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Estimate The Level of Deoxynivalenol and Several Biochemical Factors in Iraqi and Imported Wheat

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ABSTRACT

Fusarium species infect cereal crops worldwide and cause the important diseases. *Fusarium* head blight and crown rot in wheat. *Fusarium* pathogens reduce yield and some species also produce trichothecene mycotoxins, such as deoxynivalenol (DON), during infection. These toxins play roles in pathogenesis on wheat and have serious health effects if present in grain consumed by humans or animals. The aim of present study was to evaluate the extent of mycotoxins contamination and their occurrence by measuring the deoxynivalenol (DON) levels in local and imported wheat grain and other biochemical parameters such as moisture ,Ash ,fatty acid ,protein, hydrolysis enzyme (protease) before and after 3 months storage according to types in all stores. The results showed that Deoxnivalenol significantly increased ($p<0.05$) before and after 3 months storage according types in all stores as well as the moisture, Ash, alkaline protease, and fatty acid were significantly increase before and after 3 months

The current study conclude that the increase in the .storage according types in all stores occurrence of colon irritation and abdominal pain, headache as well as nausea may be due to the contamination of wheat with DON and exclusively being the form of a nutrition item when the chief components of the people in Iraqi

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INTRODUCTION

Wheat *(Triticum aestivum L.*) is one of the important food crops in the world, a cereal grain in the grass family is counted among the 'big three' cereal crops which is used for human food and livestock feed, bread wheat have two types varieties cultivation winter and spring wheat difference at their flowering time the spring wheat is much shorter than the winter wheat's [1,2]. Deoxynivalenol is a trichothecenes which is a large group of sesquiterpenoid antibiotics that inhibit protein synthesis in eukaryotes also referred to as vomitoxin. The DON affects animal and human health causing diarrhea, vomiting, gastro-intestinal inflammation, and immunomodulation it dose well in moist and wet conditions, trichothecenes biosynthesis begins with the formation of trichodiene, which undergoes multiple oxygenation, cyclization and esterification reactions [3-6].Reactive oxygen species are produced in both unstressed and stressed cells in various locations chloroplasts, mitochondria and peroxisomes. Free radicals are very unstable and react quickly with other compounds (protein and lipid) [7-8]. Polyunsaturated fatty acids that contain two or more double bonds are particularly susceptible to oxidation by free radicals and other highly reactive species malondialdehyde has been known as the end product of peroxidation of membrane lipids [9]. Proteases constitutes a complex group of enzymes that hydrolyze protein and plays an important nutritional and organizational role in nature. Proteases are protein digesting enzyme that are classified according to the optimum PH into neutral, acidic and alkaline proteases [10-11].

MATERIALS AND METHODS

Experiment was conducted using wheat (*Triticum aestivum L.*) genotype fareed taken from different places in Iraqi stores and different species of [Domestic and Imported wheat] from harvesting of crop year 2013. Moisture Content[The procedure of AACC (2000) method No. 44-15A] was followed for the estimation of moisture content in each sample. The moisture content was determined in each flour sample by drying 3 g flour

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sample at a temperature of 105± 5 °C till constant weight[12].The ash content in each flour sample was determined as a total inorganic matter by following the procedure given in AACC (2000) method No. 08-01. The moisture content was determined in each flour sample by drying 5 g flour sample at a temperature of 550-600 °C till grayish ash formed[13].Fatty acid was determind by adopting procedure described in AOCAC(2005) method No. (940.22) [14]. The soluble sugars were extracted from the dried wheat flour sample by alcohol extraction. Five hundred mg of sample was placed in a conical flask. Ethyl alcohol (80%) was added to it and boiled for 5 min on a hot water bath. The contents were cooled to room temperature and the supernatant was transferred to a volumetric flask. The extraction was carried out thrice and the final volume was made up to 10 ml. Total sugars were estimated in alcohol free extract using Nelson-Somogyi's method. After hydrolyzing 1 ml of the alcohol-free extract with 1N HCl at 50-600C, it was cooled and neutralized with 1N NaOH followed by 0.1N HCl using phenolphthalein as an indicator. The neutralized extract was made up to 5 ml with distilled water. To one ml of neutralized extract, one ml of alkaline copper reagent was added. It was kept on the boiling water bath for 20 min. After cooling, one ml of arsenomolybdate reagent was added with immediate shaking and the final volume was made up to 15 ml. Absorbance was measured at 510 nm in a spectrophotometer using standard curve of D-glucose against a reagent blank. Reducing sugars were estimated in the alcohol-free extract using Nelson-Somogyi's method (Sadasivam and Manikam, 1992)[15] .Protein concentration was determined by the method of Lowry *et al.,* 1951 using bovine serum albumin (BSA) as standard protein. The amount of the soluble protein was calculated from the standard curve as mg of protein per ml of test samples[16].Activity of protease was measured by the method of casein digestion by using the tyrosine as standard [17].

