



COMPARISON OF MYCOTOXINS CONTENT FOUR IMPORTED WHEAT TO EGYPT

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ABSTRACT

Wheat is comprised the most important grain and single largest crop by area in Egypt. Despite this fact, Egypt is heavily dependent on imports wheat to meet its consumption needs. The objective of the present study was to determine some mycotoxins (aflatoxin B1, total B1, B2, G1, and G2, ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZON)) in wheat grains imported from four countries (France, Romania, Russia and Ukraine). The tested five samples were obtained from batches imported at five different times of the year. The results showed that Ukrainian wheat was the best which contained the least mycotoxins values. Total B1, B2, G1 and G2 of Ukrainian wheat ranged from 0.767 to 1.60 $\mu\text{g.kg}^{-1}$, while OTA, DON and ZON were ranged from 0.50 to 1.367, 48.90 to 222.33 and 40.47 to 67.17 $\mu\text{g.kg}^{-1}$, respectively.

Key words: Wheat grain, Aflatoxin, deoxynivalenol, Ochratoxin, zearalenone.

INTRODUCTION

Wheat is considered of to be the greatest importance among cereals because of its processing characteristics; it is basically classified into hard, soft, and durum categories. Wheat quality cannot be simply defined since it changes depending on the workers (from the farmer to those in the processing industry) and end-use (from flour to bread, pasta, or cookies) (Marie and Ivan, 2009). Wheat is one of the most important sources of nutrition in Egypt. Resulting from its economic and social dimensions and feeding habits, the Egyptian population depends mainly on baladi bread as a source of food meals being considered relatively cheap compared to other alternative sources such as rice and potatoes (Dawoud, 2017). Wheat is the dominant crop in many countries including Egypt, as it is used for human food and livestock feed. Quality of wheat depends not only on its adaptability and high yield

potential but also on the gluten protein fraction which confers the viscoelastic properties that allow dough to be processed into bread, pasta, noodles, and other food products. Wheat also contains essential amino acids, minerals, vitamins, beneficial phytochemicals and dietary fiber components which are important to the human diet (Shewry, 2009).

The domestic wheat production is estimated by about eight million ton produced from three million feddan. While the annual wheat consumption is fluctuated between 12 to 14 million tons. In other words, the gap between production and consumption lies between four to six million tons annually (Anas, 2015; Gaber and Mamdouh, 2015; FAO, 2017).

Mycotoxins are natural food and feed contaminants, mainly produced by moulds of genera *Aspergillus*, *Penicillium* and *Fusarium*. The number of mycotoxins known to exert toxic effect on human and

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animal health is constantly increasing, as well as the legislative provisions made to control their presence in food and feed (**Zinedine *et al.*, 2006**). Currently, more than 400 mycotoxins are identified in the world. Considering their heat stability, these substances constitute a potential risk for human and animal health. The chemical and biological properties of mycotoxins and their toxic effects are extremely variable. These effects are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, hepatotoxicity and immunotoxicity. Mycotoxins are not only dangerous for the health of consumers, but they also deteriorate the market able quality of the contaminated products, causing heavy economic losses (**Zinedine and Manes, 2009**). Aflatoxins (AFs) B1, B2, G1 and G2 are produced by various strains of *Aspergillus*, mainly by *A. flavus*, *A. parasiticus*, *A. nomius* and *A. tamarii*. They have immunotoxic, mutagenic and carcinogenic effects (**Moss, 1998**). Aflatoxins are often present in cereals (maize, sorghum, rice, and wheat), oilseeds, spices and nuts. Aflatoxins were classified as group 1 carcinogen by the International Agency for Research on Cancer (IARC). Aflatoxin B1 is the most frequent and the most toxic one (**IARC, 1993**). Ochratoxin A is a secondary fungi metabolite mainly produced by *Penicillium verrucosum*, *Aspergillus ochraceus* and *Aspergillus carbonarius* (**Eskola *et al.*, 2001**). These fungi are natural opportunist biodeterioration agents of carbohydrate-rich agricultural commodities. Therefore, this mycotoxin can occur in a large variety of commodities, such as cereals, dried fruits, coffee, beer, wine, and because of carry-over effect, in milk, blood, liver, kidney, and poultry meat from animals fed with contaminated feed (**Mantle, 2002**). Zearalenone is an estrogenic mycotoxin produced by several species of the fungal genera *Fusarium graminearum* and *Fusarium culmorum*. It widely exists in maize, barley, wheat, oats, sorghum and sesame seeds, as well as in hay and corn

silage, which are prime ingredients in many food products for humans and animals (**CAST, 2003**). ZON is classified by IARC under group 3 carcinogen (**IARC, 1999**). Deoxynivalenol (DON) is a member of the trichothecene family of mycotoxins. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* and *Fusarium culmorum*, both of which are important plantpathogens commonly found in cereals and other crops (**JECFA, 2001**). DON is commonly detected in cereals and grains, particularly in wheat, barley, maize and their byproducts. Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world, and its occurrence is considered to be an indicator of the possible presence of other, more toxic trichothecenes (**Lombaert, 2002**).

