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ORIGINAL ARTICLE

An Investigation of Fumonisins Quantities correlation in Wheat/flour used for Bakery obtained from North, West and South Regional Provinces of Iran

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ABSTRACT
Wheat is one of the most important grains consumed in the world. Food contamination by toxigenic molds increased attention over the last three decades, which impact on food safety. Mycotoxins are secondary metabolites produced when they grow on agricultural products before or after harvest or during transportation or storage. 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 was done by ELISA (Kits and Rida Screen fumonisins analysis R-Bio-Pharm GmbH) on the samples so that fumonisins to be analyzed. a significant degrees of correlation between the numerical differences between the processed wheat flour toxin values and fumonisin in wheat grains (NPar-Wilcoxon Signed Ranks Test for FFum-WFum; Z;2.480a, Asymp-Sig; 0.013, Pearson Correlation; -0.077, Sig; 0.793) and its misalignment quite reasonable, (Z:-2.480a, Sig: 0.013) 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 according to the standard value of 2µg/kg for feed could be serious attention to the cumulative effects of toxin, a serious risk and should not be overlooked about the cities and provinces where there. The maximum values 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 in superior territories in Iran.
Keywords: Fumonisins, Wheat, Flour, Territories of Iran

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INTRODUCTION

Mycotoxins are a natural food and feed contaminants, Currently, are identified in the world, considering their serious problem, these substances constitute a potential risk to human and animal health, are not only dangerous for the Public Health, but they also deteriorate the marketable quality of the contaminated conventional and organic cereal products, causing tremendous economic losses[2]. Food contamination by toxigenic molds increased attention over the last three decades, which impact on food safety. Wheat is one of the most important grains consumed in the world. Rice and wheat, barley and soybean are considered as the most important staple food for the human population Worldwide, in particular in the Middle East. This grain is the second highest worldwide production after corn [13]. Although contamination of rice with fumonisins has been reported in the United States and it has been studied extensively in the European Union little information has come from Asia [14,16]. As 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. Currently, 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 fumonisins. During growth cereals are exposed to mycoflora geographically the most frequent "field fungi", *Fusarium* species, colonies before the harvest, the summary of the IARC evaluation of *Fusarium mycotoxins* is reported by several Fusarium species[10].. Ingestion of food contaminated with mycotoxins can cause mycotoxicosis which is an acute or chronic toxicity The types of fumonisins include FB1FB2 and FB3, which are known to contaminate. The European Commission regulates levels of fumonisinin U.S. Food and Drug Administration specifies significantly higher limits of all three total fumonisins (FB1+FB2+FB3)However, low amounts are synthesized during crop growth, whereas the highest amounts are produced by *Fusarium* during storage [4]. Fumonisins, the TCs and ZEA are hazardous for human and animal health. Epidemiological evidence indicates a link between human esophageal cancer and ingestion of *Fusarium* verticillioides -contaminated corn [30]. FB1, in cereals was 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 [2,13,31].There are no confirmed biomarkers for human exposure to FB1, whereas higher concentrations interfere with the health status. Major objectives on mycotoxin produced by genus *Fusarium* in cereals contribute to determine the distribution and level of fumonisins, DON and zearalenone in milled fractions and wheat milling performance study[5]. Therefore, a rapid and sensitive technique for routine assay 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 thin layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC) and capillary electrophoresis (CE). A number of liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods detect a large range of mycotoxins and their metabolites in a variety of food and feed commodities. Immuno affinity column use for mycotoxins has increased because of its simple cleanup procedure and lower detection level of mycotoxins. Additionally, simple extraction procedures including Quick, Easy, Cheap, Effective, Rugged, and Safe coupled to LC/MS or LC/MS/MS have been increasingly used as alternatives to traditional derivatization and extensive cleanup [8]. Over the last years, the importance and application of immunoassays, especially enzyme-linked immune sorbent 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 [7]. Several studies carried out in Erupean/transcontinental countries, reported higher incidence of fumonisins in cereals and in animal feeding stuffs as a potential risk to human and animal health. Among several hundreds of mycotoxins, fumonisins are Fusarium toxins and are among the most important mycotoxins regarding food safety [1]. The presence of different species of Fusarium, in particular food substrates is relevant not only because the potential role of such fungi in these product decays during storage and marketing but also because of the fact that some strains can produce mycotoxins. Fumonisin analogs are known, and the most abundant analog in nature is fumonisin B1 (FB1), followed by FB2 and FB3 [2,3]. Furthermore, an association between high rates of human esophageal cancer and high concentration of fumonisin in cereals has been reported in different countries [5]. In 2001, the Joint FAO /WHO Expert Committee on Food Additives and Contaminants (JECFA) established a Provisional Maximum Tolerable Daily Intake (PMTDI) for fumonisins (the sum of FB1, FB2 and FB3) of 2.0 μg/kg of body weight per day and in 2002, the International Agency for Research on Cancer (IARC) determined that the FB1 belongs to Group 2B, i.e. a possible human carcinogen [7]. However, there is evidence that they can occur in crops such as and medicinal plants [12]. Current regulations of fumonisins in foods and feeds set by countries from Europe, Asia, Africa and America and reported by FAO, the risks of fumonisin B1 have been evaluated by The World Health Organization's International Programe on Chemical Safety (IPCS) and the Scientific Committee on Food(SCF) of the European Commission. They determined a tolerable daily intake (TDI) for FB1, FB2, FB3, alone or in combination of 2 μg/kg body weight too [22]. 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/milles and flours as one of the important risk factors (toxin fumonisins) in Superior territories in Iran.