RESULTS AND DISCUSSION

Tables (1), (2) showed the mean and standard deviation of moisture % before and after 3 months storage according types of wheat and storage stores , the present study shows a non-significant(p>0.05) differences in the mean between different types of wheat except the local second class wheat and harmed(damaged) wheat (p<0.01) and according to the types of wheat ,also shows a non-significant differences(p>0.05) in the mean according to the storage stores in all types of wheat except two stores Al- Essaouira store and Salman pak stores(p< 0.01) .[18-19]

Table 3-3: The mean and standard deviation of moisture % before and after 3 months storage according types in all stores .

moisture %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	11.04±0.96	12.91±0.92	0.063	p> 0.05 (NS)
Local Second Class Wheat	10.97±0.99	13.25±0.80	0.002	p< 0.01 (S)
Australian wheat Origin	12.60±0.50	13.98±0.74	0.060	p> 0.05 (NS)
Turkish wheat Origin	12.50±0.46	13.22±0.81	0.052	p> 0.05 (NS)
Russian wheat Origin	12.73±0.57	13.96±0.67	0.054	p> 0.05 (NS)
Harmed(Damaged) wheat	14.34±0.32	18.28±1.08	0.003	p< 0.01 (S)

Table 3-4: The mean and standard deviation of moisture % before and after 3 months storage according stores in all types .

moisture %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	12.18±1.44	14.06±1.62	0.059	p> 0.05 (NS)
Al- Doura store	12.09±1.24	14.25±2.39	0.078	p> 0.05 (NS)
Al- Essaouira store	12.98±1.11	15.73±1.97	0.006	p< 0.01 (S)
Al-Taji store	12.87±0.98	14.74±2.12	0.080	p> 0.05 (NS)
Salman pak store	12.98±0.77	15.14±1.88	0.026	*p< 0.05 (S)*
Al-Kut store	12.64±1.11	14.01±2.09	0.188	p> 0.05 (NS)
Al- Nasiriya store	11.76±1.82	13.33±1.64	0.151	p> 0.05 (NS)
Basra store-Al	11.90±2.19	14.18±2.18	0.101	p> 0.05 (NS)

After 3 months of storage, higher relative humidity in atmosphere caused the flour moisture to increase up to end of storage. However, the changes were more in samples having higher initial moisture content. The moisture in Al- Essaouira and Salman pak stores increased throughout the storage period.Tables (3)and (4) showed a non-significant differences Ash (p>0.05) in the mean between different types of wheat The ash content in wheat and flour has significance important for milling millers need to know the overall mineral content of the wheat to achieve desired or specified ash levels in flour. Since ash is primarily concentrated in the bran, ash content in flour is an indication of the yield that can be expected during milling ash content also indicates milling performance by indirectly revealing the amount of bran contamination in flour [20]

Table 3-5: The mean and standard deviation of ash % before and after 3 months storage according types in all stores

Ash %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	1.04±0.08	1.19±0.11	0.169	*p*> 0.05 (NS)
Local Second Class Wheat	1.11±0.09	1.14±0.07	0.395	*p*> 0.05 (NS)
Australian wheat Origin	1.13±0.22	1.26±0.14	0.187	*p*> 0.05 (NS)
Turkish wheat Origin	2.07±0.06	1.99±0.35	0.524	*p*> 0.05 (NS)
Russian wheat Origin	1.12±0.09	1.28±0.31	0.218	*p*> 0.05 (NS)
Harmed(Damaged) wheat	1.05±0.05	1.16±0.04	0.310	*p*> 0.05 (NS)

Table 3-6: The mean and standard deviation of ash % before and After 3 months Storage according stores in all types .