A wide gap in self-sufficiency between the production and consumption of food occurred in the light of the increasing population, rising standards of living, declining of trade in food grains and high prices in the market (**Gerber, 2014**). Cereals and other crops are very susceptible to fungal attacks during its growth in the field and also storage. Depending on environmental conditions and other factors, a fungal attack may result in mycotoxin contamination of the crop. These toxins are stable compounds and do not completely degrade at high temperatures (**Kabak, 2009**); therefore, mycotoxins can contaminate processed foods and enter the human food chain through cereal-derived foods. The main mycotoxins, due to their toxicity and prevalence, are aflatoxins (AFs) and ochratoxin A (OTA) and to a lesser extent, zearalenone (ZON), among others.

MATERIALS AND METHODS

Raw Materials

Four types of imported wheat were used in their assessment, namely French wheat,

Romanian wheat, Russian wheat and Ukrainian wheat. Samples were drawn from imported wheat shipments when they reached the ports of (Alexandria, El Dekhela and Damietta), the biggest ports in Egypt receiving imported wheat. The samples drawn in five importing intervals including 1st interval (July-August/2014), 2nd interval (September- October / 2014), 3rd interval (November-December / 2014), 4th interval (January-February/ 2015) and the 5th interval (March-April/2015). Thus, the aim of this study is to detect some mycotoxins (aflatoxin B1, total B1, B2, G1, and G2, ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZON)), in wheat grain imported from four countries (France, Romania, Russia and Ukraine).

Mycotoxin Determination

Reagents preparation

Phosphate buffered saline (PBS) was prepared by dissolving potassium chloride (0.2 g), potassium phosphate dibasic (0.2 g), sodium phosphate dibasic (1.16 g) and sodium chloride (8 g) in 900 ml water. The pH of the solution was adjusted to 7.40 with HCl or NaOH, and two drops of Tween 20 were added. Finally, the volume was adjusted to one liter

Standard solutions

A stock standard solution containing 500 µg/l of AFB1, AFG1 and OTA, 125 µg/l of AFB2 and AFG2 and 20 mg/L of ZON and DON were prepared by diluting different standard solution volumes of each mycotoxin in a mixture of acetonitrile and methanol (50:50; *V/V*). Working standard solutions of AFB1, AFG1 and OTA (100, 10 and 1 µg/l), AFB2 and AFG2 (25, 2.5 and 0.25 µg/l), ZON and DON (4000, 400 and 40 µg/l) respectively, were prepared by dilution from this stock standard solution. All prepared solutions were stored at -20°C and maintained at room temperature and in darkness for 30 min before their use. Calibration samples were prepared by

evaporating a given volume of the working standard solution under vacuum at 40°C in rotary evaporator (Gene Vac). The residue was then dissolved in 150 µl of a mixture (40:60) of acetonitrile - methanol (50:50) and water, both acidified with 0.5% formic acid. The acetonitrile extract of wheat samples were evaporated and dissolved in the same way.

Sample preparation, mycotoxins extraction

The method used for mycotoxin extraction from wheat samples is based on that which was described by **Göbel and Lusky (2004)**, with some modifications. Three hundred grams of wheat were ground and screened, using a sieve size of 0.75 mm. 10g of milled sample were extracted with 50 ml of a mixture of acetonitrile-water (60:40, *V/V*) in an orbital shaker for 30 min. The extract was filtered and then 10 ml of the filtrate were mixed with 40 ml of PBS. The mixture was centrifuged (6249 xg) at 4°C for 15 min. Fifteen milliliters of the supernatant were transferred to an AOZ immunoaffinity column (Vicam), the flow rate was adjusted to 0.9 ml per min pre-conditioned with 3 ml of water and 10 ml of PBS. After the sample had passed, the column was washed with 5 ml of PBS and 15 ml of water. Finally, the column was dried with air and the mycotoxins were eluted with 3 ml of acetonitrile, after maintaining in contact with acetonitrile and column antibodies for 5 min. The extract was evaporated to dryness in rotary evaporator and the residue was re-extracted as previously indicated. The sample was maintained at 4°C in the chromatograph tray until its analysis and mycotoxins were determination by HPLC with fluorescence detection (FLD) (**María et al., 2011**).

Recovery of mycotoxins

The value of recovery percentage was determined by comparing the peak areas of wheat blank samples spiked with different levels of mycotoxin compounds with the

peak areas of the same standards prepared in methanol (the first preparation standard) then take sequence dilutions 50:50 methanol and acetic acid. The mean detector response of different mycotoxin samples post-extracted with those prepared by adding the compound to extracted mycotoxin free wheat extract at the corresponding concentrations. All samples were established from an average of three replicate measurements.

