

EVALUATIONS OF ZEARALENONE CORRELATION IN WHEAT/FLOUR OBTAINED FROM NORTH, WEST AND SOUTH REGIONAL PROVINCES OF IRAN USED FOR BEAKERY

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ABSTRACT

Zearalenone (Mycotoxin F2) is a mycotoxin produced by genus *Fusarium* in cereals. This mycotoxin is a non-steroidal estrogenic-like that causes reproductive problems in animals (infertility, abortion) and hyper estrogens in man. Since *Fusarium* species are commonly associated with cereals can produce several secondary toxic metabolites the samples collected from provincial premier and preparation of cell extracts then toxin estimation were done by ELISA, so that zearalenone to be analyzed. The wheat pollution were in the range of 8.700 ppb , Minimum; 11.140 ppb, Maximum; 19.840 ppb comparisonly of flour by Range ; 6.710, Minimum; 14.920, Maximum ; 21.630 have health treating toxin levels according to the standard values for man and animal feed hence could be serious attention to the cumulative effects of toxin, a serious risk and should be overlooked about the conducted cities and provinces. The maximum values off respectively were more than standards up to 50% , so a serious risk are considered. The aim of this study was to determine the contamination of wheat grains in one of the chemically risk factors (toxins zearalenone) in superior territories in Iran.

KEY WORDS: Zearalenone, Wheat, Flour, Territories of Iran

Introduction

Food contamination by toxigenic molds increased attention over the last three decades. Wheat is one of the most important grains consumed in the world. Most grains, such as wheat, maize, bean and rice can be infested by fungi genera can produce toxic secondary metabolites namely mycotoxins, which impact on food safety. Currently, more than mycotoxins have been identified in the world but the most important groups of mycotoxins are the major health concern for humans and animals and occur quite often in food, including: AFs , OTA , trichothecenes (deoxynivalenol, nivalenol), ZEA and fumonisins. During growth Substrate moisture (>20 %), air temperature and relative humidity (< 90 %) provide “field fungi” excellent environmental conditions for development before the harvest (Cvetnic *et al.* , 2004). Zearalenone, an estrogenic metabolite, commonly occurs with DON in cereal crops, its derivatives (a-zearalenol , b-zearalenol , zearanol , taleranol and zearalanone) can be produced by *Fusarium* spp. However , low amounts are synthesized during crop growth whereas the highest amounts of Zearalenon ZEA are produced by *Fusarium* during storage (Hazmi., 2010). Zearalenone induces feminization at dietary

concentrations of less than 1 ppm, whereas higher concentrations interfere with conception, ovulation, implantation, fetal development and the viability of newborn animals. Previous studies conducted on the effect of milling on fusaria toxins in wheat showed that they were a hard effect on zearalenone each year for all districts have been characterized by above-normal total precipitation for most of the spring and summer of the years, heavy and frequent rains saturated fields resembling Such a condition favors growth and toxin production by *F.graminearum* produces DON and zearalenone. Major objectives on mycotoxin produced by genus *Fusarium* in cereals contribute to determine the distribution and level of DON and zearalenone in milled fractions and wheat milling performance study (Trigo-Stockli *et al.*, 1996). The presence of ZEA in food stuffs may cause hyper estrogenism in women, as they are highly sensitive to the action of estrogenous hormones (Hazmi., 2010). Therefore, a rapid and sensitive technique for routine assays of mycotoxins in foods is necessary. There are several types of chromatography methods available for mycotoxins analysis. Traditionally the most popular methods used for mycotoxins analysis are chromatography and capillary electrophoresis require extensive sample preparation and are expensive. Methods for the detection of mycotoxins are mainly based on chromatography and Immunochemistry (Jeroen *et al.* , 2013). Over the last years, the importance and application of immunoassays, especially enzyme-linked immunosorbent assay (ELISA) has grown significantly. ELISA test kits became very popular recently due to their relatively low cost and easy application and their results could be compared with those obtained by other conventional methods such as TLC and HPLC (Feizy *et al.*, 2014). Several studies carried out in European/transcontinental countries, reported the highest incidence of ZEA in cereals and in animal feeding stuffs. Bottalico (1998) reported the occurrence of ZEA/derivatives at levels up to 2758g.kg⁻¹ and up to 175g.kg⁻¹ in cereal grains worldwide and in European countries (respectively). ZEA was first regulated in 1990 decades, by the year 2003 ZEA was regulated by more and more countries. Limits for ZEA in cereals currently vary from 50 to 1000µg/kg. Current regulations of ZEA on foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO(2004). Toxicity of Zearalenone/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-betaestradiol was also reported. The estrogenic potency of ZEA has been shown to be several times higher than that of other environmental estrogens in various test assays (Hazmi., 2010). The aim of this study was to determine the contamination of wheat grains and flour as one of the important risk factors (toxins zearalenone) in superior territories in Iran.

