logrieco et al., 2002).

Zearalenone (ZEA) is considered as a natural contaminant of food with significant Fusarium infection of cereal origin components. Biosynthesis of the compound was observed after infection by several species of Fusarium (F. graminearum, F. culmorum, F. crookwellense, F. equiseti and F. semitecutem) in cereals such as corn, rice and wheat (Bennett and Klich, 2003).

The concentration of accumulated ZEA in cereals depends on several factors such as the substrate, temperature, duration of Fusarium growth and strain of fungal species. Moreover, the humid tropical environment promotes microbial proliferation on food and feedstuffs and finally mycotoxin biosynthesis (Nuryono et al., 2005). Zearalenone and its derivatives (a-zearalenol, b-zearalenol, zeranol, taleranol and zearalanone) can be produced by Fusarium spp. in corn stems infected by Fusarium in the field (Mirocha et al., 1979; Bottalico et al., 1985) and in rice culture (Richardson et al., 1985). However, the highest amounts of Zearalenone none are produced by Fusarium during storage whereas low amounts are synthesized during crop growth (Cheeke, 1998).

Several studies carried out in Europe and in transcontinental countries, reported the high incidence of ZEA in cereals and in animal feeding stuffs (Bottalico, 1998; Muller et al., 1998; Scudamore et al., 1998; Scudamore and Patel, 2000).

Bottalico (1998) reported the occurrence of ZEA and its derivatives at levels up to 2758 µg.kg⁻¹ and up to 175 µg.kg⁻¹ in cereal grains worldwide and in European countries (respectively). In the United Kingdom, maize for
human consumption and maize products used in animal feed have been found to have high concentrations of ZEA and its derivatives at ranges of 4 to 584 µg.kg\(^{-1}\) and 55 to 1400 µg.kg\(^{-1}\) (respectively) (Scudamore et al., 1998; Scudamore and Patel, 2000).

ZEA was regulated in 1996 by 6 countries, by the year 2003 ZEA was regulated by 16 countries. Limits for ZEA in cereals currently vary from 50 to 1000 µg/kg. Current regulations of ZEA in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO (2004).

Toxicity of Zearalenone and its metabolites, described as estrogenic properties, is related to the chemical structure of the mycotoxins, a structure similar to naturally occurring estrogens (estradiol, estrone and estriol). Interaction of such compounds with human estrogen receptors in competition with 17-bestradiol was also determined ZEA level and the source of the wheat.

MATERIALS AND METHODS

**Materials**

A total of 30 wheat grain samples (12 local and 18 imported) representing 8 production areas (2 local areas, Najran Province and Qassim Province as well as 6 importing areas Dubai, Australia, Sharjah, Syria, Oman and Egypt) were collected during 2008 from food stores and mills located at Jeddah, Saudi Arabia. Each sample was transferred to the laboratory immediately and was stored at low temperature (4°C) in a dark place.

**Methods**

**Mycological analysis**

Wheat grain samples (20 to 30 g) were collected and stored at 4°C for mycological analysis. Each sample was thoroughly mixed and a 10 g sub-sample was taken randomly for the analysis. The seeds were completed to 100 ml distilled water and shacked for 15 min at 200 rpm. Two dilution series of 1:10 to 1:10\(^5\) in sterile distilled water were prepared.

Aliquots (1 ml) of each dilution were dispensed into individual sterile 9 mm diameter Petri dishes, mixed with potato dextrose agar (PDA) (15 ml), allowed to cool and incubated at 25°C in dark for 7 days. Developed fungal colonies were microscopically identified and the number of colonies expressed as colony forming units per gram of the ground sample (cfu.g\(^{-1}\)). The fungal species were identified according to Raper and Fennell (1965), Von Arx (1974), Nelson et al. (1983), Mislivec et al. (1992) and Nelson (1992).