Calibration curve and quantification

Calibration curve for each mycotoxin compound was prepared by spiking blank wheat extract at 0.1, 0.2, 1, 5, 10, 20 and 100 $\mu\text{g l}^{-1}$. Similarly, control solutions (0.5, 50, and 100 $\mu\text{g l}^{-1}$) were prepared. The calibration standards were obtained by serial dilution of working solutions with methanol, to yield final concentrations of 4.0, 5.0, 6.0, 8.0, 10 and 20 $\mu\text{g l}^{-1}$ for each mycotoxin compound for analysis of wheat sample and the quality control solutions were 5, 10, and 20 $\mu\text{g l}^{-1}$. The internal standard solution was prepared by dilution of the stock solution with methanol to a final concentration of 20 $\mu\text{g l}^{-1}$. The external standard method was applied for quantitative analysis. Standard curves were obtained by unweighted linear regression of the peak-area of the mycotoxin compound versus its known concentration. The linear regression equation of the standard curve was used to calculate the concentration of both of the control and unknown samples by peak area matching to concentration axis.

Statistical Analysis

Data analyzed by using completely randomized design (analysis of variance) and producer using MSTAT-C package differences pattern (**Michigan University, 1983**). Means were compared by using each of least significant differences (LSD) (**Steel and Torrie, 1980**) and Duncan's multiple range test (**Duncan, 1955**) at the 5% level.

RESULTS AND DISCUSSION

Aflatoxin B1 (AFB1) Content

Aflatoxin B1 (AFB1) contents ($\mu\text{g.kg}^{-1}$) in wheat samples were recorded as $\mu\text{g.kg}^{-1}$ and were presented in Table 1.

From the results it could be noted that, AFB1 contents of French wheat all over the importing studied intervals was the least among all other wheat originated from Ukraine, Romania and Russia, as the French wheat samples' values of AFB1 ranged from 0.167 to 0.400 $\mu\text{g.kg}^{-1}$ with an average of 0.288 $\mu\text{g.kg}^{-1}$. These results are in accordance with the results of **Odoemelam and Osu (2008)** who found that AFB1 in wheat was in the range of 0.17 to 0.2 $\mu\text{g.kg}^{-1}$.

Higher ranges of AFB1 (0.420, 0.572 and 0.300 $\mu\text{g.kg}^{-1}$) were found in Russian, Romanian and Ukrainian wheat samples respectively. Within the French wheat samples, 1st interval got the minimum value of (AFB1) 0.167 $\mu\text{g.kg}^{-1}$, while 3rd interval had the maximum value of AFB1 (0.400 $\mu\text{g.kg}^{-1}$), respectively.

The values of AFB1 of the imported Ukrainian wheat were in the range of 0.133 to 0.467 $\mu\text{g.kg}^{-1}$ with an average of 0.300 $\mu\text{g.kg}^{-1}$. Within this wheat origin (Ukraine) fifth interval scored the minimum value of AFB1 (0.133 $\mu\text{g.kg}^{-1}$), followed by second interval (0.233 $\mu\text{g.kg}^{-1}$). The maximum value of (AFB1) was found in the 3rd interval with score 0.467 $\mu\text{g.kg}^{-1}$.

The values of AFB1 of the imported Russian wheat samples were in the range of 0.367 to 0.500 $\mu\text{g.kg}^{-1}$ with an average of 0.420 $\mu\text{g.kg}^{-1}$, and the minimum AFB1 values were that of first and fourth intervals with similar score 0.367 $\mu\text{g.kg}^{-1}$. A maximum value of AFB1 was that of the 2nd interval with score 0.500 $\mu\text{g.kg}^{-1}$.

Table (1): Aflatoxin (B1) content of four imported wheat in five intervals from Jul. 2014 to Apr. 2015 ($\mu\text{g.kg}^{-1}$)

Origin	Intervals					Interval mean \pm SD
	Jul. / Aug.	Sep. / Oct.	Nov. / Dec.	Jan. / Feb.	Mar. / Apr.	
French Wheat	0.167 ^{fg} \pm 0.12 ¹	0.240 ^{e-g} \pm 0.05	0.400 ^{c-e} \pm 0.10	0.333 ^{c-1} \pm 0.06	0.300 ^{d-g} \pm 0.10	0.288 ^c \pm 0.11
Romanian wheat	0.400 ^{c-c} \pm 0.10	0.593 ^{ab} \pm 0.06	0.400 ^{c-e} \pm 0.10	0.766 ^a \pm 0.12	0.700 ^a \pm 0.10	0.572 ^a \pm 0.18
Russian wheat	0.367 ^{c-c} \pm 0.12	0.500 ^{bc} \pm 0.10	0.467 ^{b-d} \pm 0.12	0.367 ^{c-c} \pm 0.06	0.400 ^{c-c} \pm 0.17	0.420 ^b \pm 0.11
Ukrainian wheat	0.300 ^{d-g} \pm 0.10	0.233 ^{f-g} \pm 0.12	0.467 ^{b-d} \pm 0.12	0.367 ^{c-c} \pm 0.06	0.133 ^g \pm 0.06	0.300 ^c \pm 0.14
Interval mean \pm SD	0.309 ^b \pm 0.13	0.392 ^{ab} \pm 0.17	0.434 ^a \pm 0.10	0.458 ^a \pm 0.20	0.383 ^{ab} \pm 0.24	0.395 \pm 0.18
LSD 0.05						
Among [Origin (O.)]	Among [interval (I.)]		Interaction (O. X I.)			
0.0116 ^{**}	0.0113 [*]		0.0118 ^{**}			

