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Prevalence of Microbial Contamination and Aflatoxins (B1) in Flour Producing Silos

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Abstract

Background: The microbial growth and fungal growth associated with the process of storing and grinding wheat is one of the main reasons affecting consumer health. The current study aimed to measure bacterial, fungal and mycotoxins prevalence in Iraqi silos and mills, which May affect the health of silo workers and public health.

Methods: The study included 40 samples of local wheat grains and 40 samples of wheat flour were collected randomly for the summer seasons from 4 silos in Baghdad, Maysan silo and Al- Basra silos in the south, and Erbil silo and Kirkuk silo in the northern region during July and August 2022.

Results: The results of the study showed the spread of many types of pathological spores that were isolated from wheat flour and local wheat, and also the chemical analysis indicated the presence of mycotoxins the following silos (Al-Dora, Al-Taji, Al-Khan, Maysan, Al- Basra, Kirkuk) in a percentage affecting human health.

Conclusions: The statistical analysis data shows significant difference ($p \leqslant 0.05$) for local wheat and local flour, the temperature and moisture associated with storage and grind respectively, most specimens of domestic wheat and the flour produced from them contain more total coliforms and fungi than the permissible limit.

Introduction

Wheat (bread wheat) (*Triticum aestivum L.*) is one of the main agricultural commodities in world trade represent the main requirements for human and animal consumption. It must meet the growing need for it as the world's population increases, reaching more than 9 billion by 2050 [1], the global wheat production is about 715 million tons annually which ranks as second in consumption after corn (one billion tons/annually), Mold growth is one of the most common causes of microbial spoilage and loss of grain quality during storage and stored goods can gain moisture from environments with high relative humidity, they may lead to fungal infections and increase the accumulation of mycotoxins [2]. Fungi is among the most important organisms as preferred enzymes are outside the cell. There are many studies and researches have shown the secondary metabolites known as mycotoxins which considered as a major cause of damage in grains of sand silos that can lead to poisoning food and animal feed [3]. The fungal mycotoxins pass to the center of flour via mall pockets in the grain. This process will increase the mycotoxins concentrations level to above an acceptable limit. [4], Aflatoxins B1 is one of the most dangerous types of adrenal toxins and considered a strong carcinogen for both humans and animals [5], fungi (eg, *Apergillus* spp., *Penicillium* spp. *Fusarium* spp.) and bacteria (eg, *Salmonella* spp. *Bacillus cereus*) contaminate the flour and their products may cause many diseases [6].

The current study aimed to measure microbial contamination (fungal and bacterial) and also to measure the percentage of mycotoxins prevalent in Iraqi local wheat and local flour.

Methods

Collection of Samples

The samples were collected during the 2022 summer season. The 40 samples were randomly from (Al-Dora Silo, AL-Taji Silo, Al-Rusafa Silo, Al- Khan Silo), also in (Maysan Silo, Al- Basra Silo, Kirkuk Silo and Erbil Silo) wheat sample varieties were Local wheat and flour produced from it.

Bacterial isolation

Five grams of the sample are weighed and suspended in 45 ml of distilled water, and were left to homogenize for 15 minutes, and after that only 1 ml is taken and distributed in sterile petri dishes nutritious agar and MacConkey agar to obtain a pure subculture, these subculture plates analyzed and identified by the compact Vitek 2 system [7].

Fungal isolation

The wheat grains was planting in the Petri dishes (diameter 90 mm, 10 grains) containing Potato dextrose agar(PDA) and plates incubated at 25 °C that were carried out for (5-7) days. Another way to isolate the fungus is to take one gram of each sample dispensed in 9 ml of sterile D.W, and leave for 15 minutes, then the mixture is transferred to sterilized Petri dishes and cooled Sabouraud dextrose agar (SDA) to 45 °C And after pouring it was incubated at 25 °C for 5 days as explained by [8].

Mycotoxins

Mycotoxins are toxic by-substances produced by some fungal species such as *Aspergillus, Fusarium, Penicillium,* the growth of these fungi producing mycotoxins which when ingested, they cause a serious effect and damage [9]. Hundreds of these toxins have been discovered, however, the most important and widespread are the influential that affect human and animal health like aflatoxin, okratoxin, patulin, fumonisin, zearalenone, nivalenol and deoxynivalenol, however mycotoxins appear as a result of contamination of crops with mold before and after harvesting [4].

