Al-Muthanna J. For Agric Sci Print ISSN: 2226-4086 Online ISSN:2572-5149
Vol. 10 , Issue. 02. 2023

https://muthjas.mu.edu.iq/ http://doi.org/10.52113/mjas04/10.2/1

Detection of microorganisms and afla toxins (B1) in local wheat grains and local flour produced in Iraq

Iman K.L. Al-Zubaydi [1], Prof . Dr . Kadhim Hashim Yassin [2], Lubna A.J. Rasheed [3]

[1] Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq
Emankhalil87644@gmail.com ORCID ID: https://orcid.org/0009-0002-1768-9580
[2] Ibn Khaldun University College and Al-Mustansiriyah University, Baghdad, Iraq
khzwaini@uomustansiriyah.edu.iq ORCID ID: http://orcid.org/0000-0002-7481-7270
[3] Department of Environmental Engineering, Al-Mustansiriyah University Baghdad, Iraq
dr.lubna@uomstansiriyah.ed.iq ORCID ID: http://orcid.org/0000-0001-8741-0069

Received on15 /5/2023 Accepted on 31/7/2023 Published on 15/12/2023

Abstract

It can cause scattered dust and usually loaded with various germs that adversely affect the health of silos workersin silos. The main objective of the study is to measure microbial contamination. (bacterial, fungal, mycotoxins), which are widely prevalent in Iraqi silos and mills and negatively It affects the health of silos workers in particular and the general health of the community. The study included 40 samples of wheat grains and 40 samples of wheat flour, which were randomly collected for the winter seasons from 4 silos in Baghdad, silo Maysan and siloAL- Basra, and silo Erbil and silo Kirkuk during December and January 2022. The results indicated that the most isolated types of wheat and wheat flour are pathological spores in addition to the presence of a percentage of microbial toxins, and the chemical analysis with HPLC during the winter season showed the presence of toxic mycotoxin contamination for a number of silos, and the khan was about (76.6 ppb) by 12.5%, while the silos (cycle, coronary) were about 37.5%, with statistically significant differences ($p. \leq 0.05$). The results of the statistical analysis indicated that there were statistically Statistically significant differences ($p \leq 0.05$) were recorded for wheat and flour in (temperature and humidity associated with storage,) and most of them samples contained more Coliforms and Mold than the permissible limit, while some of the samples contained an allowable percentage of mycotoxins.

KEYWORDS: Temperature and Moisture, Microorganisms Contamination, B1 afla toxin

Introduction

Wheat (bread wheat) (Triticumaestivum L.) It is considered one of the main commodities and plays a key role in global trade. (7), 715 million tons per year is the total world production per year, thus ranking second after corn in consumption and about (billion tons / year), and The most famous microbes are Widespread and highly common damage caused by loss of grain quality during storageperiod is the growth of the mold. (1).

A large number of research and studies have shown secondary metabolites known as mycotoxins are one of the main causes of damage to grains stored in silos, which may lead to food and feed poisoning.. The importance of fungi is due to the fact that their enzymes are extracellular (9). Most fungi have the ability to produce high concentrations of toxins if they have the right conditions and are characterized by their ability to contaminate large quantities of stored grains in proportions exceeding the permissible limits. (8)

Mycotoxins are toxic by-substances produced from some fungi such as Aspergillus, Fusarium, Penicillium, when eating food products containing these toxins, causing a serious risk to consumer health (15). Many types of these toxins Which have been discovered, and the most important and widespread are those that negatively affect health in general. of organisms such as Aflatoxin, Ocratoxin, Patulin, Fumioncin, Zeraline, Nivanol and Diboxinvalenol, although they may appear in the food chain as a result of crop contamination with mushrooms in the field and sometimes after harvest. (8).

