ABSTRACT

Wheat is one of the most important grains consumed in the world. Food contamination with toxigenic moulds increased attention over the last three decades, which impact on food safety. Fumonisins, the TCs and ZEA are hazardous for human and animal health during normal food processing, therefore, a rapid and sensitive technique for routine assay of mycotoxins in foods is necessary. Attention to this subject that bread is one of the most widely used food substances in cereal series, it is important to continue to monitor the occurrence of these mycotoxins in cereals and cereal products. 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 (Kits and Rida Screen T-toxin/Fumonisin analysis R-Bio-Pharm GmbH) on the samples so that T-toxin/Fumonisin to be analyzed. According to the amount of fumonisin toxins measured in grain and flour samples comparing to the amount of toxin observed in bread flour maintain compliance found that the amount of FT/FFum (NPar-Wilcoxon Signed Ranks Test for F tox - FFum; Z: -2.480a, Asymp-Sig: 0.013, Pearson Correlation :0.266, Sig:0.358) for T-toxin correlations in wheat flour incomparison have no significant correlation despite reverse relations for the both, but not statistically significant was supporting by the Pearson statistical determinations a significant correlation for the two variables incontrast were statistically meaningful in numerical differences (P<0.05 ). The determinative pollutions of wheat flour samples were in the Mean; 28.764 ppb, Range; 30.100 (Min; 11.100, Max; 41.200 ppb), Var; 65.922, Std. Dev; 8.119, Skewness; -0.614, Kurtosis; 0.590 for T toxin and for Fumonisin (Mean; 21.422 ppb, Range; 5.670 (Min; 19.290,Max; 24.960 ppb),Var; 2.623,Std.Dev; 1.620;Skewness; 0.712, Kurtosis; 0.209) as Statistic definitions were showed a considerable disalignmented correlation specially at a Pearson Correlation; 0.408/0.266, Sig; 0.358, Sig; 0.001-0.013 values. Thus according to the standard values for feed  and food could be serious attention to the cumulative effects of toxins, a serious risk and that should not be overlooked about the cities and provinces where conducted .The maximum values of found respectively were more than standards 50% up to 100%, so a seriousrisk are considered.