Statistical Analysis

We used the program (statistical analysis) - SAS (2018). The program revealed the differences and their effects for the study groups in the parameters of study. T-test and Least Significant Difference in -LSD test (Analysis of Variation-ANOVA) which was used to compare evidence. Chi-square test, also it was used, for moral comparison in percentage, (0.05 and 0.01) probability in this study.

Results

The study was carried out between 2022, and 2023, as a part of PhD postgraduate work; the study came out are depicted below in tables and figures.

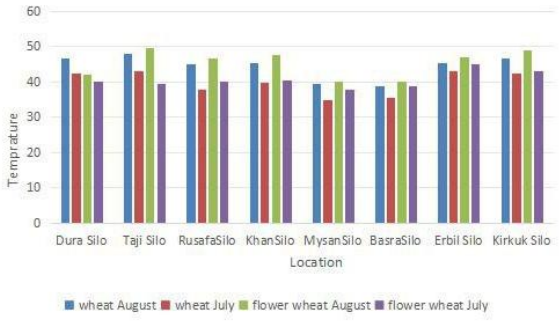

Figure 1: Effect of location and temperature in summer months.

Location\ Silo	Type Local	Bacterial contaminations	total bacterial count (CFU\g
Al Dora	wheat	*Pantoea* spp. *Bacillus cereus*	1.33×10^6
	Flour	*Bacillus cereus* *Staphylococcus haemolyticus*	6.2×10^6
Al Taji	wheat	*Enterobacter cloacae* *Serratia plymuthica*	0.68×10^6
	Flour	*Enterobacter cloacae* *Serratia plymuthica*	4.8×10^6
Al Rusafa	wheat	*Bacillus cereus* *Kytococcus sedentarius* *Staphylococcus Kloosii*	0.81×10^6
	Flour	*Kytococcus sedentarius* *Enterobacter cloacae*	6.2×10^6.
Al Khan	wheat	*Bacillus cereus* *Enterobacter cloacae*	2.30×10^6
	Flour	*Bacillus cereus* *Enterobacter cloacae complex, Pantoea* Spp.	5.8×10^6
Maysan	wheat	*Sphingomonas paucimobilis* *Staphylococcus gallinarum*	0.85×10^6
	Flour	*Enterococcus faecalis* *Sphingomonas paucimobilis*	3.18×10^6
ALBasra	wheat	*Lactococcus garviae* *Staphylococcus lentus*	0.88×10^6
	Flour	*Lactococcus garviae* *Enterobacter cloacae*	6.48×10^6
Erbil	wheat	*Enterobacter concerogenus*	0.5×10^6
	Flour	*Enterobacter concerogenus* *Enterococcus faecalis*	4.76×10^6
Kirkuk	wheat	*Ralstonia Pickettii* *Enterobacter cloacae*	0.5×10^6
	Flour	*Ralstonia Pickettii* *Enterobacter cloacae*	3.96×10^6

Table 1: Identification of Bacteria (local wheat and flour) summer 2022 and total bacterial count (CFU\g).

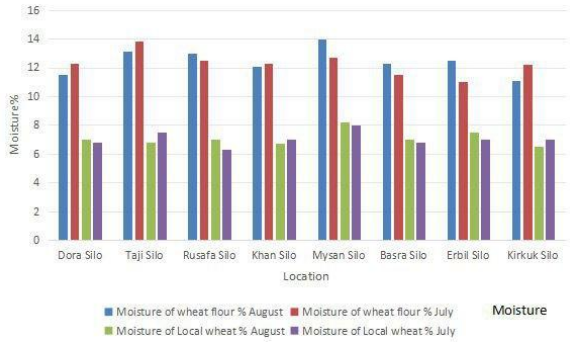

Figure 2: Effect of Location and Moisture / summer months

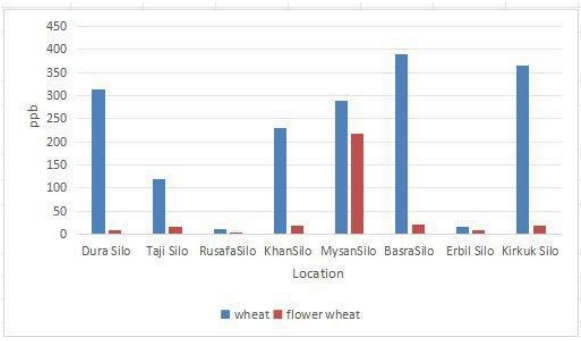

Figure 3: Quantitative determination of aflatoxin B1 in local wheat and flour in the summer season / 2022