Aflatoxin (B1) is classified among the most dangerous types of deadly toxinsnd is a powerful carcinogen (11), fungi (e.g., *Apergillus spp, Fusarium spp, Penicillium spp*) and Bacteria (e.g., *Salmonella spp Bacillus cereus*) contaminate flour and its products and also the presence of these bacterial species more than the permissible limit causes many diseases., (16)

The main objective of this study is to measure microbial contamination associated with storage conditions including temperature and humidity and also To measure aflatoxin B1 in Iraqi wheat and flour**.**

MATERIAL AND METHODS

COLLECTION OF SAMPLES

Forty samples were collected randomly. from silos (Dora, Taji, Rusafa, Khan), and also from (Maysan, Basra, Kirkuk, Erbil,) and samples were collected for the winter season 2022. Domestic wheat included only at the time of research, flour produced from the grinding process to compare microbial contamination before and after milling.

ISOLATION OF BACTERIA

Weigh five grams for each sample and then hang it in 45 ml of distilled water, and then leave it to homogeneity within 15 minutes, after which only 1 ml of stuck Then divide into suitably sterilized Petri dishes (nutritious agar and McConki agar) until pure isolation is obtained, and by the Vitek 2 system to diagnose it definitively. (4).

ISOLATION OF FUNGAL

Wheat samples are planted in petri dishes (diameter 90 mm, 10 grains) containing (grapes agar) (PDA) and the dishes were incubated for (5-7) days at 25 ° C.There is a second The fungus was isolated by taking one gram for each sample added to 9 ml of sterile D.W with a light shake of the mixture, and then leave the mixture for 15 minutes., and then transfer to sterile Petri dishes and cool (dextrose agar Sabord) (SDA) to 45 ° C and is poured and incubated at 25°C for 5 days, Morphology characterization of fungal species and microscopic observation was done , The average number of colonies developing in each sample, the percentage of the presence of each sex and species, the frequency of isolation for each sex and the type of fungal load of the samples were calculated. (18).

$$\%\text{frequency of species} = \frac{\text{Number of species appearance in the sample}}{\text{total number of the species appearance}} \times 100\%$$

Detection of mycotoxins using HPLC technology

The examination was carried out in the laboratories of the Ministry of Science and Technology - Department of Environment and Water and according to the method provided using a high-performance liquid chromatography device (SYKAM model) (German origin), where the carrier phase was used: acetic tontrile: distilled water: (30: 70) The separation column was: (C- 18) ODS (25cm * 4.6mm) to separate the mycotoxins. A fluorescence detector (ex=365nm, em= 445nm) was used to detect the mycotoxins, where the flow rate of the carrier phase was: 0.7 ml. / min.

STATISTICAL ANALYSIS

We used the program (Statistical Analysis) - SAS (2018) and through this program the differences and their effects on the elements of the current study and also in the study parameters were revealed. The T-test and the less significant difference in the LSD (Analysis of Variance-ANOVA) test used here to compare evidence. The chi-square test, through which the moral comparison was made in percentage, (0.05 and 0.01) probability in this study.

RESULTS

Identification of Bacteria & Fungi for Winter Season.

The results obtained for the winter season . 2022 in the table, which shows a variety of contaminated microorganisms in samples of local cereals and wheat flour, which included pathogenic bacteria and fungi in 4 silos in Baghdad from (Dora , Taji , Rusafa , Khan), Maysan silo, Basra silo, Erbil silo and Kirkuk silo**.** Microbial growth was diagnosed by studying the microscopic properties of bacterial isolates by gram dye and fungal isolates by lactophenol, so the built-in Vitek2 system was used to confirm the identification of isolated bacteria as well as the diagnosis of fungi based on morphology under the microscope

Identification Of Bacteria (Grain and Wheat flour) winter \2022 and total bacterial (Table 1) (count (CFU\g

Location	Type	Isolated bacterial specie	total bacterial (count (CFU\g
Al- Dora silo	wheat	*Pantoea Spp.*	1.95×10^6
	Flour	*Pantoea Spp. Sphingomonas paucimobilis*	9.7×10^6
AL –Taji silo	wheat	*Ralstonia insidiosa Sphingomonas paucimobilis*	4.5×10^6
	Flour	*Ralstonia insidiosa Enterobacter cloacae Sphingomonas paucimobilis*	9.9×10^6
Al-Rusafa s ilo	wheat	*Serratia plymuthica Ralstonia insidiosa Ralstonia Pickettii*	1.54×10^6
	Flour	*Sphingomonas paucimobilis Ralstonia Pickettii*	7.1×10^6
Al- Khan silo	wheat	*Sphingomonas paucimobilis Pantoea Spp.*	1.17×10^6
	Flour	*Pantoea Spp. Sphingomonas paucimobilis Enterobacter cloacae complex*	5×10^6
Maysan silo	wheat	*Ralstonia insidiosa Staphylococcus Kloosii*	0.86×10^6
	Flour	*Ralstonia insidiosa Ralstonia Pickettii Enterococcus faecalis. Sphingomonas paucimobilis*	5×10^6
Basra	wheat	*Sphingomonas gallinarum*. *Sphingomonas*	0.68×10^6

