EVALUATION OF ZEARALENONE MYCOTOXIN IN EDIBLE OILS DISTRIBUTED IN KERMAN-SHAH CITY BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Introduction: Zearalenone as mycoestrogen find in human food and animal food. Estrogenic properties depending on the Biotransformation level, in this regard, there are some reports of food contamination by mycotoxins such as ZEA. This study has investigated ZEA mycotoxin in edible oils.

Materials and methods: 104 random samples of 8 different brands edible oil (corn, sunflower, mix) collected and evaluated from Kermanshah stores in 2013. Determination of ZEA levels was done by using high performance liquid chromatography and fluorescence detector. Detection and quantification was limits of the method and order of micrograms per kilogram.

Results: Zearalenone levels in all samples was positive and samples levels was less than the limit specified by the Standards and Industrial Research Institute of Iran. The minimum and maximum ZEA mycotoxin, respectively, with a sunflower oil average 2.67µg/kg Bw and E brand of corn oil with average was 70.78 µg/kg Bw. results of ANOVA significant contamination of corn oil, sunflower and blend oil (P < 0.001).

Conclusion: Due to the increased levels of ZEA in corn oil liquid than other oils tested and on the other hand, the consumption of food contaminated with mycotoxins Fungal toxins receive provisional tolerable daily intake is much higher than Expert Committee on Food Additives of the FAO / WHO determined therefore, prevention of mycotoxin contamination ours to control pre-and post-harvest And consideration of relevant experiences and practices is essential to a great extent.

Key words: Edible Oil, Zearalenone mycotoxin, HPLC.

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Introduction

Today, considering the quality of food consumed from all aspects is very important. Mycotoxins are secondary metabolites of fungal toxins that create contamination in higher organisms and are produced by more than one hundred species of molds. One of the fungal mycotoxins is called Zearalenone which is an estrogenic toxin and is produced by Fusarium and its relevant species on crops like Wheat, Barley, Maize and Sorghum.

The toxin is evident in cereals after the harvest and in products produced through their processing. Its further growth is in warm and temperate situation. Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have declared the maximum amount of ZEA in breakfast cereals to 50 micrograms per kilogram of body weight. Complications related to the toxin in animals such as pigs, include reproductive disorders, infertility, miscarriage, reduction in size of testicles, reduction of strength and increased volume of
mammary gland in pigs\textsuperscript{(10-11)}. Cooper Goodman from Italy has studied the relevance of the toxin to the increased breast size and sexually precocious puberty in girls due to consumption of corn contaminated by ZEA mycotoxin\textsuperscript{(12)}. The role of lipid oils in human nutrition is one of the most important fields of research in nutrition science. The benefits and risks of edible oil intakes have always been proposed in scientific resources and mass media. After starch and sugar, fats are the main suppliers of energy needed by the human body and due to their role in human life, are known as essential consumable goods. Fats are one of the main components of feeding people around the world. Each individual needs 45 grams of fat (animal or vegetables) per day. About 15 percent of your needed calories is provided by fat\textsuperscript{(13-15)}.

Several studies are done around the world about the levels of ZEA mycotoxin in Wheat, Corn, Barley, and other grains. However, the studies about the amount of ZEA mycotoxin in edible oils are limited to a few ones in Germany. In 2005, Coopon Stein from Germany reported the mean levels of ZEA about 197\(\mu\)g/kg and the maximum amount of 921\(\mu\)g/kg after the analysis of 77 samples of edible oil in 38 samples of corn oil\textsuperscript{(16)}.

In 2009 Majrius et al from Germany, reported the levels of ZEA in corn oil and canola oil about 370 to 386 \(\mu\)g/kg in 2.8% of samples\textsuperscript{(17)}. Marjit Skoolen Burger in 2008, declared the levels of ZEA between 116-1730 \(\mu\)g/kg in 14 samples among 110 gathered samples of oil\textsuperscript{(18)}. Regarding to the fact that there has been no studies upon this issue so far, the aim of this study is to determine the levels of ZEA in edible oils.