INTRODUCTION

Mycotoxins are natural food and feed contaminants, in conventional cereal production, considering to constitute a potential risk for human and animal, are not only dangerous for the Public Health, but they also deteriorate the, causing tremendous economic losses (Solarska et al., 2009, Rashedi et al., 2012) increased attention over the last three decades, which impact on food safety (CVETNíc et al., 2004). The most important Fusarium mycotoxins are Fumonisins, TCs such as T-2, HT-2, DON, DAS, FUS-X, NIV, diacetylvalenol, neosolaniol and ZEA, are common mycotoxins throughout the world, mainly associated with cereal crops, in particular corn, wheat, barley, rye, rice and oats (Ghazvinian et al., 2011, Shephard et al., 2000, Mankeviciene et al., 2011) Infection of cereal grains with Fusarium species can trigger serious human and animal diseases (Jereon et al., 2013, Mankeviciene et al., 2011). However, low amounts are synthesized during crop growth whereas the highest amounts are produced by Fusarium during storage (Hazmi, 2010, Trigo et al., 1996, Sadeghi et al., 2012, Rashedi et al., 2012). Fusarium species are probably the most prevalent toxin-producing fungi of the northern temperate regions and are
commonly found on cereals grown in the temperate regions of America, Europe and Asia might occur more frequently in the warmer and subtropical part of the world (Mankeviciene et al., 2011, Trigo et al., 1996). Fumonisins, the TCs and ZEA are hazardous for human and animal health as they are commonly found on cereals grown in the temperate regions of Europe, America and Asia. The toxin is probably the most important of the northern moderate regions (Ghazvinian et al., 2011). The toxin is commonly found world-wide on cereals products are prevalent used in feed and farm animals may thus consume relatively high amounts as Wheat is one of the most important grains consumed in the world. Epidemiological evidence indicates a link between human esophageal cancer and ingestion of Fusarium contaminated corn (WHO 2001). These are in cereals associated with the incidence of a high rate of human esophageal cancer in Africa, in northern Italy, in Iran, the Southeastern of the United States and with promotion of primary liver cancer in certain endemic areas of the People’s Republic of China (Feizy et al., 2014, Pleadin et al., 2012, ). There are no confirmed biomarkers for human exposure (WHO 2001), whereas higher concentrations interfere with the health status. Major objectives on mycotoxicity produced by genus Fusarium in cereals contribute to determine the distribution and level of TCs, Fum,Don and Zea in milled fractions and wheat milling performance study (TRIGO-STOCKLI et al., 1996) although the allegation that TCs were responsible for the reported symptoms is controversial (Ghazvinian et al., 2011). Following By now, causing most concern are T-2, which is the most acute toxic TC, HT-2, and NIV (Jereon et al., 2013, Feizy et al., 2014, Ghazvinian et al., 2011). Generally, occurs together cereals products found in various cereal crops such as wheat, corn, barley, oats and rye and processed grains (malt, beer and bread) (Jereon et al., 2013). Since T-2 tetraol is the most stability metabolite, it is the most appropriate metabolite for diagnostic testing (Marasas et al., 1996). T-2-contaminated products can cause severe effects in humans/animals at the same time it may even result in death (Trigo et al., 1996) and cytotoxic effect. The ATA Committee noted that IARC (1993) concluded that no data were available on the carcinogenicity to humans (Feinberg et al., 1989), KARAMI et al., 2008, Solarska et al., 2009, Mallmann et al., 2001). DON is a mycotoxin that commonly contaminates cereal-based foods worldwide. It is detected often at the ppm level (Mallmann et al., 2001, Remza et al., 2014). DON is generally found in various cereal crops such as wheat, barley, oats, rye, rice and corn. Natural occurrence of DON in cereals is certainly prevalent and surveys from South America, Canada, China and many countries of Europe have showed contamination levels in excess of 50% in oats, barley and wheat with mean concentrations as high as 9 mg/kg in barley (Feizy et al., 2014, Ghiasian et al., 2006). DON is the most often occurring TC and is prevalent in crops used for food and feed production (Solarska et al., 2009, Riazipour et al., 2012, Feizy et al., 2014). There have been reports that in Asia of illness in humans, associated with the consumption of cereals contaminate with DON and possibly much lower doses of other TCs (Feizy et al., 2014). In 1993, IARC placed DON in Group 3, not classifiable as to its carcinogenicity to humans (Trigo et al., 1996, Tanaka et al., 2007). A provisional maximum tolerable intake (PMTDI) of 1 μg/kg body weight (BW) was set by (Mallmann et al., 2001). NIV occurs more often in years with dry and warm growing seasons. NIV is more frequently reported in Europe, Australia and Asia than in America. Both mean levels and incidence of positive samples of NIV are lower than for DON even in the Nordic countries and Europe (Egmond et al., 2004, Tanaka et al., 2010, Marasas et al., 1996, Khosravi et al., 2013, Alizadeh et al., 2012). Toxic effects of DAS in humans and animals seemed similar, Also, the hematopoietic system appeared extremely sensitive, showing, as teratogen and esophageal hyperplasias. It is not mentioned very frequently in the research, as the main interest for TCs concerns DON, T-2 and HT-2 (Riazipour et al., 2012, Anne et al., 1995). ZEA is a non-steroidal, estrogenic mycotoxin, produced by Fusarium spp. are of two general types: 1) the nonsterogenic TCs, including DON, NIV, T-2, and DAS; 2) the mycoestrogens, including ZEA and zearalenol (Rashedi et al., 2012). The most important characteristic of Fusarium species is their ability to synthesize ZEA, and its co-occurrence with certain TCs raise important point regarding additive and/or synergism in the etiology of mycotoxicoses in animals (Schollenberger et al., 2006). TCs are stable compound both during storage/milling and the processing/cooking of food. It does not degrade at high temperatures. ZEA is found, especially, as a contaminant in corn. Also, it may occur problems in specific animals and possibly in humans (Trigo et al., 1996, Sadeghi et al., 2012, Khosravi et al., 2013). Any compound with activity may be genotoxic and/or carcinogenic, may be an important etiologic agent of intoxication in young children or fetuses exposed (Feizy et al., 2014). ZEA was evaluated by the International Agency or Research on Cancer in 1993, based on inadequate data in humans and limited evidence in experimental animals, allocated, together with other Fusarium toxins, in group 3 (not classifiable as to their carcinogenicity to humans). Therefore, a rapid and sensitive technique for routine assay of mycotoxins in foods is necessary. There are several types of chromatographic methods available for mycotoxins analysis. Methods for the detection of mycotoxins are mainly based on chromatography and immunochemistry. Traditionally the most popular methods used for mycotoxins analysis are thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE) (Jereon 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 comparable 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 high incidence of Fusarium toxins in cereals and in animal feeding stuffs. Mycotoxin contamination in cereals is a potential risk to human and animal health. Among several hundreds of mycotoxins, T, Fum and Zea toxins are among the most important mycotoxins regarding food safety (Anne et al., 1996). Furthermore, an association between high rates of human esophageal cancer and high concentration in cereals has been reported in different countries (Trigo et al., 1996). Wheat and rice are as the most important staple food for the human population Worldwide, in particular in the Middle East These grains are of the highest worldwide production as well as corn (Pleadin et al., 2012). Although contamination by the legal limits vary significantly both from country to country and by mycotoxin type and matrix; the determination methods need to provide accurate and reproducible results both within and between laboratories. Current regulations of fusarium toxins in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO (2004). The risks of fusarium toxins have been evaluated by The World Health Organization’s International Programme on Chemical Safety (IPCS) and the Scientific Committee on Food (SCF) of the European Commission. They determined a tolerable daily intake (TDI) for Fumonisins, TCs such as T-2, HT-2, DON, NIVand ZEA, alone or in combination of μg/kg/body weight (Riazipour et al., 2012). Cereal products are important in our food chain and economy. Therefore, foodstuffs need to be controlled/analyzed during food processing and all mycotoxin analyses for the entire food chain has importance for human health. It is important to continue to monitor the occurrence of these mycotoxins in cereals and cereal products. The aim of this study was to determine the contamination of wheat grains as one of the important risk factors in Superior territories in Iran.