AL silo		*paucimobilis* *Sphingomonas lentus*	
	Flour	*Sphingomonas paucimobilis. Enterococcus faecalis*	7×10^6
Erbil silo	wheat	*Ralstonia insidiosa* *Enterobacter cloacae complex*	5.27×10^6
	Flour	*Enterobacter cloacae complex* *Enterococcus faecalis*	9.90×10^6
Kirkuk silo	wheat	*Sphingomonas paucimobilis* *Pantoea Spp.*	3.88×10^6
	Flour	*Sphingomonas paucimobilis. Ralstonia Pickettii* *Enterococcus faecalis*	8.65×10^6

A different group of bacteria found in samples of wheat grains and flour produced from these grains the growth of these types of microorganisms may be due to the closed conditions inside the storage silos of grain, as well as the biological processes that occur inside the grain from the growth of seed reaps and others, which need extensive studies in this regard.

shown in Table 1. For samples throughout the winter season 2022 is the *Pantoea Spp*. Isolated group of wheat grains and flour produced from them*Pantoea Spp.* (3). as well as bacteria species belonging to the Enterobacteriaceae family *Rahnella aquatilis* Gram include negative isolated from a sample of wheat found in freshwater and soil and isolated from humans which represent as pathogenic bacteria also transmitted by snails and some beetles(10) Furthermore, *Enterococcus faecalis* and isolated from wheat grains in both; Al-Taji silo, Erbil silo, Maysan silo, and Kirkuk silo were selective gram-negative bacteria isolated from a sample of wheat found in freshwater and soil and isolated from humans which represent as pathogenic bacteria also transmitted by snails and some beetles, (6)). It is likely that most of the sources of these pathogenic bacteria isolated in this study are the result of irrigating agricultural land with water loaded with sewage waste and animal waste., (14). From the results obtained which noted in the Table 6 the maximum value of bacteria growth for the winter season was detected in wheat flour of Erbil silo was 5.27×106 CFU/g in the moisture content 13.5% and temperature 15°C, as well as in mixing mills and wheat flour 9.9×106 CFU/g in the M.C13.5%and temp. 16°C While the minimum value of bacterial growth in local wheat in AL-Basra silo were 0.68×106 CFU/g in M.C 8 .7% and temp.5°C, and minimum value of bacterial growth in wheat flour AL-Khan silo 5×106 CFU/g in M.C 14.3% and temp10.5°C respectively**.**

Table 2: Identification of (wheat and flour grain) in winter \2022 and Frequency of fungi Species

Location	Type	Fungal Contamination	Frequency of Species
Al- Dora Silo	Local wheat	*Asp. niger,* *Penicillium spp.* *Aspergillus ochraceus*	25.50 28.30 46.20
	Flour	*Asp. Niger* *Penicillium spp.* *Asp. Fumigatus*	33.30 35.45 31.25
Taji silo	wheat	*Asp. flavus ,* *Rhizopus spp.*	77.78 22.22