Materials and Methods

Fresh wheat samples harvested from the early May to late September from 7 superior wheat cultivating shores, for every one hundred of samples provided 100g were randomly selected in order to sample measurements per 10 tonnes of origin, sample control, sample stock and the sample was prepared for flour, and Wheat samples were then taken and milling process were done after combination. Releasing toxins in solution using solvent extraction, separation was done with the solvent containing 40ml methanol, 40ml ethanol and 20ml of acetone up to 20ml For each 10g chopped/mailed sample at first which transferred to a falcon tube container will previously 20ml NS and 20ml of solvent Extract to be shaken for 30minutes and heading and then transferred to a

water bath to reduce values to 10ml and then extracts separated using a filter paper WhatmanNo.1 flat that operating with simultaneous transfer of 10ml of deionized distilled water to wet the filter and also dilute the extract and speeding the movement take place. Finally 50 microliters were used for ELISA testing.

Elisa Determination

To detect aflatoxin levels in the fungal biomasses and the culture medium samples using the Competitive ELISA Procedure as described by R-Bio-Pharm GmbH was used and measured at the observance of 450nm (Rosi et al., 2007).

Results

The total number of wheat samples collected from the North, West and South of Iran, in seven provinces, including Khuzestan, Golestan, Ardebil, Zanzan, Kermanshah, Hamedan, where 14 out of 14 cities and shopping centers (Figure1). According to sampling distribution criteria that is indictable the number of samples obtained from regions shows the Northern belongs a frequency of 71.4 percent, the highest, Westerns by a frequency of 21.4% finally the lowest Southern bring its frequency of 7.1 percent. According to the amount of the normal distribution zearalenone measured in grain samples were Range:8.700, Minimum:11.140, Maximum:19.840 (Skewness; 0.758, Kurtosis; -0.508) comparing to the amount of toxin normal distribution Range:6.710, Minimum:14.920, Maximum:21.630 (Skewness;0.539, Kurtosis; -0.102) observed in bread making flour maintain compliance with the standards and practices conserving National average nutritional values approvals (authorising the mixing wheat flours) found that the amount of toxin in wheat and flour have no significant correlation despite reverse relationbut not statistically significant Supporting by the Pearson statistical determinations a significant correlation (NPar-Wilcoxon Signed Ranks Test for FZea-Wzea; Z; -2.542a, Asymp-Sig; 0.011, Pearson Correlation; -0.099 Sig: 0.737). In examining sample numbers/obtained measurements of toxin shown, concerning the highest Zearalenon measured zone were at interval ranges and because the number of samples have been accumulated tended the higher range of the curve to the right, a normal curve is resulting in drawn (Figure 2).