MATERIALS AND METHODS

Fresh wheat samples harvested from the early May to late September 2014 from 7 superior wheat cultivating shores, including the southern provinces (Khoozestan), Western (including Kermanshah, Hamedan) and Northern (including Zanjan, Ardebil, Mazandaran, Golestan), for every one hundreds of samples provided that, after preparation, drying / adjusting humidity, mixing and re-mixing for each four particular samples of 100g per 10 tone of origin were randomly selected in order to sample measurements, sample control, sample stock. The sample was prepared for flour, and Wheat samples were then were taken and processed were done by the Laboratory mills, Releasing toxins in solution using solvent extraction separation were done with the solvent containing 40 ml methanol, 40 ml ethanol and 20 ml of acetone up to 20 ml For each 10 g chopped/miled sample at first which transferred to a falcon tube container will previously 20 ml NS and 20 mL of solvent Extract to be shaken for 30 Minutes and heading and then transferred to a water bath to reduce values to 10 ml, and then extracts separated using a filter paper Whatman No.1 flat that operating with simultaneous transfer of 10 ml of deionized distilled water to wet the filter and also dilute the extract and speeding the movement take place. Finally 100 micro liters were used for ELISA testing. To detect toxin 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 absorbance of 450 nm (Rosi et al., 2007).

RESULTS

Of wheat flour samples collected from the North, West and South of Iran, in seven provinces, including 14 cities and shopping centers (Figure 1), according to sampling distribution criterias that is indicateable the number of samples obtained from regions shows, the Northers belonging a frequency of 71.4 percent, the highest, Westerns by a frequency of 21.4 % finally the lowest are in Southern bring its frequency of 7.1 percent.