Materials and methods

This is a Cross-sectional study. 104 samples of three types of oil (sunflower, corn and mix (sunflower, soybeans, and rapeseed) in 8 different brands from A to H with three different production dates and three times of repetition were gathered in Kermanshah City. Sampling from supplying centers of different areas of Kermanshah was done randomly. For measuring ZEA in edible oil samples; at first, 5gr of sample was dissolved in 50 mg of normal-hexane and then was mixed with 50 ml of Acetonitrile / water by the ratio of (25/75) within a volume in a container of 250 ml in a shaker (made by Iran Khodsaz) for an hour. Then it was put in a centrifuge for separation of phases with spinning period of 3400 rpm for 10 minutes. Then the sub layer poured in a decantation funnel (made in Iran) with 50 ml of normal-hexane and the sub layer was detached again and was isolated in evaporator under vacuum at 40\(^\circ\)C until complete evaporation of the solvent. The remaining was dissolved in 2.5 ml of ethanol solution and got transmitted through immune-affinity column (Made by New Column, England) ZEA containing the antibodies got transmitted. Column was washed with Methanol and ZEA mycotoxin was separated and 5.0 ml of it was injected to HPLC device to measure the levels of ZEA (Knuer model, Germany). Zearalenone determination was performed by high performance liquid chromatography with C18 reverse phase silica gel column (4.6*250 mm, particle size 5 mm) equipped with fluorescence detection. Excitation and emission wavelengths of 274 and 465 nm were set from the mobile phase of water - acetonitrile - acid acetic (495-495-12, V / V / V) at a flow rate of 6.0 ml per minute and injection volume of 100 ml was used respectively. The detection of ZEA mycotoxin was confirmed through injecting the sample sap and measuring it through measuring the sub layer during deterrence period and comparing them with the calibration curve. Concentration of ZEA in oils was determined regarding to the standard curve. To determine the efficacy of the method, two doses of 20 and 50 micrograms per kilogram of ZEA were prepared and were added to the sample of oil, then the preparation, extraction was done, and extraction percentage was 15. Finally, after the classification of information, analyzing the data was done using Excel and SPSS software and ANOVA. All the materials were related to Merck, Germany.

Results

The results of analysis by high performance liquid chromatography showed that the contamination by ZEA exists in all samples. The maximum amount of ZEA was related to corn oil and the minimum was related to sunflower oil. Samples were normal in appearance (Tables 1-3).

10.18 percent of the samples (11 samples out of 108 samples) have a high contamination rate of 50 mg/kg respectively. ANOVA test results in the contamination of corn and sunflower oil and mix one shows the contamination rate as significant (\(P<0.001\)). However, the difference in ZEA mycotoxin in sunflower oil and mixed is not significant.
(P<0.05). T independent test showed that the contamination of corn oil in significantly higher than sunflower and mixed (sunflower, soybeans and rapeseeds) (P< 0.001). The contamination of all samples was below the national standard of Iran and Europe Union (Figure.1).

The lowest level of contamination was for sunflower oil and the highest was for corn oil. Corn and sunflower oils were the lowest contaminated by ZEA mycotoxin, but no significant difference between the contamination of sunflower oil and the mixed (sunflower, soybeans, rapeseed) (P<0.005). Among different brands, the lowest amount of ZEA is related to sunflower oil brand C with an average of 2.1 micrograms and the maximum amount is related to corn oil brand E with an average of 65.2 mg (Figure 2).

Table 1: The mean and standard deviation of ZEA in sunflower oil.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Mean of ZEA (Micrograms/Kilogram)</th>
<th>Standard Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.67</td>
<td>0.22</td>
<td>0.048</td>
</tr>
<tr>
<td>B</td>
<td>2.67</td>
<td>0.2</td>
<td>0.042</td>
</tr>
<tr>
<td>C</td>
<td>2.67</td>
<td>0.17</td>
<td>0.028</td>
</tr>
<tr>
<td>D</td>
<td>2.24</td>
<td>0.18</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Table 2: The mean and standard deviation of ZEA in corn oil.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Mean of ZEA (Micrograms/Kilogram)</th>
<th>Standard Deviation</th>
<th>Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>22.5</td>
<td>1.57</td>
<td>2.46</td>
</tr>
<tr>
<td>E</td>
<td>70.78</td>
<td>1.18</td>
<td>1.39</td>
</tr>
<tr>
<td>F</td>
<td>31.2</td>
<td>0.85</td>
<td>0.72</td>
</tr>
<tr>
<td>G</td>
<td>37.88</td>
<td>1.81</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 3: The mean and standard deviation of ZEA in mixed edible oils (sunflower, soybeans, and rapeseed).