Location\ Silo	Type of wheat	Fungal Contamination	Frequency of Species%
Al Dora	Local wheat	*Rhizopuss* pp. *Aspergillus fumigatus*	55.56 44.44
	Wheat Flour	*Aspergillus fumigatus* *Aspergillus flavus*	22.22 77.78
Al Taji	Local wheat	*Aspergillus f lavus* *Aspergillus fumigatus* *Aspergillus niger* *Rhizopus* spp.	45.60 18.13 24.50 11.77
	Wheat Flour	*Aspergillus flavus* *Aspergillus fumigatus* *Aspergillus niger* *Rhizopus* spp.	50.45 11.13 11.7 26.72
Al-Rusafa	Local wheat	*Aspergillus flavus* *Aspergillus ochraceus*	75.45 24.55
	Wheat Flour	*Rhizopus* spp. *Aspergillus flavus* *Aspergillus fumigatus.*	24.50 49.35 26.15
Al-Khan	Local wheat	*Aspergillus flavus* *Aspergillus niger*	75.45 24.55
	Wheat Flour	*Aspergillus flavus* *Aspergillus niger* *Rhizopus* spp.	45.60 34.22 20.18
Maysan	Local wheat	*Aspergillus fumigatus* *Rhizopus* spp.	50 50
	Wheat Flour	*Aspergillus fumigatus* *Aspergillus ochraceus*	78.46 21.54
Al Basra	Local wheat	*Aspergillus flavus* *Rhizopus* spp.	75.77 24.23
	Wheat Flour	*Rhizopus* spp. *Aspergillus flavus* *Aspergillus fumigatus*	35.50 46.78 17.72
Erbil	Local wheat	*Aspergillus niger* *Penicillium* spp.	22.38 77.62
	Wheat Flour	*Aspergillus flavus* *Aspergillus niger* *Penicillium* spp.	78.76 10.24 11
Kirkuk	Local wheat	*Rhizopus* spp. *Penicillium* spp.	75.97 24.03
	Wheat Flour	*Aspergillus fumigatus* *Penicillium* spp. *Rhizopus* spp.	35.50 17.72 46.78

Table 2: Identification of (local wheat and flour) in summer 2022 and Frequency of fungi Species

Discussion

Identification of Bacteria in local Wheat and flour

The results obtained in Table 1, represents the variety of contaminated of bacteria that were found in samples of local wheat grains and wheat flour. The most prevalent pathogenic contamination in the samples throughout the summer season 2022 is the *Bacillus cereus* group that was isolated in both, local wheat, and local wheat flour. It is a gram-positive which has the advantage of growing in temperature ranging from 4-40°C [10].

In a previous study on pathogens of *B. cereus* a grain analysis indicate that bacteria from the *B. cereus* group were present in 89.6% of grain species that were analyzed as mentioned by these results are consistent with the current study. Bacillus cereus has higher antibacterial effect on gram positive bacteria as depicted by [11], Furthermore, *Enterobacter concerogenus* that were isolated from local wheat in; Taji Silo, Khan Silo, Maysan Silo, and Basra Silo were selective anaerobic gram-negative bacteria that are usually considered as opportunistic pathogens even in hospitals which may cause osteoporosis, acute and cancer infections[12],It has been reported as an opportunistic pathogen in immunocompromised patients in patients with primary diseases such as

cancer, leukemia, renal failure and cirrhosis [13]. Sources of these pathogenic bacteria that were isolated from wheat grains and flour samples may be due to the result of the use of irrigation of agricultural land with sewage wastes, that are hazardous, also *Staphylococcus haemolytic*us are gram-positive, selective anaerobic cells , optimal growth occurs between 30-40 °C found among normal plants and isolated from hospital and are opportunistic bacteria. It has the ability to produce intestinal toxins and hemolysin. The ability to form biofilms is one of the most important factors of virulence [14], The maximum value of bacteria growth for the summer season was detected in local wheat in Al basra silo was 0.88×10^6 CFU/g , as well as in mixing mills and wheat flour 6.48×10^6 CFU/g . While the minimum value of bacterial growth in local wheat in Kirkuk silo were 0.5×10^6 CFU/g and minimum value of bacterial growth in wheat flour 3.96×10^6 CFU/g

Identification of fungi in Local wheat and flour

The results obtained in Table 2 showed the isolation of different genera of fungi contained in silos, and the largest number of fungi was presented as follows; *Aspergillus spp., Rhizopus spp.,* and *Penicillium spp.*, and the reasons for the presence of these species in wheat grains is because that wheat was newly harvested during summer season 2022**.**