• Data are expressed as means (3 replicates) \pm SD

• Averages of origin in the same column having the same superscripts are not significantly different, same averages in interval row which having same superscripts are not significantly different at $P \leq 0.05$.

The highest AFB1 value were obtained by the Romanian wheat samples with a range of 0.400 to 0.766 $\mu\text{g.kg}^{-1}$ and an Average of 0.572 $\mu\text{g.kg}^{-1}$, and minimum AFB1 values were that of first and third intervals with similar score 0.400 $\mu\text{g.kg}^{-1}$. The maximum value of AFB1 was that of the 2nd interval with score 0.593 $\mu\text{g.kg}^{-1}$.

Among intervals, first interval was the least in AFB1 as it contained an average of 0.309 $\mu\text{g.kg}^{-1}$, followed by fifth and second intervals with averages of 0.383 and 0.392 $\mu\text{g.kg}^{-1}$. That may be related to the high temperature in the first interval which didn't allow the growth of molds.

EOS (2015) stated that AFB1 content should not exceed 2 $\mu\text{g.kg}^{-1}$, and the obtained results indicated that all wheats from Ukraine, Russia, Romania and France are acceptable. Ukrainian wheat contained the least AFB1 contents are compared with those imported from Romania and Russia. Also the first and fifth intervals achieved the lowest level of AFB1 content.

All results Analyses of aflatoxins in wheat were performed and all the analyzed samples were lower than limit of detection and also agree with Zinedine *et al.* (2006).

Total Aflatoxin (B1, B2, G1, and G2) Contents

Total Aflatoxin (B1, B2, G1, and G2) contents expressed as ($\mu\text{g.kg}^{-1}$) of wheat samples imported from France, Romania, Russia and Ukraine were all recorded as $\mu\text{g.kg}^{-1}$ and were presented in Table 2.

Ukrainian wheat samples contained the least amounts of total AF (B1, B2, G1 and G2) with the range of 0.767 to 1.600 $\mu\text{g.kg}^{-1}$. The average of the five intervals of Ukrainian wheat samples was 1.160 $\mu\text{g.kg}^{-1}$ which showed to be the lowest comparing to 1.547, 1.743 and 2.147 $\mu\text{g.kg}^{-1}$ for Russian, French, and Romanian wheat samples, respectively. Within the Ukrainian wheat samples, 5th interval (Mar/Apr) got the lowest total contents of AF (B1, B2, G1, and G2), recorded 0.767 $\mu\text{g.kg}^{-1}$, while the third interval showed the highest value (1.600 $\mu\text{g.kg}^{-1}$) of total AF (B1, B2, G1, and G2).

Russian wheat showed higher values of total AF (B1, B2, G1, and G2) being (0.800-2.233 $\mu\text{g.kg}^{-1}$) with an average of 1.547 $\mu\text{g.kg}^{-1}$, the first interval of Russian wheat sample had the lowest total AF contents of all samples (0.800 $\mu\text{g.kg}^{-1}$) while

Table (2): Total AF (B1-B2-G1-G2) of four imported wheat from France, Romania, Russia and Ukraine

Origin	Total AF (B1,B2,G1 and G2) ($\mu\text{g.kg}^{-1}$)					Interval mean \pm SD
	Jul. Aug	Sep. Oct	Nov. Dec	Jan. Feb	Mar. Apr	
French Wheat	0.450 ^k \pm 0.03 ^l	3.233 ^a \pm 0.25	1.767 ^{de} \pm 0.15	1.733 ^{de} \pm 0.15	1.533 ^{e-g} \pm 0.21	1.743 ^b \pm 0.93
Romanian wheat	2.733 ^b \pm 0.15	2.200 ^c \pm 0.36	0.900 ^{ij} \pm 0.10	3.167 ^a \pm 0.35	1.733 ^{de} \pm 0.15	2.147 ^a \pm 0.84
Russian wheat	0.800 ^{jk} \pm 0.10	2.000 ^{cd} \pm 0.26	1.467 ^{e-g} \pm 0.15	1.233 ^{gi} \pm 0.25	2.233 ^c \pm 0.21	1.547 ^b \pm 0.56
Ukrainian wheat	0.933 ^{hj} \pm 0.15	1.233 ^{gi} \pm 0.06	1.600 ^{ef} \pm 0.26	1.267 ^{fh} \pm 0.25	0.767 ^{jk} \pm 0.15	1.160 ^c \pm 0.34
Interval mean \pm SD	1.229 ^d \pm 0.93	2.167 ^a \pm 0.78	1.434 ^{cd} \pm 0.37	1.850 ^b \pm 0.85	1.567 ^c \pm 0.57	1.649 \pm 0.78