	Flour	*Asp.s flavus* , *Rhizopus spp.* *Penicillium spp.*	77.78 11.11 11.11
Al- Rusafa Silo	wheat	*Penicillium spp.*, *Rhizopus spp.*	55.56 44.44
	Flour	*Rhizopus spp.* *Penicillium spp.* *Asp. flavus*	13.64 11.11 75.25
Al- Khan silo	wheat	*Asp.s flavus*, *Asp. fumigtaus*	75.40 24.60
	Flour	*Asp.flavus*, *Asp. fumigtaus* *Rhizopus spp.*	77.78 11.11 11.11
Maysan silo	wheat	*Asp.flavus*, *Rhizopus spp.*	77.78 22.22
	Flour	*Aspergillus flavus*, *Penicillium spp.* *Asp. ochraceus*	77.78 11.11 11.11
AL- Basra silo	wheat	*Penicillium spp.*, *Asp. fumigtaus*	75.45 24.55
	Flour	*Penicillium spp.*, *Asp. ochraceus* *Asp. fumigtaus*	50.40 25.60 25
Erbil silo	wheat	*Asp. fumigtaus*, *Rhizopus spp.*	50.65 49.35
	Flour	*Asp. flavus*, *Asp.ochraceus* *Asp. fumigtaus*	77.78 11.11 11.11
Kirkuk silo	wheat	*Asp. fumigtaus*, *Penicillium spp.*	65.20 34.80
	Flour	*Asp. fumigtaus*, *Penicillium spp.* *Asp. ochraceus*	45.60 22.30 32.10

The fungi referred to in Table 2 and isolated from Iraqi silos, and the results showed that the most present genera are; *Asp spp.*, *Rhizopus spp*. and *Penicillium spp.*, which are classified among the dominant fungi in the poor storage of grain in general, knowing that wheat was newly harvested during the winter 2022, The most prevalent was *Asp.spp*, while the dominant genus was *Asp. fumigtaus* and *Asp.Flavus*, a number of fungi distinguished by rapid growth, resistance to low heat and low water, nevertheless *Rhizopus spp*., Penicillium appeared largely for grain and flour grain samples, a study conducted in the Bulgarian state (17), A number of fungal species have emerged that have been accurately isolated such as; *ASP. spp.* and *Pen. spp*. and *Rhizopus spp*. isolated from grain-loaded fungi, correspond to the results of Kumar's study (2).

Temperature and its effect on the storage of spelt grains and flour

Heat in winter with location The statistical analysis of the high value of LSD revealed significant differences ($p \leq 0.05$) between local Wheat grains and grains flour produced from it in the winter season during the months of December and January 2022 in each of the silo (Dora, Taji, Khan, Maysan) while no significant differences ($p \leq 0.05$) with the value of LSD for each of the silo (Rusafa, Erbil, Kirkuk, Basra).

Table 3: Wheat affected by temperature and location / winter months					
Location	**temperature of wheat C^0**		**temperature of flour C^0**		**LSD value**
	December	**January**	**December**	**January**	
Al Dora Silo	15	18.5	14.1	19.5	4.69 *
Taji Silo	10.2	15.3	11	16	4.55 *
Al Rusafa Silo	12.2	16	15	13.2	3.91 NS
Al Khan Silo	9	14.3	10.5	13	4.62 *
Maysan Silo	5.3	7	6.7	6	2.91 NS
Basra Silo	4.7	5	4.4	6.1	2.88 NS
Erbil Silo	16	20	15	17	4.74 *
Kirkuk Silo	18	19	19.1	17.7	2.86 NS
LSD value	4.78 *	5.13 *	4.97 *	4.91 *	---
* ($P \leq 0.05$) , NS: Non-Significant.					

Reference to the results in Table 3, the low The temperature for the period in winter 2022 in the silos and mills, In addition, low temperatures help in The rapid growth of fungi and their ability to produce toxins, including aflatoxin poison, in contrast, the optimum temperature for the growth of important fungi is about 20-30 C While the degree measured in the current study was lower than this rate, however, many fungi were diagnosed, the reason may be due to the presence of spores in the stored grain or sometimes to biological conditions specific to the growth of the seed embryo and other factors that need to be researched and studied., Knowing aspergillosis, especially flavos, grows faster than wheat grains when the appropriate humidity is available to a temperature of up to 45 ° C. (12).

moisture and its effect on the storage of spelt grains and flour

Heat in winter with location The statistical analysis of the high value of LSD revealed significant differences ($p \leq 0.05$) between local wheat and flour produced from it in the winter season during the months of December and January 2022 in each of the silo (Dora, Taji, Khan, Maysan) while no significant differences ($p \leq 0.05$) with the value of LSD for each of the silo (Rusafa, Erbil, Kirkuk, Basra).