![Figure 1: Distribution frequency of obtained wheat flour samples Fum-T toxin,of the different cities](image)

NPar-Wilcoxon Signed Ranks Test for Ftox - FFum; Z; -2.480a, Asymp-Sig.; 0.013, Pearson Correlation; 0.266, Sig; 0.358

According to the amount of T toxin measured in flours(Mean; 21.422, Range; 5.670 (Min; 19.290,Max; 24.960), Var; 2.623, Std. Dev; 1.620; Skewness; 0.712, Kurtosis; 0.209) maintain compliance with the standards and practices conserving National average nutritional values approvals, the amount of toxins in wheat flours have no significant correlation despite reverse relation, but not statistically significant Supporting statistical determination and significant correlations. In examining
wheat flour samples numbers/obtained measurements of toxin shown, normalized distribution frequency of obtained samples for T,Fum. toxin, in different ranges concerning the highest T toxin measured zone were at interval ranges of 10-50ppb and Fum(19-25ppb) because the most number of samples have been accomulatet tended the higher range of the curve to the right, a normal curve were resulted. Counter currently a significant degrees of correlation between the numerical differences between the processed wheat flour T/Fum (Z; -2.480a,Asymp-Sig.; 0.013, Pearson Correlation ;0.266, Sig;0.358 and NPar-Wilcoxon Signed Ranks Test for Ffox - FFum; Z; -2.480a, Asymp-Sig.; 0.013, Pearson Correlation ;0.266, Sig;0.358) toxin values and their disalignment quite reasonable, entirely due to the presence of toxin-producing agents in the process of harvesting, handling, storage, meal preparation/mixing and suspективely to take place in the packages and also the increased further.

Figure. 2: Normalized distribution frequency of obtained wheat flour samples T toxin

Mean; 28.764, Range; 30.100 (Min; 11.100, Max; 41.200),Var; 65.922,Std.Dev.; 8.119, Skewness; -0.614, Kurtosis; 0.590

Figure. 3: Normalized distribution frequency of obtained wheat flour samples Fumonisin

Mean; 19.151, Range; 6.780 (Min; 16.560, Max; 23.340),Var ; 4.306,Std.Dev.; 2.075; Skewness; 0.886, Kurtosis;-0.291