LSD 0.05

Among [Origin (O.)] 0.23**	Among [interval (I.)] 0.23**	Interaction (O. X I.) 0.36**
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- Data are expressed as means (3 replicates) \pm SD
- Averages of origin in the same column having the same superscripts are not significantly different, same averages in interval row which having same superscripts are not significantly different at $P \leq 0.05$.

the fifth interval had the highest contents in all tested samples ($2.233 \mu\text{g.kg}^{-1}$).

The total values of total AF content of the Romanian wheat samples were in the range of 0.900 to $3.167 \mu\text{g.kg}^{-1}$ with an average of $2.147 \mu\text{g.kg}^{-1}$. Within this wheat samples. The third interval scored the lowest total value of AF content ($0.900 \mu\text{g.kg}^{-1}$). Were, the highest AF total value was that of the 4th interval being $3.167 \mu\text{g.kg}^{-1}$.

Interval with the lowest contents of total AF was that of the first interval followed by the third interval with no significant differences with fifth interval followed by fourth interval and maximal contents was that of the second interval (Sep/Oct).

EOS (2015) stated that total values of AFB1, AFB2, and AFG1 should not exceed than $4 \mu\text{g.kg}^{-1}$, and the obtained results showed that all wheats imported from Ukraine, Russia, Romania and France are acceptable.

Ochratoxin A (OTA) Content

Table 3 shows the Total ochratoxin A (OTA) content expressed as $\mu\text{g.kg}^{-1}$ of wheat samples imported from four origin

countries (France, Romania, Russia and Ukraine).

From the results, it could be reported that OTA content of the Ukrainian wheat was the least among all other wheat samples, as it had OTA content ranged from 0.5 to $1.367 \mu\text{g.kg}^{-1}$ with an average of $1.047 \mu\text{g.kg}^{-1}$ which showed to be lower comparing to average values of 1.153, 1.207 and $1.467 \mu\text{g.kg}^{-1}$ for Russian, French, and Romanian wheat samples, respectively. Within the Ukrainian wheat samples, the first interval got the minimum $0.5 \mu\text{g.kg}^{-1}$ OTA value, while the fifth interval had the maximum $1.367 \mu\text{g.kg}^{-1}$ OTA among content.

French and Russian wheat came above Ukrainian ones regarding the OTA content with no significant differences $P \leq 0.05$. French and Russian wheat contained the ranges of 1.067 to $1.367 \mu\text{g.kg}^{-1}$ and 0.367 to $1.5 \mu\text{g.kg}^{-1}$, respectively, with averages of 1.207 and $1.153 \mu\text{g.kg}^{-1}$, respectively., OTA contents were significantly lower than that of the Romanian wheat samples which contained an average of $1.467 \mu\text{g.kg}^{-1}$ of OTA with the range of 0.633 to $2.400 \mu\text{g.kg}^{-1}$. Within the Romanian wheat, the

Table (3): Ochratoxin A (OTA) of four imported wheat in five intervals from Jul. 2014 to Apr. 2015 ($\mu\text{g.kg}^{-1}$)

Origin	Intervals					Interval mean \pm SD
	Jul/Aug	Sep/Oct	Nov/Dec	Jan/Feb	Mar/Apr	
French Wheat	1.067 ^{ef} \pm 0.12 ¹	1.067 ^{ef} \pm 0.12	1.367 ^{c-e} \pm 0.12	1.167 ^{ef} \pm 0.29	1.367 ^{c-e} \pm 0.06	1.207 ^b \pm 0.19
Romanian wheat	0.633 ^{gh} \pm 0.15	1.567 ^c \pm 0.25	0.700 ^g \pm 0.10	2.033 ^b \pm 0.25	2.400 ^a \pm 0.10	1.467 ^a \pm 0.75
Russian wheat	1.333 ^{c-f} \pm 0.06	1.267 ^{c-f} \pm 0.25	1.500 ^{cd} \pm 0.17	0.367 ^h \pm 0.12	1.300 ^{c-f} \pm 0.20	1.153 ^b \pm 0.44
Ukrainian wheat	0.500 ^{gh} \pm 0.10	1.200 ^{d-f} \pm 0.20	1.033 ^f \pm 0.15	1.133 ^{ef} \pm 0.12	1.367 ^{c-e} \pm 0.15	1.047 ^b \pm 0.33
Interval mean \pm SD	0.883 ^c \pm 0.36	1.275 ^b \pm 0.26	1.150 ^b \pm 0.35	1.175 ^b \pm 0.64	1.609 ^a \pm 0.49	1.219 \pm 0.49

LSD 0.05

Among [Origin (O.)] 0.20**	Among [interval (I.)] 0.20**	Interaction (O. X I.) 0.31**
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- Data are expressed as means (3 replicates) \pm SD
- Averages of origin in the same column having the same superscripts are not significantly different, same averages in interval row which having same superscripts are not significantly different at $P \leq 0.05$.

maximum OTA content was that of the 5th interval being $2.4 \mu\text{g.kg}^{-1}$ which also was the maximal value among all imported wheat samples.