**Extraction of ZEA**

ZEA was extracted and determined in wheat samples according to the AOAC (2007). Wheat samples (50 g) was ground and placed in 500 ml flask with 25 g diatomaceous earth, 250 ml chloriform and 20 ml H\(_2\)O. The mixture was shacked for 15 min, then filtered through filter paper and 50 ml of the filtered extract was collected. The extract was transferred to 250 ml separating funnel and 10 ml NaCl was added and 50 ml of 2% NaOH solution was then mixed and vigorously shacked for 1 min. The lower CHCl\(_3\) layer was discarded and 50 ml of citric acid was then added. ZEA was extracted with 50 ml CH\(_2\)Cl\(_2\). After shaking, the lower phase was drained through 40 g anhydrous Na\(_2\)SO\(_4\) and 50 ml of CH\(_2\)Cl\(_2\) was used to re-extract the aqueous phase. The combined CH\(_2\)Cl\(_2\) extract was evaporated to dryness under nitrogen and the dry film was then dissolved in 0.5 ml mobile phase.

**ZEA determination using HPLC analysis**

ZEA was separated and determined using HPLC Agilent 1100 system equipped with quaternary pump model G1311A and autosampler model G1329A. C18 column (150 x 4.6 mm), 5 µm. Methanol, CH\(_3\)CN, and H\(_2\)O (1 + 1.6 + 2 v/v) was used as mobile phase at flow rate 2 ml.min\(^{-1}\). ZEA was detected using fluorescence detector set at 236 nm (excitation) and 418 nm (emission).

**RESULTS AND DISCUSSION**

The occurrence of fungal contamination in wheat grains in Saudi Arabia market was frequently studied; however, the potential presence of ZEA in Saudi market wheat was not investigated carefully. The fact that the source of ZEA contamination may be mainly due to the country source of wheat production and may not be due to contamination source during storage triggered the idea of exploring the correlation between the detected ZEA level and the wheat production source.

After surface disinfection, the fungal microflora...
contaminating the wheat grain samples were isolated after incubation at 25°C in dark for 7 days. Out of the 30 collected samples only 18 samples (60%) showed fungal contamination Table (1). The mean of the total fungal count was $3 \times 10^3$ colony/g and it was as high as $8 \times 10^3$ colony/g (for samples imported from Oman) and as low as $2 \times 10^2$ colony/g (samples imported from Australia and Sharjah). The local samples from Qassim were the second high fungal count ($6 \times 10^3$ colony/g).

In the 18 fungal infected samples, 11 species were purified and identified. They were mainly assigned to six genera namely Aspergillus, Penicillium, Alternaria, Emericella, Eurotium and Acremonium (Table 2). The Aspergillus species were the most dominant species in the infected samples representing 70.33% of the total infected species. This genus contained six different species namely Aspergillus flavus, A. versicolor, A. parasiticus, A. carneus group, A. niger, A. tamari. The A. flavus was the most dominant species (38.1%) compared to other isolated Aspergillus species and was also the most dominant species in the infected samples.
Table 3. Level of zearalenone in local and imported wheat grains from food stores and mills located at Jeddah, Saudi Arabia.

<table>
<thead>
<tr>
<th>Type</th>
<th>Local</th>
<th>Imported</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>No. of wheat types</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>No. of positive samples</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>% of positive samples</td>
<td>25%</td>
<td>50%</td>
</tr>
<tr>
<td>Range of Zearalenone (µg.kg$^{-1}$)</td>
<td>ND - 4,000</td>
<td>ND - 10,000</td>
</tr>
<tr>
<td>Overall mean (µg.kg$^{-1}$) ± SE</td>
<td>996 ± 520.2</td>
<td>1663 ± 900.5</td>
</tr>
</tbody>
</table>

Figure 1. HPLC chromatogram of zearalenone standard.

dominant species (26.79%) compared to the total isolated species. *A. versicolor* and *A. parasiticus* were the second dominant species with a percentage of 14.97% of the isolated *Aspergillus* species and 10.53% of the total isolated species. *A. carneus* group was the lowest percentage of *Aspergillus* species with 6.8% of *Aspergillus* species and 4.78% of the total infection percent (Table 2). It was also observed that the infection percentage of all the other isolated species was as low as the percentage of *A. carneus* group except for the *Alternaria alternata* that was 9.56% of the total infection.