Table 4: Wheat affected by moisture and location / winter months					
Location	Moisture of wheat %		Moisture of lour %		LSD value
	December	January	December	January	
Al Dora Silo	8.0	9.0	13.7	14.0	3.78 *
Taji Silo	8.1	9.0	14.0	14.2	3.55 *
Al Rusafa Silo	8.5	9.0	14.1	14.0	3.81 *
Al Khan Silo	8.9	9.5	13.5	14.3	3.69 *
Maysan Silo	9.5	9.0	14.1	14.3	3.61 *
Basra Silo	9.0	8.7	14.0	13.8	2.98 *
Erbil Silo	8.8	9.1	13.5	12.1	3.06 *
Kirkuk Silo	9.5	8.7	13.5	12.7	2.95 *

LSD value	2.15 NS	2.17 NS	2.08 NS	2.20 NS	---
* (P≤0.05), NS: Non-Significant.					

Humidity in winter The results recorded in the table showed that the highest percentage of moisture in wheat grains is the silo and mill of Erbil, where the percentage of moisture of the grains for the month of January 9.5 and for the month of February 9) As for the flour produced, the humidity for the month of January was about 14.1 and 14.3 for the month of February, while the lowest percentage of humidity was close between the Khan silo in Baghdad province and the Basra silo in Basra province affect moisture content, in agreement with a study conducted by (13) in which the moisture tint in Wheat grains and grains flour ranges between 9% - 13%.

Presence of Aflatoxin (B1) in Iraqi Wheat grains and grains flour samples

The chemical analysis process was performed using HPLC device for all the samples included in the study, where all the samples were taken and before the laboratory transplant to ensure the presence of Mycotoxin, and several types of Mycotoxins were investigated and according to standerd available in the local markets ,and Table (5) shows the chemical analysis with HPLC used to analyze samples of samples taken from silos included in the study.

Table 5 Determination of fungal aflatoxin toxin (B1) in wheat cereal and cereal flour samples 2022/winter

location	In wheat B1 concentration(ppb)	In flour B1 concentration (ppb)
Al- Dora Silo	5.8	241.7
Al- Taji Silo	10.05	136.2
Al- Rusafa Silo	10.72	18.75
Al- Khan Silo	76.6	331.6
Maysan Silo	3.05	16.45
Al- Basra Silo	5.03	18.07
Erbil Silo	9.59	17.95
Kirkuk Silo	10.30	18.06

Results of the winter season of Silo Alkhan The presence of B1 poison contamination in local wheat (76.6 ppb) by 12.5%, while the rest of the silos did not show any percentage of pollution with the mentioned poison, while the percentage of contamination of flour produced for silos (Dora, Taji,) was about 37.5%, as shown in the table above, and the reason for the emergence of this large percentage of pollution may be due to the quality of the water used in the process of processing wheat before the grinding process or to the poor process of storing grain in the mill and the type of silo Storage and mill .

Conclusions

. It is important for silo workers to follow safety guidelines, use personal protective equipment (PPE) and receive appropriate training. Regular medical examinations and regular periodic visits to hospitals and health centers can help reduce the risks of direct handling of microbial contaminants and flying dust from transporting, storing and grinding grain and preserving the health of the community.

