DETERMINATION OF ZEARALENONE LEVELS IN CONSUMED RICE SAMPLES IN IRAN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (2015)

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Abstract
Aflatoxin, Fumonisin, Zearalenone and Ochratoxins as mycotoxins afford the carcinogenic, nephrotoxicity, estrogenic problems especially kidney and liver cancer in human and animal. Rice and wheat is important foodstuff that used as feed for world's population. Considering the high rate consumption of rice and its derivatives in human diet, this study was to evaluate the Zearalenone levels in consumed rice samples in Kermanshah city by High Performance Liquid Chromatography. For this aim, 42 samples rice randomly selected from 10 Iranian and 4 imported brands from different parts of city. Results revealed that all samples were contaminated to zearalenone and the zearalenone average in Iranian and imported rice were 4.43 and 3.88 µg/kg respectively. Given the zearalenone risks for human health, it is recommended the control of zearalenone production during harvesting, preserving, and storage of rice. Therefore, implementing health regulation and monitoring their enforcement, determine the maximum allowable amount of mycotoxins in food and attention of public health and nutrition specialists to this issues is most important.

Keywords: Zearalenone, Rice, HPLC.

Introduction
Rice is one of the most consumed foods of the people of Iran. Thus, quality control is essential, especially in terms of the absence of toxic substances6. Zearalenone is a non-steroidal estrogenic mycotoxin that produced by many spices of Fusarium. This fungus mainly grows on cereal especially maize, barley, oats, wheat and rice2-3. Zearalenone mostly produce in moderate temperate, tropical and subtropical conditions4. The fungus that produces zearalenone is considered as store-growth fungus. Corn will be infected to this fungus at store, while wheat and barley at farm6. Despite the low acute toxicity of zearalenone, but has main role in domestic animals poisoning especially pigs and sheep.

As well as in over doses afford animal infertility and sexual dysfunction. Therefore, its presence in animal feed has long been regarded as a problem in the livestock industry6,7. Human can exposed to this mycotoxin by swelling, inhalation and skin8. Rice is the one oldest cultivated crop in the world. Main cultivating place of rice is in Southeast Asian countries and mainly India and China. This product has an important role in nutrition (main food of more than half of the world's population) and employment of people in the Iran and world. 35 to 80% of daily calories of 3 billion people in Asia provided by rice9. More than 100 developed and undeveloped countries are rice consumer or producer, 50 to 70% of the income of undeveloped countries is related to buy rice, as well as rice has a great importance in Iranians diet10.
However, rice cultivation area in the world is less than wheat cultivation, but their production is equal. Unlike wheat that two-third of its production is in developed countries, almost all world rice production is in undeveloped world countries\(^{11}\). According to the Iranian people taste, rice is the most essential daily needs of people and as necessary goods in the consumption basket of Iranian households. Several studies were conducted regarding to the amount of zearalenone toxin in wheat, corn, barley and other grain in different parts of the world. However studies of zearalenone toxin in rice are limited to a few studies in Brazil and Korea. Because the store of Iranian and imported rice for long time (before consumption), would be prepared a favorable condition for fungal growth. Therefore, improper storage and preservation causes the contamination of rice to fungus toxin. Hence, considering the rice is one of the most widely consumed food in Iran and according to the fact that there is no study in this case, so, the aim of this study is to determine the level of zearalenone in rice in Consumed Rice Samples in Iran by High Performance Liquid Chromatography.

Materials and methods

This is descriptive - analytical study. 10 brands of Iranian and 4 brands of imported rice were collected randomly from markets which located in northern, central and southern of Kermanshah city (3 samples were taken from each region), each sample triplicates analyzed and in a total 126 samples were collected. zearalenone with 10 ppm concentration was used as standard solution. Then, 10 mL of this solution (10 ppm) was added to 100 mL of methanol 50% in a flask to obtain the 1 ppm concentration. Subsequently, the 5, 20, 50, 100 and 200 ppb of zearalenone were prepared of diluted solution for providing of cure calibration of HPLC (Knuer model, Germany) system. Each of 5 concentration repeated three times for determining RT (Peak viewing time) of Zearalenone.

Two rice samples with 20 and 50 ppb of zearalenone concentration were used for evaluate of mentioned method as follow: 250 and 100 mL of standard solution (10 ppm) were added to two rice samples with 25gr weight and zero concentration of zearalenone. All stages of preparation and extraction that used for actual samples also were repeated for these two samples. For zearalenone measurement in rice sample, 25 gr of milled sample transferred into 250 mL of Erlenmeyer flask and then 125 mL of methanol added and was blended at high speed for 5 min (extraction phase). Filtrate was obtained with filter paper. Then 300 ml phosphate buffer (pH7.2) added to 75 mL of filtrate solution and passed through the Buchner funnel and filter flask (0.2µ), for impurities removal. Then 25 mL of filtrate solution with rate of 2 ml/min passed through Immunoaffinity columns (IAC) which contain particular antibodies. During of this process, the existing toxin in filtrate solution (antigen) is attached to the anti-bodies of the column. Ultimately, column was washed with 10 mL distilled water graduated pipette.