The fact that *Aspergillus* species was the most dominant species in the wheat samples under study was reported by different investigators in different areas around the world especially in the warm and subtropical regions (Mantle, 2002; Hocking and Pitt, 2003). Also the total fungal count that was reported in the current study and ranged from 200 to 8000 colony/g was also reported in Germany in a study on whole wheat flour and white flour where they find that the count ranges from 1730 to 1830 colony/g and the most dominant species is belonging to *Aspergillus* and *Penicillium* genera (Weidenborner et al., 2000). *Aspergillus* and *Penicillium* are the dominant species in reports of fungal isolation from wheat and corn flour in Egypt, Australia, Spain and Algeria (Abdel-Hafez et al., 1990; Berghofer et al., 2003; Cabañas et al., 2008 and Riba et al., 2008).

ZEA was detected in 40% of the total samples. Only 25% of the local samples were positive to ZEA with a maximum level that was reached 4,000 µg.kg$^{-1}$ (Table 3). The percentage of positive samples were higher in the imported samples (50%) and were higher in ZEA maximum level (10,000 µg.kg$^{-1}$) as well as the overall mean (1663 µg.kg$^{-1}$) compared to the local samples (Table 3). It should be noted that the HPLC method that was used in this study to detect ZEA was sensitive enough to detect low concentration of ZEA as low as 2 µg.kg$. Figure 1 showed the HPLC chromatogram of ZEA standard where Figure 2 showed the highest (10,000 µg.kg$^{-1}$) ZEA concentration that was detected in the samples under study.

Table 4 indicated that positive samples from local
production areas where only in the Najran province while all samples from Qassim province were negative to ZEA. Although samples from both local areas showed the same percentage of positive samples of fungal infection (50%) and both of them showed no Fusarium infection. It should be noted that the dominancy of Aspergillus and the absence of Fusarium in the collected samples is an indication of the effect of the warm storage condition in Jeddah not the effect of the cold condition that normally exist during wheat cultivation where the Fusarium is normally among the dominant fungal species in wheat grown in the temperate zone and in the subtropical areas during winter season throughout Europe, Africa, Asia and the Americas (Hajieghrari, 2009; Kammoun et al., 2010; Brown et al., 2010). In Suadi Arabia Fusarium species is found in wheat as well as in other crops in different regions in the Kingdom including Qassim and Najran (El-Meleigi et al., 1990; Al-Kherb et al., 1996). This mean that the presence of Fusarium in some areas (namely Najran and Qassim) is not necessarily means the presence of Fusarium toxins in wheat samples as indicated in this study by the presence of ZEA in the Najran samples without any detection of Fusarium infection (Table 4).

Although, the imported samples has higher percentage of positive samples and higher ZEA level, however, the data in Table (4) showed that the highest level was obtained from wheat samples imported from Dubai. Although Dubai, is not a producing country but it rather a focal trading point in the Middle East region which means that the source of this contaminated wheat is unknown and may be formed in the countries of production.

Comparing the fungal infection data in Table 2 with the ZEA level in Table 4, it was noticed that none of samples have Fusarium infection which means that the source of infection is most likely due to Fusarium that was occurred in the field or during storage in the production regions. Sanchis and Magan (2004) reported that there is no correlation between ZEA production and its fungal biomass suggesting the important influence of environmental stress on toxin production which often unrelated to total fungal biomass. On the other hand, the absence
of toxigenic fungi does not guarantee that a commodity is free of mycotoxins, since the toxins may persist long after the moulds have disappeared (Shapira and Paster, 2004).

ZEA contamination in wheat is a world wide problem where several reports documented the presence of ZEA in wheat samples of Brazil, Switzerland, Netherlands, Bulgaria and Germany in a level ranged from 2 to 2000 µg.kg⁻¹ (Furlong et al., 1995; Bucheli et al., 1996; Tanaka et al., 1990; Perkowski et al., 1990; Vrabcheva et al., 1996; Schollenberger et al., 2005). The Fact that the European Union sets a maximum level for ZEA of 100 µg.kg⁻¹ in most unprocessed cereals and grains (Lawley et al., 2008) and that 20% of the investigated samples in this study exceed that limit more than 20 folds indicated the real hazard of ZEA contamination not only in the imported samples but also in the local samples. This result should trigger the close attention to control such hazard at the local basis and to restrict the regulation for the imported commodities. Also, the absence of Fusarium and the presence of ZEA should drew the attention to the importance of the routine monitoring of the mycotoxins as the existing hazards and the importance of the detection of the mycotoxicogenic fungi as indication of the existing and the potential hazards during storage.

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REFERENCES


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