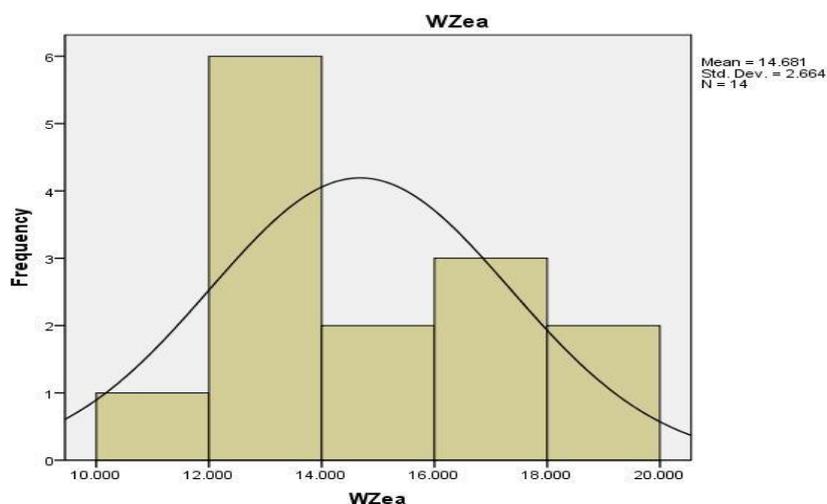


Figure 1: Normalized distribution frequency of obtained wheat samples Zea-toxin, of the different Ranges.

Range: 8.700, Minimum: 11.140, Maximum: 19.840, Skewness; 0.758, Kurtosis: -0.508

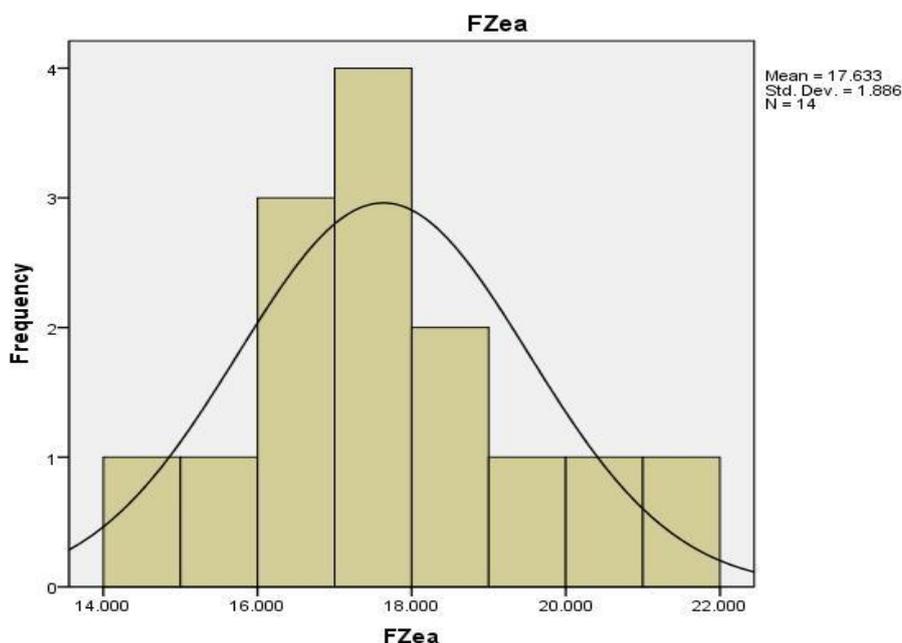


Figure 2: Normalized distribution frequency of obtained wheat flour samples Zea-toxin, of the different ranges

Range: 6.710, Minimum: 14.920, Maximum: 21.630, Skewness; 0.539, Kurtosis; -0.102
NPar-Wilcoxon Signed Ranks Test for FZea-Wzea; Z; -2.542a, Asymp-Sig.; 0.011, Pearson Correlation; -0.099 Sig: 0.737

Counter currently a significant degree of correlation between the numerical differences between the processed wheat flour toxin values and zearalenone in wheat and its disalignment quite reasonable ($Z:-2/54$, $Sig:0/01$) entirely due to the presence of toxin-producing agents in the process of harvesting, handling, storage, meal preparation and suspectively to take place in the packages and also the Increased further. However, the per capita consumption of wheat flour in bread and bread made especially for the cumulative effect of the toxin, that not be negligible.

Discussion

In moderate climates, the occurrence of *Fusarium* and their toxins in cereals is predisposed primarily by wet and cold vegetation periods requisite preventive measures against the multiplication of fungi and toxin production include tearing of well-dried grains at optimal conditions. An inevitable part of the preventive measures are regular foodstuffs monitoring with Mycological and Mycotoxicological examinations. Contamination of feed with a *Fusarium* toxin can lead to impaired immune functions, metabolism disorders, decreased performance, and increased susceptibility to adverse environmental influences. Having carcinogenic potential and poisonous effects, mycotoxins are considered to be one of the most important regulatory issues.