Ash %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	1.34±0.43	1.35±0.40	0.938	*p*> 0.05 (NS)
Al- Doura store	1.24±0.44	1.32±0.40	0.743	*p*> 0.05 (NS)
Al- Essaouira store	1.28±0.37	1.38±0.38	0.627	p> 0.05 (NS)
Al-Taji store	1.28±0.44	1.35±0.39	0.776	*p*> 0.05 (NS)
Salman pak store	1.22±0.44	1.30±0.41	0.763	*p*> 0.05 (NS)
Al-Kut store	1.22±0.39	1.38±0.40	0.501	*p*> 0.05 (NS)
Al- Nasiriya store	1.20±0.42	1.29±0.37	0.720	*p*> 0.05 (NS)
Basra store-Al	1.27±0.40	1.33±0.39	0.648	*p*> 0.05 (NS)

Tables (5) and (6) showed the mean and standard deviation of protein concentration [mg/ml] before and after 3 months of storage according types and according to the stores, the present study shows a significant decrease in the protein concentration between different types of wheat(p<0.01)except harmed wheat (p<0.05), these result may be due to the length of storage period by the fungi , also shows a significant differences in the mean according to the storage stores in all types of wheat except Al- Nasiriya store(p>0.05) .The significant decrease in protein concentration after 3 month storage due to the different storage conditions between the stores while all types of wheat shows less protein concentration with the time of storage the normal value as reported was (1.5-2.7 mg/ml) experimentally that the stress environments drought and increased temperature effects on grain protein content which is used as a good marker of baking performance[21,22].

Table 3-13: The mean and standard deviation of protein concentration [mg/ml] before and after 3 months storage according types in all stores.

Protein Con. [mg/ml]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	1.26±0.03	0.90±0.07	0.001	*p*< 0.01 (S)
Local Second Class Wheat	1.24±0.04	0.93±0.07	0.002	*p*< 0.01 (S)
Australian wheat Origin	1.32±0.05	0.98±0.09	0.001	*p*< 0.01 (S)
Turkish wheat Origin	1.31±0.05	0.94±0.14	0.001	*p*< 0.01 (S)
Russian wheat Origin	1.28±0.06	0.95±0.18	0.003	*p*< 0.01 (S)
Harmed(Damaged) wheat	0.79±0.17	0.61±0.11	0.020	*p*< 0.05 (S))

Table 3-14: The mean and standard deviation of protein concentration [mg/ml] before and after 3 months storage according stores in all types.

Protein Con. [mg/ml]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	1.16±0.22	0.88±0.15	0.025	*p*< 0.05 *(S)*
Al- Doura store	1.17±0.16	0.85±0.16	0.016	*p*< 0.05 *(S)*
Al- Essaouira store	1.18±0.28	0.75±0.14	0.007	*p*< 0.01 (S)
Al-Taji store	1.24±0.15	0.92±0.13	0.003	*p*< 0.01 (S)
Salman pak store	1.15±0.32	0.75±0.16	0.022	*p*< 0.05 (S)
Al-Kut store	1.25±0.16	0.90±0.11	0.002	*p*< 0.01 (S)
Al- Nasiriya store	1.22±0.21	1.11±0.24	0.373	*p*> 0.05 (NS)
Al- Basra store	1.21±0.14	0.93±0.10	0.002	*p*< 0.01 (S)

Tables (8), (9) showed The significant increase in neutral protease specific activity [U/gm] before and after 3 months storage according types in all stores due to long storage period caused the protein to consumption and digested by the protease which break it down into smaller amino acid protease breaks peptide bonds and hydrolyze the protein and form a bulky cluster of enzymes the high increased in protease enzyme in all types of wheat which is an indicator of increased the risk of fungal infection they employ the enzyme to break down protein and provide the energy required for growth. [23-25]

Table 8: The mean and standard deviation of alkaline protease specific activity [U/gm] before and after 3 months storage according types in all stores.