MATERIALS AND METHODS

Fresh wheat samples harvested from the early May to late September from 7superior 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, wheat collected for use, drying/adjusting humidity, mixing and re-mixing for each samples were done ,four samples of 100g were randomly selected in order to sample measurements per 10 tone of origin, sample control, sample stock and the sample was prepared for flour, and Wheat samples were then were taken and process were done by the laboratory mills, after combination. Releasing toxins insolution using solvent extraction separation were done with the solvent containing 40 ml methanol, 40ml ethanol and 20ml of acetone up to 20 ml For each 10g chopped/milled sample at first which transferred to afalcon tube container will previously 20ml 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 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 50micro liters were used for ELISA testing. To detect fumonisins 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.

RESULTS

The total number of wheat samples collected from the North, West and South of Iran, in seven provinces. According to sampling distribution criteria that is indictable the number of samples obtained from regions shows, the Northern 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 fumonisins measured in grain samples comparing to the amount of toxin observed in bread flour maintain compliance with the standards and practices conserving National average nutritional values approvals (auto rising the mixing wheat flours) found that the amount of toxin in wheat and flour have no significant correlation despite reverse relation, (PC;-0.077) but not statistically significant (Sig;0.793) Supporting by the Pearson statistical determinations a significant correlation (P< 0/05).In examining wheat flours samples numbers/obtained measurements of toxin (Range; 5.670, Minimum; 19.290, Maximum; 24.960) shown, the skewness0.539, Kurtosis ;-0.102 in comparison to wheat grain samples in which were measurements of toxin (Range; 6.780, Minimum; 16.560, Maximum; 23.340) concerning Skewness;0.886, Kurtosis;-0.291 at the highest fumonisins measured zone were at interval ranges of 16-24/25ppb, and because the most number of samples have been accumulate tended the higher range of the curve to the right, a normal curve is resulting in drawn (Figure 1,2).

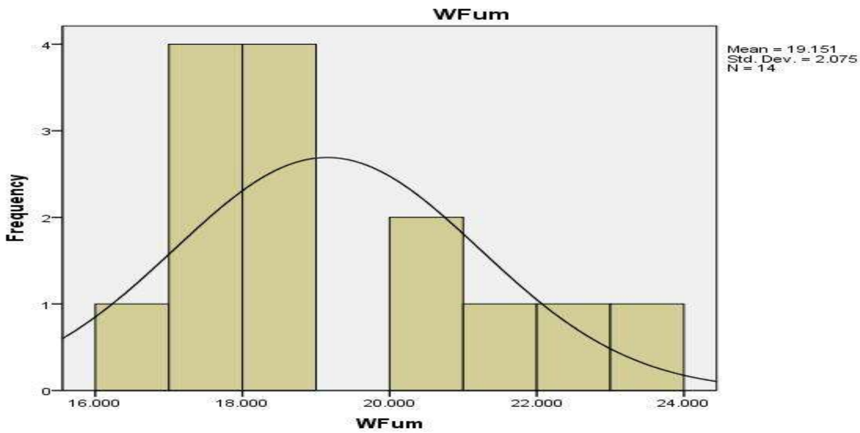

Figure 1: Normalized distribution frequency of obtaining wheat samples Fum-toxin, of the different ranges
Range; 6.780, Minimum; 16.560, Maximum; 23.340, Skewness;0.886, Kurtosis;-0.291

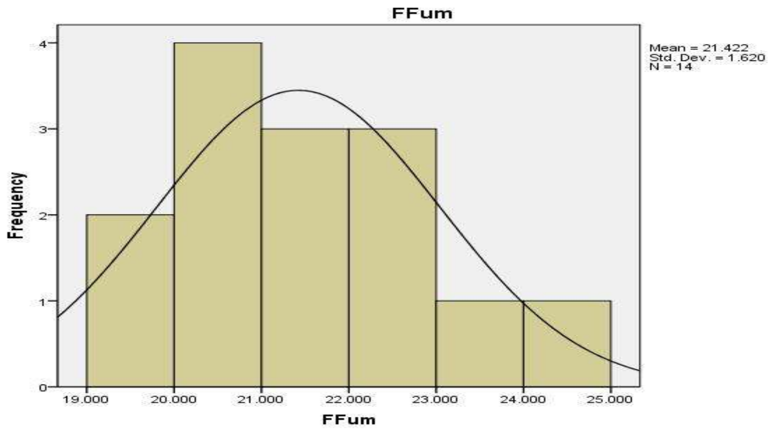

Figure 2: Normalized distribution frequency of obtaining wheat flour samples Fum-toxin, of the different ranges
Range;5.670, Minimum;19.290, Maximum;24.960, Skewness;0.539, Kurtosis;-0.102