Contamination of feed with mycotoxins is often a worldwide problem since there is no universal procedure that removes most of the mycotoxins without any effect on the nutritional value or not make it more expensive to produce (Egmond et al., 2003, Ghiasian et al., 2006). Unfortunately, a limited number of mycotoxins including aflatoxins, fumonisins, zearalenone and ochratoxins have only been measured only in export products, but they are not usually checked in foodstuffs for domestic consumption in Iran. In relation to the results of previous research for DON and ZEA in Croatia and also with the published data worldwide, it can be concluded that a certain number of feed samples in this research had significantly high concentrations also comparing the obtained concentrations of ZEA with the maximum recommended concentrations of these mycotoxins in feed the results indicated an increased contamination of whom feed with DON and ZEA with mean concentrations, higher than recommended, respectively. DON and ZEA could be determined with great variety of concentrations, of DON generally was higher than the recommended in about 10% of samples (Pleadin et al., 2012). A higher ZEA concentration than the maximum recommended was determined in about 2% of the total number of samples, determined in the western then the northern part of the country. In this study, it was also observed that the samples in which lower concentrations were determined have predominantly more concentrations of ZEA or both samples always could be detected, or mostly the results indicate on both higher concentrations as in our study performed on processed wheat flour obtained by mixing imported wheat crops. The wheat pollution was in the range of 8.700ppb, Minimum:11.140 ppb, Maximum:19.840ppb comparison of flour by Range; 6.710, Minimum:14.920, Maximum:21.630 has health threatening toxin levels, according to the standard values of man and animal feed hence could be serious attention to the cumulative effects of toxins, a serious risk and should be overlooked about the conducted cities and provinces. Zearalenone side-effects on health are undeniable, due to the chronic and acute effects of zearalenone for consumers is qualified to provide sufficient information about its exposure to the general population. In the past studies, zearalenone mycotoxin in Tafton, Sangak, Barbari and Lavash bread distributed in Kermanshah (Iran western territory) indicated that all samples were contaminated with zearalenone and the most bread samples had contamination higher than of

Europe standards, but had consonant with Iran national standard, such amount of higher than standard was not observed surprisingly confirm our results about the original wheat crops and processed wheat flour for bread making. Kazemi et al in 2009 studied storage wheat in the Azerbaijan state perceived that 90% of samples were polluted by mycotoxins. In the Yazdanpanah study on the assessment encounter of Tehran's population of the zearalenone mycotoxin of 72 samples of rice, bread, popcorn and wheat flour were, according to the Iran standard samples below the maximum tolerable ZEA contamination in the food that is similar to results obtained from our study. In the Golestan state study by Karami et al 8.6% of the wheat samples were ZEA-contaminated, unlike our study the extent of the contamination was not wide. Mean and range of pollution were determined respectively, that was to be less than the recommended amount of zearalenone in wheat. According to sampling distribution criteria that is indictable the number of samples obtained from regions shows, the Northerns belonging a frequency of 71.4 percent, the highest, Westerns by a frequency of 21.4 % finally the lowest Southern bring its frequency of 7.1 percent. According to the amount of the normal distribution zearalenone measured in grain samples were Range ; 8.700 (Skewness; 0.758, Kurtosis; -0.508) comparing to the amount of toxin normal distribution Range; 6.710 (Skewness; 0.539, Kurtosis; -0.102) observed in bread making flour maintain compliance with the standards and practices conserving National average nutritional values approvals (authorising the mixing wheat flours) found that the amount of toxin in wheat and flour have no significant correlation despite reverse relation but not statistically significant Supporting by the Pearson statistical determinations for significant correlations (NPar-Wilcoxon Signed Ranks Test for FZea – Wzea ; Z ; -2.542a , Asymp-Sig: 0.011, Pearson Correlation;-0.099 Sig:0.737). In examining sample numbers/obtained measurements of toxin shown, concerning the highest zearalenon measured zone were at interval ranges, and because a number of samples have been accumulating tended the higher range of the curve to the right, a normal curve is resulting in drawn (Figure 2). According to the JECFA average of absorption this toxin of all the samples is less than the to tolerable daily uptake. Daily intake of zearalenone reasonably showed that such breads are recognized dangerous of view and have stringent security to eliminate or reduce this toxin is thought by the authorities since not aggregation in occasion the effects of mycotoxins on human health , economic status and sensitivity to the toxin has caused the standard employed for each country is different. Few studies have examined the contamination of zearalenone in cereals. A survey of zearalenone mycotoxin contamination of cereals and other crops in other countries has led to different results. Schollenberger showed that only two cases were free -fusarium pollution toxins and other sample were contaminated with one or more mycotoxins of sample of wheat, barley, corn and corn products, shows many similarities to have been done in such a way so that the high prevalence of mycotoxins in samples had evidence. In the Chelkowski study to determine zearalenone in wheat field wheat crops before harvest in central, northern and southern Netherlands were studied. Fusarium species were found in 48% of surveyed samples. In our study wide scale contamination of original wheat crop grains and processed wheat flour for bread making to Zearalenone is a potent indicator in lack of proper storage conditions of grain and flour. Unfortunately, the origin of the wheat crops and processed wheat flour for bread making samples contaminated with zearalenone that were examined in this study exactly, indicating that is the post harvested products from the state or other states and other countries imported. Daily intake of zearalenone is considered hazardous and should be stringent those results suggesting that the type of bread and flour in terms of contamination, showed no significant differences and was accounted