Alkaline Protease [U/gm]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	0.282±0.06	0.431±0.03	0.013	*p*< 0.05 (S)
Local Second Class Wheat	0.292±0.06	0.452±0.03	0.015	*p*< 0.05 (S)
Australian wheat Origin	0.316±0.05	0.458±0.03	0.041	*p*< 0.05 (S)
Turkish wheat Origin	0.336±0.05	0.444±0.03	0.043	*P*< 0.05 (S)
Russian wheat Origin	0.312±0.04	0.449±0.04	0.035	*p*< 0.05 (S)
Harmed(Damaged) wheat	0.429±0.04	0.710±0.06	0.008	*P*< 0.01 (S)

Table 9: The mean and standard deviation of alkaline protease specific activity [U/gm] before and after 3 months storage according stores in all types .

Alkaline Protease [U/gm]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	0.321±0.06	0.479±0.10	0.011	*p*< 0.05 (S)
Al- Doura store	0.298±0.07	0.489±0.10	0.004	*p*< 0.01 (S)
Al- Essaouira store	0.284±0.09	0.511±0.11	0.003	*p*< 0.01 (S)
Al-Taji store	0.334±0.08	0.464±0.10	0.036	*P*< 0.05 (S)
Salman pak store	0.314±0.06	0.512±0.16	0.016	*p*< 0.05 (S)
Al-Kut store	0.354±0.05	0.469±0.10	0.030	*P*< 0.05 (S)
Al- Nasiriya store	0.338±0.07	0.511±0.12	0.011	*p*< 0.05 (S)
Al- Basra store	0.380±0.04	0.489±0.09	0.026	*p*< 0.05 (S)

Tables (10), (11) showed a significant increase in the mean between different types of wheat and also shows a significant differences in the mean according to the storage stores in all types of wheat due to higher temperature and long period of storage which caused to hydrolysis of glycosides by microorganisms and fungi[19]

Table 10: The mean and standard deviation of fatty acid % before storage and after 3 months storage according types in all stores.

Fatty acid %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	23.74±1.90	34.73±1.66	0.006	*p*< 0.01 (S)
Local Second Class Wheat	24.52±1.17	35.70±1.86	0.008	*p*< 0.01 (S)
Australian wheat Origin	31.48±1.25	41.14±1.03	0.012	*p*< 0.05 (S)
Turkish wheat Origin	29.54±1.22	40.36±0.88	0.030	*P*< 0.05 (S)
Russian wheat Origin	31.25±1.93	42.22±0.90	0.018	*p*< 0.05 (S)
Harmed(Damaged) wheat	44.06±3.21	59.32±1.95	0.001	*P*< 0.01 (S)

Table 11: The mean and standard deviation of fatty acid % before storage and after 3 months storage according stores in all types.

Fatty acid %	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	29.69±6.50	42.42±7.82	0.018	*p*< 0.05 (S)
Al- Doura store	30.79±7.88	43.18±9.38	0.021	*p*< 0.05 (S)
Al- Essaouira store	33.24±8.14	43.45±8.60	0.033	*p*< 0.05 (S)
Al-Taji store	30.29±6.89	41.44±10.35	0.024	*p*< 0.05 (S)
Salman pak store	33.55±8.13	43.01±7.65	0.044	*p*< 0.05 (S)
Al-Kut store	30.03±7.58	41.90±10.42	0.046	*p*< 0.05 (S)
Al- Nasiriya store	29.51±6.65	40.65±8.43	0.029	*p*< 0.05 (S)
Al- Basra store	29.02±7.28	41.89±9.02	0.022	*p*< 0.05 (S)

Tables (12), (13) show the mean and standard deviation of total carbohydrate [mg/100ml] before and after 3 months storage according types in all stores and according to storage stores . The present study shows a significant differences in the mean of total carbohydrate between different types of wheat in all stores showed a significant differences($p<0.01$) in the mean of total carbohydrate between different types of wheat in all stores. Carbohydrates are important components of storage and structural materials in the plants [26-28].

Table 12**:** The mean and standard deviation of total carbohydrate [mg/100ml] before storage and after 3 months storage according types in all stores

Total carbohydrate [mg/100ml]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	2.49±0.16	1.59±0.10	0.002	*p*< 0.01 (S)
Local Second Class Wheat	2.48±0.13	1.58±0.11	0.002	*p*< 0.01 (S)
Australian wheat Origin	2.47±0.09	1.61±0.09	0.001	*p*< 0.01 (S)
Turkish wheat Origin	2.51±0.12	1.59±0.12	0.003	*p*< 0.01 (S)
Russian wheat Origin	2.48±0.11	1.55±0.11	0.004	*p*< 0.01 (S)
Harmed(Damaged) wheat	2.34±0.14	0.84±0.08	0.0008	*p*< 0.001 (S)

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Table 13**:** The mean and standard deviation of total carbohydrate [mg/100ml] before and after 3 months storage according stores in all type.