DISCUSSION

An inevitable part of the preventive measures is regular food stuffs monitoring with mycological and mycotoxicological examinations. In moderate dimates, 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 toring of well-dried grains at optimal conditions. In 1999, the worldwide contamination of Fusarium mycotoxins (DON, NIV, ZEA, DAS, T-2, HT-2) in cereal grains have been reported by Placinta et al. (Schollenberger et al., 2006). In 2000, mycotoxin contamination (DON, NIV, ZEA) in rice have been suggested by Tanaka et al. (Pleadin et al., 2012). Between 2003 and 2005, the studies of DON, T-2 toxin, ZEA and FUS (FB1+FB2+FB3) in cereal samples collected from European and Mediterranean markets and Asian-Pacific region have been reported by Binder et al (Kariam et al., 2008). The limit values of Fusarium mycotoxins in cereal and cereal products (in the USA, EU) are given (Hazmi et al., 2010, Leslie et al., 2006, Solarska et al., 2009). Having carcinogenic potential and poisonous effects, mycotoxins are considered to be one of the most important regulatory issues. However, based on available information on the occurrence of fusarium toxins, FDA accepted that typical levels found in products intended for human consumption are much lower than the recommended levels (Trigo et al., 1996). In countries with adequate information about mycotoxin occurrence, regular tests to control foodstuffs and detect widespread and serious toxins are currently being performed and this leads to the exclusion of products with higher than allowable limits (Shephard et al., 2000; Tanaka et al., 2007). In 2001 the SCOOP (Scientific Co-operation on Questions relating to Food) have been reported data of Fusarium toxins (DON, NIV, FUS-X, T-2, HT-2, DAS, ZEA) in cereals (wheat, corn, barley, oat, rye) collected from 12 countries (The Netherlands, Norway, Portugal, Sweden, UK, Italy, Germany, France, Finland, Denmark, Belgium, Austria). Between 2003 and 2005, the studies of DON, T-2 toxin, ZEA and fumonisins (FB1+FB2+FB3) in cereal samples collected from European and Mediterranean markets and Asian-Pacific region have been reported by Binder et al. The limit values of Fusarium mycotoxins in cereal and cereal products (in the USA, EU) are given by (Hazmi et al., 2010). Unfortunately, in Iran a limited number of mycotoxins including Aflatoxins, Fumonisins, Zearalenone and, Ochratoxins are only being measured only in export products, but they are not usually checked in foodstuffs for domestic consumption (Egmond et al., 2003, Ghiasian et al., 2006). Contamination of feed with Fusarium 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. With the aim of minor losses in the industry, considerable attention is paid to the prevention of mycotoxins contamination, and studies on different types of raw materials and compound feed, depending on various factors, are of great importance. In general, there is a lack of investigations on the presence of mycotoxins in food and feed. (Pleadin et al., 2012). In relation to the results of previous research and also with the published data worldwide, it can be concluded that a certain number of feed samples in this research had significantly high Fumonisins concentrations, also, comparing the obtained concentrations of T,Fum and ZEA with the maximum recommended concentrations for these mycotoxins in feed the results indicated an increased contamination of who feed with Fumonisins ,T and ZEA, with mean concentrations of more higher than recommended for food and feed, respectively ( Pleadin et al., 2012). A higher Fusarium mycotoxins concentration than the maximum recommended was determined in about 60% of the total number of samples, with a maximum concentration of T (50 ppb), Fum ( 25ppb) determined in the northern then the other part of the country. In this study it was also observed that the samples in which the low concentrations of ZEA were determined have predominantly more concentrations of T toxins and Fumonisins, or both mycotoxins 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. In the past studies, investigated in by ELISA method indicated that all samples were contaminated, also results showed that most samples had contamination higher than of Europe standards but had consonant with Iran national standard, such as amount of higher than standard was not observed surprisingly confirm our results about the original wheat crops and processed wheat flour for bread making. According to the JECFA average of absorption this toxins of all the samples is less than the tolerable daily uptake, reasonably showed that such are recognized dangerous of view and have stringent security to eliminate or reduce this toxin is thought by 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 fumonisins in cereals. Alizadeh et al. 2012, reported that Fumonisin B1 (FB1 a toxic and carcinogenic mycotoxin produced in cereals due to fungal infection) contamination of rice and corn samples and its relationship with the rate of esophageal cancer (EC) in a high risk area in northeastern Iran geographical subdivisions of Golestan province were measured by TL and HPL chromatographies. The mean level of FB1 and the proportions of FB1 contaminated samples were compared between low and high EC-risk areas of the province, respectively even found high levels of correlations between FB1 contamination in rice and the risk of EC. Therefore, fumonisin contamination in commonly used staple foods, especially rice, may be considered as a potential risk factor for EC in this high risk regions. Daily intake of Fusarium toxins are 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 were 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 in wheat flour samples of various aspects can be considerable seriously. Thus, according to the results of those study indicate that the extent of contamination flour to the fumonisins toxin. In case of contamination with levels above the limit of the cycle is eating out (Sadeghi et al., 2014). The occurrence of mycotoxins produced by Fusarium spp. in small cereal grains, particularly in wheat flour and maked breads, is of great concern worldwide, because their presence in processed 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 (Chehri et al., 2010). Although the distribution concentration in the ranges considered, are not significantly correlated are countercurrent (Figure 1). But it should be noted that most concentrations were in the range of which may indicate endemic fungal causative agents of fumonisin in the conducted geographical areas (Figure 2 and 3). Given that the largest amount of toxin production observed in the range of ppb, therefore, this suggests the possibility of Fusarium infection in all studied wheat fields or ware houses for temporary maintenance or transportation process (Figure 2, 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 of country (Figure 1). Based on the results of samples collected from the milling process there are no significant differences, although pollution levels are above the limit. According to the results of this research can be said that of all the major steel-producing Fusarium toxin, T toxin in first step then Fum and finaly Zea or Don, are at intervals after planting and cultivation remains, and in the longer term remains and can cause contamination of farm and food products there foryears. This level of contamination varies according to geographical regions, but in the process of turning wheat into flour specially mixing ,Contamination by toxins 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 ingredients, 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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REFERENCES


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