the lowest and the highest contamination levels of the toxin. Attention to this subject that bread is one of the most widely used food substances in cereal series, over prevalence contamination to zearalenone in wheat samples of various aspects can be considerable seriously. Thus, according to the results of those studies indicate that the extent of contamination flour to the zearalenone toxin. In case of contamination with levels above the limit of the cycle is eating out. The occurrence of mycotoxins produced by *Fusarium* spp. In small cereal grains, particularly in wheat, is of great concern worldwide, because their presence in processed feeds and foods seems unavoidable. Consequently, they have been associated with chronic or acute mycotoxicoses in a lesser extent, in humans. Our results are in agreement with other studies in the USA, Canada, Argentina and Europe, in studies conducted in this investigation, the data relating to the north of the country, with 71.4%, the West 21.4% and south of the country with 7.1% and in terms of results and the toxins zearalenone with conduction study are consistent, Chehri et al., 2010, (Figure 1 and Table 1). The frequency of the samples studied in this investigation has shown the Northern regional location of sample collection have contributed to the production of wheat, which is consistent with conduction study. Although the distribution of zearalenone concentration in the ranges considered, are not significantly correlated are Countercurrent (Table 3, Figure 3). But it should be noted that most of zearalenone concentrations were in the range of 12-14ppb, which may indicate endemic fungal causative agents of Zearalenone in the conducted geographical areas. The maximum values of found respectively were more than standards up to 50%, so a serious risk are considered. The aim of this study was to determine the contamination of wheat grains in one of the chemical risk factors (zearalenone toxin) in Superior territories in Iran. (Figure 3). Given that the largest amount of toxin production observed in the range of 12-14ppb, therefore, this suggests the possibility of fusarium infection in all studied wheat fields or warehouses for temporary maintenance or transportation process. Storepitt here (Figure 3 and Table 1). The highest possible average toxin production due to the plurality of samples collected from the area north and south and then to the West country (Figure 1 and 2). Based on the results of samples collected from the milling process, there are no significant differences, although pollution levels above the limit. According to the results of this research can be said that of all the major steel-producing fusarium toxin, zearalenone, is at intervals after planting and cultivation remains, and in the longer termed mains and can cause contamination off an arm and food products there for years. This level of contamination varies according to geographical regions, but in the process of turning wheat into flour. Contamination by toxins such as zearalenone toxin may be somewhat reduced and sometimes increased. Comparing the results of studies in other countries, it can be concluded that the major items of potential contamination of food due to fungi and toxins exist and should be harvested at all items. Human nutrition in gradients, apply to the use of international standards and conditions for shipping they keep creating. Another interesting point is that the harvest at the end of the line. Production or the food to be less time consuming, less chance of infection.

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