EOS (2015) stated that the importing Ukrainian wheat OTA should not exceed $5 \mu\text{g.kg}^{-1}$ max, and the obtained results showed that all imported wheats from Jul. 2014 to Apr. 2015 are acceptable, but the first interval from Jul. Aug 2014 was the best interval as it contained the lowest OTA content.

Deoxynivalenol (DON) Content

Deoxynivalenol (DON) contents (measured as $\mu\text{g.kg}^{-1}$) of the imported wheat samples, from four origins (France, Romania, Russia and Ukraine) within five importing intervals are presented in Table 4.

The results in Table 4 show that, DON content of Ukrainian wheat samples was the least comparing to that of French, Romania and Russia, as the DON contents in Ukrainian wheat ranged from 48.90 to $222.33 \mu\text{g.kg}^{-1}$ with an average of $93.88 \mu\text{g.kg}^{-1}$ comparing to the average values of

105.700 , 117.512 and $156.200 \mu\text{g.kg}^{-1}$ for Russian, French, and Romanian wheat samples, respectively. Within the Ukrainian wheat samples, the 5th interval (Mar. April) contained the minimum DON content being $48.90 \mu\text{g.kg}^{-1}$, while the first interval (Jul. Aug.) contained the maximum DON content ($222.33 \mu\text{g.kg}^{-1}$).

The average of DON contents for the different intervals of Russian wheat samples was higher than that of the Ukrainian wheat. DON contents are ranged between 57.67 and $200.43 \mu\text{g.kg}^{-1}$ with an average of $105.7 \mu\text{g.kg}^{-1}$. Within the Russian wheat samples, 5th interval got the minimum value of DON recorded $57.67 \mu\text{g.kg}^{-1}$ while the 2nd interval gave the maximum DON contents $200.43 \mu\text{g.kg}^{-1}$.

The content of DON of the French wheat ranged between of 70.33 to $249.33 \mu\text{g.kg}^{-1}$ with an average of $117.512 \mu\text{g.kg}^{-1}$. The French wheat at the first interval scored the minimum content of DON with value of $70.33 \mu\text{g.kg}^{-1}$, while the maximum DON of French wheat was that of the 5th interval (Mar. Apr; 2015) being $249.33 \mu\text{g.kg}^{-1}$.

Table (4): Deoxynivalenol (DON) content of four imported wheat in five intervals from Jul. 2014 to Apr. 2015 ($\mu\text{g.kg}^{-1}$)

Origin	Intervals					Interval mean \pm SD
	Jul. / Aug.	Sep. / Oct.	Nov. / Dec.	Jan. /Feb.	Mar. Apr.	
French Wheat	70.33 [±] 1.53 ¹	90.12 [±] 0.12	92.08 [±] 5.21	85.70 [±] 3.84	249.33 [±] 6.03	117.512 [±] 68.76
Romanian wheat	184.33 [±] 7.37	123.67 [±] 8.14	189.00 [±] 3.61	222.67 [±] 6.43	61.33 [±] 4.16	156.200 [±] 59.41
Russian wheat	88.67 [±] 1.53	200.43 [±] 2.38	70.73 [±] 1.36	111.00 [±] 10.67	57.67 [±] 2.35	105.700 [±] 52.59
Ukrainian wheat	222.33 [±] 3.51	79.43 [±] 1.34	60.57 [±] 5.65	58.17 [±] 2.82	48.90 [±] 1.71	93.88 [±] 67.33
Interval mean \pm SD	141.415 [±] 66.62	123.413 [±] 49.61	103.095 [±] 53.27	119.385 [±] 65.52	104.308 [±] 87.64	118.323 \pm 65.18

LSD 0.05Among [Origin (O.)]
0.20**Among [interval (I.)]
0.20**Interaction (O. X I.)
0.31**

- Data are expressed as means (3 replicates) \pm SD.
- Averages of origin in the same column having the same superscripts are not significantly different, same averages in interval row which having same superscripts are not significantly different at $P \leq 0.05$.

The maximum DON content was that of the Romanian wheat, as it ranged between of 61.33 to 222.67 $\mu\text{g.kg}^{-1}$ with an average of 156.2 $\mu\text{g.kg}^{-1}$. Wheat imported from Romania in the fifth interval scored the minimum DON content which was 61.33 $\mu\text{g.kg}^{-1}$, while the maximum was that of the 4th interval (Jan. Feb; 2015) with score 222.67 $\mu\text{g.kg}^{-1}$.