Then 1.5 mL methanol passed through the column in order to zearalenone dissolution, subsequently column was washed with 1.5 mL distilled water again, finally the methanol that contain zearalenone injected into the column C18 of HPLC. By comparing of curve area obtained from samples and curve area of standard solution and counting dilution coefficient, the final amount of zearalenone (µ/g or ppb) was reported\(^{12}\). Data were analyzed using Excel and SPSS software (ANOVA statistical test).

Results

Data analysis indicated that all samples were contaminated with zearalenonemycotoxin. mean, minimum and maximum of zearalenone levels in rice samples is presented in Table 1-2-3.

<table>
<thead>
<tr>
<th>Zearalenone</th>
<th>Number of samples</th>
<th>Range</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>126</td>
<td>10.9</td>
<td>1.2</td>
<td>12.1</td>
<td>4.273</td>
<td>2.20354</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Minimum and maximum level of zearalenone in rice samples.

The level of zearalenonemycotoxin in 2.3% of samples (3 samples of 126 samples) was between 11.3-12.1 ppb. Results revealed that zearalenone contamination between different brands of rice (C and B) was significant (p<0.001). But there aren’t significant differences between other brands (p>0.05). Figure 1 and 2 shows the amount of zearalenone toxin in different Iranian and imported rice brands.

The lowest level of contamination in Iranian brand was related to sample B (5 ppb) and the highest level of contamination was related to sample (10 ppb).
The min and max contamination of zearalenone toxin among imported brands was related to N and M brands respectively.

Based on national standard, the concentration of zearalenone must be lower than the standard limit. (50 µg/kg) (Figure 3 and 4).

Soarez et al.\textsuperscript{13} have done separation of Aflatoxin, Ocrotoxin A, zearalenone and Astrigmatosystin in Brazilians food by chromatography method. Nuryono et al.\textsuperscript{14} determined the Zearalenone level in 89 food samples based on corn by ELISA and HPLC. The results showed that 36% of samples were contaminated by Zearalenone in the range of 5.5-526 ppb. Ibáñez et al.\textsuperscript{15} analyzed Aflatoxin, Ochratoxin and Zearalenone in 46 cereal samples in Spain. 9%, 48% and 39% of samples had

<table>
<thead>
<tr>
<th>Brand</th>
<th>Mean concentration of Zearalenone</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.83</td>
<td>0.34</td>
</tr>
<tr>
<td>B</td>
<td>2.6</td>
<td>0.24</td>
</tr>
<tr>
<td>C</td>
<td>7.45</td>
<td>0.34</td>
</tr>
<tr>
<td>D</td>
<td>3.24</td>
<td>0.23</td>
</tr>
<tr>
<td>E</td>
<td>4.45</td>
<td>0.23</td>
</tr>
<tr>
<td>F</td>
<td>4.94</td>
<td>0.38</td>
</tr>
<tr>
<td>G</td>
<td>4.2</td>
<td>0.25</td>
</tr>
<tr>
<td>H</td>
<td>4.15</td>
<td>0.33</td>
</tr>
<tr>
<td>I</td>
<td>3.25</td>
<td>0.2</td>
</tr>
<tr>
<td>J</td>
<td>5.13</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Table 2: Mean+SD of zearalenone in 25gr Iranian rice (ppb= µg/gr).

<table>
<thead>
<tr>
<th>Brand</th>
<th>Mean concentration of Zearalenone</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3.89</td>
<td>0.26</td>
</tr>
<tr>
<td>M</td>
<td>4.65</td>
<td>0.28</td>
</tr>
<tr>
<td>N</td>
<td>3.33</td>
<td>0.26</td>
</tr>
<tr>
<td>O</td>
<td>3.63</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Table 3: Mean+SD of zearalenone in 25gr imported rice (ppb= µg/gr).

The min and max contamination of zearalenone toxin among imported brands was related to N and M brands respectively.
Aflatoxin B1, Zearalenone and Ochratoxin respectively. Aflatoxin was existing just in corn based products and Zearalenone and Ochratoxin were existing in rice and wheat based samples. The study of Neguyan et al. revealed that rice samples of Vietnams were contaminated with mycotoxins includes Aflatoxin B1, Citrinin and Ochratoxin but the percentage of Aflatoxin B1 was higher than other. Reddy et al. evaluated the all rice and flour samples over India in terms of Aflatoxin B1. Results indicated all samples were contaminated. Sifo et al. evaluated the different mycotoxins including enniatins, beauvericin, fusaproliferin in 70 samples of rice. Results indicated all samples were contaminated with these mycotoxins.

**Conclusion**

Because the zearalenone is one of the important mycotoxin that have adverse effects on human health. So the use of routine testing for control of important toxins in foodstuffs and because the significant different of zearalenone contamination level in agricultural products in various regions were reported, thus further study is recommended to survey impact of geographical location on the zearalenone contamination level in agricultural products that cultivated in certain origin. Short time preservation and storage of crops in stores can prevent the growth of mycotoxins. Also, it is necessary to run appropriate agricultural methods, good manufacturing practice (GMP) and hazard analysis critical control points (HACCP) system, after and before harvesting for reduce and diminish mycotoxins contamination and give healthy food to consumer.

**References**


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