References

1. Alemayehu S, Abera FA, Ayimut KM, Darnell R, Mahroof R, Harvey J, Subramanyam B. Effects of Storage Duration and Structures on Sesame Seed Germination, Mold Growth, and Mycotoxin Accumulation. Toxins (Basel). 2023; 15(1):39. (PMID: 36668858).
2. Aloo SO, Ofosu FK, Kilonzi SM, Shabbir U, Oh DH. Edible Plant Sprouts: Health Benefits, Trends, and Opportunities for Novel Exploration. Nutrients. 2021; 13(8): 2882. (PMID: 34445042).
3. Bağcıoğlu M, Fricker M, Johler S, Ehling-Schulz M. Detection and Identification of *Bacillus cereus*, *Bacillus cytotoxicus*, *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus weihenstephanensis* via Machine Learning Based FTIR Spectroscopy. Front Microbiol. 2019; 10: 902. (PMID: 31105681).
4. Behera B, Parida, S, Dutta, S, and Thatoi H. Isolation and identification of cellulose degrading bacteria frommangrove soil of Mahanadi river delta and their cellulase production ability. Am J Microbiol Res. 2014; 2(1): 41-46. http://www.sciepub.com/AJMR/abstract/1491
5. Berthold-Pluta A, Pluta A, Garbowska M, Stefańska I. Prevalence and toxicity characterization of *Bacillus cereus* in food products from Poland. Foods. 2019; 8(7): 269. (PMID: 31331094).
6. Demir T, Baran G, Buyukguclu T, Sezgin FM, Kaymaz H. Pneumonia due to Enterobacter cancerogenus infection. Folia Microbiol (Praha). 2014; 59(6):527-530. (PMID: 24973962).
7. Ertl K and Goessler W. Grains, whole flour, white flour, and some final goods: an elemental comparison. *Eur Food Res Technol* . 2018; 244(11): 2065-2075. https://link.springer.com/article/10.1007/s00217-018-3117-1#citeas
8. Eslami M. Mashak Z, Heshmati A, Shokrzadeh M, MozaffariNejad A.S. Determination of aflatoxin B1 levels in Iranian rice by ELISA method. Toxin Rev. 2015; 34(3): 125–128. https://www.tandfonline.com/doi/abs/10.3109/15569543.2015.1074925
9. Frad AJ. Screening of AspergillusNigerfor Lipase Production. . *Al-Mustansiriyah J Sci*. 2022; 33(5): 19-23. https://mjs.uomustansiriyah.edu.iq/index.php/MJS/article/view/1099
10. Ibrahim HA, Zaki NH. The Biological Activity of Some Extracted Proteins from Bacillus spp. Isolated from Soil Against Pathogenic Bacteria. Al-Mustansiriyah J Sci. 2019; 30(4): 29-31. https://mjs.uomustansiriyah.edu.iq/index.php/MJS/article/view/685
11. Kępińska-Pacelik J, Biel W. Alimentary Risk of Mycotoxins for Humans and Animals. Toxins (Basel). 2021; 13(11): 822. (PMID: 34822606).
12. Keshri N, Truppel I, Linke M, Geyer M, Weltzien C, Mahajan P. Development of a Controlled-Ventilation Box for Modified-Atmosphere Storage of Fresh Produce. Foods. 2021 Dec 2;10(12):2965. doi: 10.3390/foods10122965. PMID: 34945516
13. Lancelot E, Fontaine J, Grua-Priol J, Le-Bail A. Effect of long-term storage conditions on wheat flour and bread baking properties. Food Chem. 2021; 346: 128902. (PMID: 33482530).

14. Lorenzo JM, Munekata PE, Dominguez R, Pateiro M, Saraiva JA, Franco D. Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description. Innovative Technologies for Food Preservation. 2018: 53–107. (PMCID: PMC7150063). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7150063/
15. Mahuku G, Nzioki HS, Mutegi C, Kanampiu F, Narrod C, Makumbi D. Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. Food Control. 2019; 96: 219-226. (PMID: 30713368).
16. Minutillo SA, Ruano-Rosa D, Abdelfattah A, Schena L, Malacrinò A. The Fungal Microbiome of Wheat Flour Includes Potential Mycotoxin Producers. Foods. 2022; 11(5): 676. (PMID: 35267309).
17. Peter Mshelia L, Selamat J, Iskandar Putra Samsudin N, Rafii MY, Abdul Mutalib NA, Nordin N, Berthiller F. Effect of Temperature, Water Activity and Carbon Dioxide on Fungal Growth and Mycotoxin Production of Acclimatised Isolates of *Fusarium verticillioides* and *F. graminearum*. Toxins (Basel). 2020; 12(8):478. (PMID: 32731333).
18. Pinotti L, Ottoboni M, Giromini C, Dell'Orto V, Cheli F. Mycotoxin Contamination in the EU Feed Supply Chain: A Focus on Cereal Byproducts. Toxins (Basel). 2016; 8(2): 45. (PMID: 26891326).