Total carbohydrate [mg/100ml]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	2.45±0.16	1.51±0.33	0.001	*p< 0.01 (S)*
Al- Doura store	2.40±0.07	1.52±0.34	0.002	*p< 0.01 (S)*
Al- Essaouira store	2.45±0.02	1.24±0.28	0.001	*p< 0.01 (S)*
Al-Taji store	2.35±0.15	1.50±0.27	0.004	*p< 0.01 (S)*
Salman pak store	2.39±0.03	1.42±0.29	0.003	*p< 0.01 (S)*
Al-Kut store	2.47±0.04	1.48±0.03	0.002	*p< 0.01 (S)*
Al- Nasiriya store	2.68±0.14	1.50±0.28	0.001	*p< 0.01 (S)*
Al- Basra store	2.49±0.08	1.52±0.33	0.003	*p< 0.01 (S)*

Table (14),(15) shows a significant increase in the mean of DON [ppm] according to the storage stores in all types of wheat except Al-Rusafa and Al-Nasiriya stores showed a non-significant differences (p>0.05) after 3 months of Harmed wheat showed a highly increased in DON levels after 3 months storage especially in Al-Doura, Al-Kut, Salman pak, Al- Nasiriya and Al- Basra stores AL-Rusafa and Al- Nasiriya stores showed the low levels of DON in the all types of wheat except harmed wheat ,this may be due to separate the harmed wheat in isolated places and far from other types of wheat compared to the other stores The increased in DON levels in wheat due to the different climate condition formed in the field (and during storage) under favorable environmental and agronomical conditions such as warm and moist during fungal infection (grain flowering) also climate change will lead to shifts in the occurrence of mycotoxins in cereal grains. [29,30]

Table 14: The mean and standard deviation of DON [ppm] before and after 3 months storage according to types in all stores .

DON [ppm]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
Local First Class Wheat	1.17±0.17	2.50±0.13	0.029	*p*< 0.05 (S)
Local Second Class Wheat	1.55±0.07	2.00±0.55	0.036	*p*< 0.05 (S)
Australian wheat Origin	1.00±0.05	2.03±0.69	0.014	*p*< 0.05 (S)
Turkish wheat Origin	1.02±0.07	1.74±0.65	0.038	*P*< 0.05 (S)
Russian wheat Origin	1.06±0.18	1.79±0.58	0.045	*p*< 0.05 (S)
Harmed(Damaged) wheat	2.41±0.48	4.25±1.01	0.025	*P*< 0.05 (S)

Table 15: The mean and standard deviation of DON [ppm] before and after 3 months storage according stores in all type.

DON [ppm]	Before storage	After 3 months Storage	Comparison of Significant	
			p Value	Sig
AL-Rusafa store	1.51±0.73	1.66±0.68	0.727	p> 0.05 (NS)
Al- Doura store	1.41±0.63	3.14±1.85	0.004	*p*< 0.01 (S)
Al- Essaouira store	1.41±0.72	2.18±0.99	0.043	*p*< 0.05 (S)
Al-Taji store	1.26±0.42	2.48±0.75	0.024	*p*< 0.05 (S)
Salman pak store	1.62±0.71	3.79±1.58	0.002	*P*< 0.01 (S)
Al-Kut store	1.25±0.43	2.90±0.55	0.008	*P*< 0.01 (S)
Al- Nasiriya store	1.24±0.40	1.25±0.55	0.911	*p*> 0.05 (NS)
Al- Basra store	1.25±0.40	3.22±1.66	0.018	*P*< 0.05 (S)

According to our information, no previous study consist of all factors that have been measured in this study and a range of grain stores in central and southern Iraq. The study showed fungal contamination in all the stores and all kinds of domestic and imported wheat and this may be due to several reasons :The observed increase in deoxynivalenol in local first class and second class wheat than the imported types may be due to the change in weather in the year of study and the highly increase in all types of wheat in the present study may be due to the retention of harmed wheat in the stocks and places neighboring to the rest of the types of wheat for a long time .

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