Regarding of the variance of time and intervals on the DON contents, it is clear that the first interval scored the maximum average 141.415 $\mu\text{g.kg}^{-1}$ followed by the second and the fourth intervals which scored 123.413 and 119.385 $\mu\text{g.kg}^{-1}$, respectively. The lowest interval in DON content was the third and fifth intervals with no significant differences between them with scores 103.095 and 104.308 $\mu\text{g.kg}^{-1}$, respectively.

EOS (2015) stated that the imported Ukrainian wheat DON content should not exceed 1250 $\mu\text{g.kg}^{-1}$ max, and the results indicated that all wheats imported in Jul. 2014 to Apr. 2015 were acceptable, Ukrainian wheat was the least among all other wheat originated from France, Romania and Russia in DON contents.

EFSA Assesses EU Limits for Deoxynivalenol in Cereals. EFSA has

recently delivered an Opinion on the risks for public health related to a possible increase of the maximum level (ML) of deoxynivalenol (DON) for certain semi-processed cereal products from 750 $\mu\text{g.kg}^{-1}$ to 1000 $\mu\text{g.kg}^{-1}$ (EFSA, 2014).

Zearalenone (ZON) Content

Zearalenone (ZON) contents ($\mu\text{g.kg}^{-1}$) of wheat samples imported from four origin countries including France, Romania, Russia and Ukraine, within five importing intervals are presented in Table 5.

As shown in Table 5 the average of Zearalenone content of the Ukrainian wheat was the least among other wheat samples, range of the ZON content was 40.47 - 67.17 $\mu\text{g.kg}^{-1}$ with an average of 51.574 $\mu\text{g.kg}^{-1}$. Among wheat imported from Ukraine, the second interval had the minimum ZON content (40.47 $\mu\text{g.kg}^{-1}$) followed by both third and first intervals with no significant differences between them (49.40 and 50.00 $\mu\text{g.kg}^{-1}$, respectively). The fifth interval had the highest ZON content (67.17 $\mu\text{g.kg}^{-1}$). The average of ZON of the French wheat was 53.306 $\mu\text{g.kg}^{-1}$, which was not significantly different from that of the Ukrainian wheat samples These results are accepted in accordance with the result of

Table (5): Zearalenone (ZON) content of four imported wheat in five intervals from Jul. 2014 to Apr. 2015 ($\mu\text{g.kg}^{-1}$)

Origin	Intervals					Interval mean \pm SD
	Jul. /Aug.	Sep. /Oct.	Nov. / Dec.	Jan. / Feb.	Mar. / Apr.	
French Wheat	25.67 ^m \pm 4.04 ¹	70.15 ^c \pm 0.17	68.71 ^c \pm 2.35	48.33 ^l \pm 3.51	53.67 ^h \pm 1.53	53.306 ^c \pm 16.91
Romanian wheat	79.33 ^b \pm 3.06	59.00 ^{ef} \pm 3.61	63.33 ^{de} \pm 4.16	86.00 ^a \pm 5.29	45.33 ^{jk} \pm 2.52	66.598 ^a \pm 15.41
Russian wheat	60.67 ^c \pm 1.15	69.40 ^c \pm 0.56	54.77 ^{fg} \pm 0.68	42.43 ^{kl} \pm 2.14	81.13 ^b \pm 1.63	61.680 ^b \pm 13.60
Ukrainian wheat	50.00 ^h \pm 2.00	40.47 ^l \pm 1.36	49.40 ^h \pm 2.16	50.83 ^g \pm 0.72	67.17 ^{cd} \pm 2.74	51.574 ^c \pm 9.10
Interval mean \pm SD	53.917 ^c \pm 20.40	59.755 ^{ab} \pm 12.62	59.053 ^{ab} \pm 8.12	56.898 ^b \pm 18.06	61.825 ^a \pm 14.33	58.290 \pm 15.03
LSD 0.05						
	Among [Origin (O.)] 3.06**		Among [interval (I.)] 2.98**		Interaction (O. X I.) 4.68**	

- Data are expressed as means (3 replicates) \pm SD
- Averages of origin in the same column having the same superscripts are not significantly different, same averages in interval row which having same superscripts are not significantly different at $P \leq 0.05$.

(Zaied *et al.*, 2012) who found that ZON in wheat was in the range of 36 to 61.31 $\mu\text{g.kg}^{-1}$.

The average of ZON content of the imported Russian wheat was higher than of French or Ukrainian wheat. The ZON content of Russian wheat was ranged from 42.43 to 81.13 $\mu\text{g.kg}^{-1}$ with an average of 61.680 $\mu\text{g.kg}^{-1}$.