Comparing the amount of ZEA mycotoxin in sunflower oil with different brands and dates of productions showed that they look normal and do not have any significant difference at ensuring level of 0.05 and the lowest amount of ZEA is related to Sunflower oil Brand C and the maximum amount was related to brands A and B, respectively. By comparing the amount of ZEA mycotoxin in corn oils with different brands and different production dates it was observed that they are normal in appearance and have a significant difference at the ensuring level of α=0.05 and the least amount was related to corn oil brand B and the most was related to corn oil brand E.

Also, given that prepared samples of corn oil were both imported and domestic productions, it was observed that the imported samples allocated more...
amount of ZEA to themselves compared to the domestic ones (P<0.00) (Figure 3).

In the comparison of the amount of ZEA in corn oil, sunflower oil and mixed with the standard of Iran and European union, all brands are lower than the standard limits of Iran. But corn oil by brand E was higher than the standard limits of National Union of Europe (Figures 4 and 5).

**Discussion**

The study showed that the contamination of oil samples with ZEA mycotoxin were positive in all cases that among the samples, the ones of sunflower oils with different brands were not different from mixed vegetable oils (soybean, sunflower, canola) regarding to the amount of ZEA by a significance level of $\alpha=0.05$, which in this case levels of mycotoxin is not considered in national standards of sunflower oil (No.1300) and mixed (No. 5950). Corn oil is considerably more contaminated by ZEA compared to other oils that are being studied but it is less than allowed limitation in national standard of Iran under the number 1447 and also the European Union for the corn oil. Imported corn oil also had more amount of mycotoxin compared to domestics and other types of oil.

Robert Copon from Germany in 2012 reported the mean levels of ZEA 194 micrograms per kilogram in his study on 12 brands of two kinds of corn oil and cannabis. Lauren and colleagues in 1997 measured three kinds of Fusarium mycotoxins in milled maize by HPLC that the amount of ZEA was reported 100 micrograms per kilogram. Also, in an evaluation done by Skulenburgh from Germany in 2005, 219 number of foods were studied that the existence of toxin in 85 samples of vegetables and fruits was reported and 7 numbers of 35 samples of oil seeds were detected as being contaminated by ZEA and T2 toxins amount of which was lower than 50 micrograms per gram. In Gromadz and Kader's study in Netherlands in 2008, they studied ZEA and its metabolism and toxic properties and they declared that this toxin is not catalyzed in production processes like milling, storage and heating.

In an evaluation done by Skulenburgh et Al in 2006 on 16 kinds of Fusarium toxins in soybean meal and soy foods, more than 5 to 7 Fusarium toxins were found in soybeans and soy foods and ZEA and T2 were identified in sunflower seeds. In the evaluation done by Rafaj et Al on mycotoxins and seeds contaminations in 2000, they reported that in consuming soybeans in food industry, there is a considerable level of mycotoxin and T2 toxins is positive, as ZEA was negative in 18% of milled sunflower seeds. Scott in Canada in 1997, during the studying of oilseeds for food consumption declared that 62% of harvested soybeans and also the foods containing soy were contaminated by 5 to 39 micrograms to kilogram of ZEA. In the study conducted in 2002 by Pakyn and colleagues, they detached some species of Fusarium fungi on soybeans. Fusarium toxins were reported to be in sunflower, canola, and linseed, palm oil (palm), olive oil, and grapes.
Conclusion

The studies were in accordance to the study of samples contaminated by ZEA in edible oils. Although the contamination of samples did not exceed the allowed limits, this amount of contamination reveals a situation in which mycotoxins are produced in crops and regarding to the consequences of the toxin, measuring the amount of mycotoxins is vital in all kinds of foods. Contamination by ZEA in edible oils can occur during the process of production in various sectors and cause harmful effects on health. Thus, according to the terms of oil production, efficient amount of light, humidity, and temperature seems necessary.

References

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