NPar-Wilcoxon Signed Ranks Test for FFum - WFum; Z; -2.480a, Asymp-Sig.; 0.013, Pearson Correlation;-0.077, Sig; 0.793

Counter currently a significant degrees of correlation between the numerical differences between the processed wheat flour toxin values and fumonisin in wheat grains (N Par-Wilcoxon Signed Ranks Test for FFum - WFum; Z; -2.480a, Asymp-Sig.; 0.013, Pearson Correlation ;-0.077, Sig;0.793) and its misalignment quite reasonable, (Z: -2.480a, Sig: 0.013) 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. A provisional maximum is fixed for tolerable daily intake (PMTDI) for FB1, B2 and B3 single or in combination, of 2 μg/kg of body weight per day on the basis of the NOEL of 0.2 mg/kg of body weight per day and safety factor of 100. 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 [9,10]. In 2001 the SCOOP (Scientific Co-operation on Questions relating to Food) has 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 has been reported by Binder et al .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[29,33]. 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 makes it more expensive to produce [13]. 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 DON and ZEA with the maximum recommended concentrations, increased contamination,of more higher than recommended for food and feed, respectively [13]. A higher fumonisin concentration than the maximum recommended for wheat and processed flours were determined in about 60% of the total number of samples, with a maximum concentration by 23 to 24ppb 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 were determined in the original wheat cropes have predominantly more concentrations of fumonisin in final processed flours, or both always could be detected in a same ranges, or mostly the results indicate on both higher concentrations as in our study performed on processing operations for wheat flour obtained by mixing silaged or stored imported wheat crops. Fumonisin side-effects on health are undeniable, due to the chronic and acute effects for consumers is qualified to provide sufficient information about its exposure to the general population. In the past studies, investigated in by ELISA method indicated that all samples were contaminated with fumonisin. 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 being polluted by forign originated wheats. According to the JECFA average of absorption this toxin of all the samples is less than the tolerable daily uptake. Daily intake of fumonisin 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 fumonisin in cereals in Iran, Khosravi et al 2013. published datas on Mycoflora profiles of fresh and stored rice grains showed that Fusarium (21.6%, 16.2%) as the second agents respectively implicated, most of the rice samples (96.7%) found to be positive for FB1 up to 56.2 mg/kg for fresh samples and to 42.8 mg/kg for stored ones indicate the need for proper surveillance and monitoring exclusively for the prevention of fungi and FB1 in rice produced in

Mazandaran province before it reaches the consumer. Survey of contamination of fumonisin mycotoxin in cereals and other crops in other countries have led to different results. Schollenberger showed that only two cases were free-fusarium pollution toxins and other samples were contaminated with one or more mycotoxins of samples of wheat, barley, corn and corn products in Germany, shows many similarities with have been done in such a way so that the high prevalence of mycotoxins in samples had evidence. 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 Golestanprovince . FB1 contamination was found in 50% and 40.9% of corn and rice samples, respectively, even found high levels of correlations between FB1 contamination in rice and the risk of EC. Daily intake of fumonisin 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 breadis one of the most widely used food substances in cereal series, over prevalence contamination to fumonisin in wheat samples of various aspects can be considerable serious. Thus, according to the results of those study indicate that the extent of contamination flour to the fumonisin toxin. In case of contamination with levels above the limit of the cycle is eating out [26]. 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 with EC. Our results are in agreement with other studies in the USA, Canada, Argentina and Europe [28].In studies conducted in this investigation, the datarelating to the north of the country, with 71.4%, the West 21.4% and south of the country with 7.1% and interms of results and the toxin fumonisin with conduction study is consistent (Figure 1and Table1).The frequency of the samples studied in this investigation have shown the Northern region location of samples collection have contributed to the production of wheat, which is consistent with conduction study. Although the distribution of fumonisin concentration in the ranges considered, are not significantly correlated is counter current, But it should be noted that most of fumonisin concentrations were in the range of 16-25ppb, which may indicate endemic fungal causative agents of fumonisin in the conducted geographical areas (Figure 3). Given that the largest amount of toxin production observed in the range of 16-25ppb, therefore, this suggests the possibility of wheat fields or warehouses pollutions for temporary maintenance or transportation process there. The highest possible average toxin produced due to the plurality of samples collected from the area north and south and then to the West of the country (Figure 1,2 and 3). 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 intoxication.

CONCLUSSION

According to the results of this research can be said that of all the major steel-producing Fusarium toxin, fumonisin, is at intervals after planting and cultivation remains, and in the longer term remains and can cause contamination of farm and food products there for years. The results of this study represent contamination of wheat samples from different parts of Is important, but the ability of a significant considered as a potential threat to human health and animals is raised. This result reveals the need of extensive epidemiological studies on the incidence, distribution and Genetic and biological diversity of the fungi with the aim of developing and implementing appropriate strategies and effective fungal contamination and mycotoxin control of human and animal foods and agricultural products reveals.

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