The content of ZON of the Romanian wheat was the highest, comparing to those of the other samples from France, Russia and Ukraine, which ranged between 45.33 to 86.00 $\mu\text{g.kg}^{-1}$ with an average of 66.598 $\mu\text{g.kg}^{-1}$. The fifth interval had the minimum ZON content (45.33 $\mu\text{g.kg}^{-1}$) followed by both the second and the third intervals had (59.00 and 63.33 $\mu\text{g.kg}^{-1}$, respectively). The maximum ZON content was that of the 4th interval (Jan. Feb; 2015) recorded 86.00 $\mu\text{g.kg}^{-1}$.

Comparatively by taking interval as the factor, the first interval had the lowest ZON content (53.917 $\mu\text{g.kg}^{-1}$) followed by the fourth, the third and the second intervals, while the fifth interval contained 61.825 $\mu\text{g.kg}^{-1}$ which was the highest average of ZON among all other intervals. The highest

value of ZON was that of Russian wheat in the fifth interval recorded 81.13 $\mu\text{g.kg}^{-1}$.

EOS (2015) stated that ZON content should not exceed 100 $\mu\text{g.kg}^{-1}$ max, and the obtained results assured that all imported wheats from Jul. 2014 to Aug. 2015 are acceptable, but the first interval from Jul. Aug; 2014 was the best one.

And all results exceeded the tolerable limit (100 mg/kg) as recommended by the European Union (European Commission, 2007).

Conclusions

The study focused on inspection and quality control of some wheat that Egypt import, recognizing how these freights correspond with the Egyptian standards. Some mycotoxins were determined and quantified in wheat grains imported from four countries at five intervals. The obtained results indicated that the Ukrainian wheat was the best among all other wheat originated from France, Romania and Russia, followed by French wheat, Russian wheat and lastly Romanian wheat in most tests used (Total (B1-B2-G1-G2), Ochratoxin A (OTA) Deoxynivalenol (DON) and

Zearalenone (ZON) and came in the second step in Aflatoxin (B1). Finally it is recommended to increase importing Ukrainian wheat.

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مقارنة بين أربعة أقماح مستوردة لمصر في محتوى الميكوتوكسينات

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يعتبر القمح من أهم المحاصيل الغذائية في مصر بالإضافة الى أنه يتم استهلاكه بمستوى عالي داخل مصر وذلك لرخصه مقارنة بالمصادر الأخرى، والإنتاج المحلي من القمح لا يكفي احتياجات المستهلكين وبالتالي تزداد الفجوة من القمح، والهدف الرئيسي من الدراسة مقارنة الأقماح المستوردة لمصر من دول مختلفة، حيث تم في هذه الدراسة مقارنة أربعة أقماح من أربعة دول هي (أوكرانيا - روسيا - فرنسا - رومانيا) خلال خمسة فترات، الفترة الأولى (يوليو - أغسطس/٢٠١٤)، الفترة الثانية (سبتمبر - أكتوبر/٢٠١٤)، الفترة الثالثة (نوفمبر - ديسمبر/٢٠١٤) الفترة الرابعة (يناير - فبراير/٢٠١٥) الفترة الخامسة (مارس - أبريل/٢٠١٥) وتم مقارنة هذه الأقماح باستخدام عدة اختبارات هي (محتوى الافلاتوكسين ب ١ - إجمالي الافلاتوكسين (ب ١، ب ٢، ج ١، ج ٢) - محتوى الاوكراتوكسين أ - محتوى الداى أوكسى نيفالينول - محتوى الزيرالينون) وجاءت النتائج كما يلي تفوق القمح الأوكراني على جميع الأقماح المستوردة من (روسيا - فرنسا - رومانيا)، حيث حقق القمح الأوكراني المركز الأول في أربعة اختبارات هي إجمالي الافلاتوكسين (ب ١ ، ب ٢، ج ١، ج ٢) حيث حقق ٠,٧٦٧ - ١,٦٠ ميكروجرام/كجم وبمتوسط ١,١٦٠ ميكروجرام/كجم، ومحتوى الاوكراتوكسين أ حيث حقق ٠,٥٠ - ١,٣٦٧ ميكروجرام/كجم وبمتوسط ١,٠٤٧ ميكروجرام/كجم، ومحتوى الداى أوكسى نيفالينول حيث حقق ٤٨,٩٠ - ٢٢٢,٣٣ ميكروجرام/كجم وبمتوسط ٩٣,٨٧ ميكروجرام/كجم، ومحتوى الزيرالينون حيث حقق ٤٠,٤٧ - ٦٧,١٧ ميكروجرام/كجم وبمتوسط ٥١,٥٧ ميكروجرام/كجم، وجاء في المركز الثاني في اختبار واحد هو محتوى الافلاتوكسين ب ١ حيث حقق ٠,١٣٣ - ٠,٤٦٧ ميكروجرام/كجم وبمتوسط ٠,٣٠٠ ميكروجرام/كجم.

الكلمات الإسترشادية: حبة القمح، الافلاتوكسين، الداى أوكسى نيفالينول، الأوكراتوكسين، الزيرالينون.

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