From the results were obtained represented variety of fungal species which isolated according to their morphological features and color, shape of mycelium and spores under the microscope the high isolation frequency fungi genera belonged to the *Aspergillus spp.* which included different species consist of *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigates* , the linked fungus that grows faster and is more resistant to high temperatures and low water have the ability to grow in a wide range temperature from low as5°C to 55°C and high moisture content also are able to grow at lower O2 levels and in the presence of elevated CO2 , which have the ability to produce the two most common aflatoxins In humans, its activity in both field crops and stored grains, moreover *Rhizopus spp.*, were common species in both wheat and flour grains. Finally, *Penicillium spp.* was less abundant in most specimens. In 2020, a study in Bulgaria [15], which shows that the isolated fungal species were; *Aspergillus spp.*, *Penicillium spp.* and *Rhizopus spp.* which were isolated from internal and external fungi loaded on the grain and the most of the fungi that have been isolated are internal and external fungi loaded on the grain and show the highest frequency ratio in wheat flour, these results are consistent with Kumar study [16].

The impact of temperature and location on wheat grain storage and wheat flour

The highest temperature was recorded during July and August 2022. In the silos and mills of Kirkuk governorate, it reached 43 ° C for the month of July and 49 ° C for the month of August, respectively, and the temperature of the flour produced was 42.6 ° C for the month of July, while it was 46.6 ° C for the month of August respectively, which is the highest compared to the rest of the silos, while the lowest temperature was recorded in the silo and mill of Erbil. The statistical analysis revealed that the value of LSD was high and there were significant differences for both local wheat and local flour wheat in both silos and mills of (Dora, Taji, Khan, Erbil, Basra and Rusafa), while there was no significant differences that were recorded for the silos and mills of (Kirkuk and Maysan), aerated cereal can be kept around four times safer than non-aerobic grains. For this, ventilation is necessary, even if the grains are dry and cold, ventilation is important in storage [17].

The impact of moisture content and location on wheat grain storage and wheat flour

The highest percentage of moisture recorded in summer in July and August 2022 was in Erbil silo, which amounted to 7% for July and 7.5% for August respectively, while for wheat flour it was 11% for July and 12.5% for August.

However, the lowest level was recorded in the Basra silo, which was 6, 8% for July and 7% for August. The moisture content of wheat flour in Kirkuk silo reached 12.2% in July and 11.1% in August. High value recorded for LSD with presence of significant differences in all silos, mills and factories with ($p \leqslant 0.05$). It is important to mention that the origin of the wheat, whether imported or local can affect the LSD, moreover, the variation in the location of the silo and mill, the long storage period, and the quality of the storage silo (built of cement or metal),and the moisture depends on the genetic composition of wheat grains and is usually affected by agricultural and climatic conditions all these factors affect the moisture content showed that the moisture content of wheat and flour ranges between 9% - 13% [18]**.**

Investigation of aflatoxin (B1) in local wheat and wheat flour samples

The results of the chemical analysis with the HPLC device shows the analysis of samples that were taken from the silos that were under study, the summer season showed the presence of poison contamination in silo of Maysan by 12.5%, while the results of flour and the same period shows the presence of poison in an influential way in (Dora, Taji, Khan, Maysan, Basra, Kirkuk) by 75%, however the presence of this contamination in the flour may be due to the high humidity, Which can be due to the lack of good sterilization of the grinding rolls with the presence of

hardened residues from the parts of wheat and flour due to continuous grinding processes , as well as not cleaning accurately, especially for narrow places, which causes dirt to collect.

The statistical analysis data shows high significant differences for local wheat and flour which is due to temperature and humidity that associated with storage and grind respectively. The grinding of local wheat grains led to the emergence of some pathogenic microbial species in the flour produced, while the percentage of mycotoxins in flour coupled with increases in the ground grains. Most specimens of local wheat and the flour produced from them contain more total coliforms and fungi and some samples were safe in terms of mycotoxins.

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Author Contributions

The researcher No. 1 collected models of local wheat and flour produced from them over the course of a whole year from silos (Dora, Taji, Khan, Rusafa) within the province of Baghdad and silo (Erbil, Kirkuk) within the northern region and silo (Maysan and Basra) within the southern region of Iraq as well as to study the physical properties related to humidity and temperature and isolation and diagnosis of microorganisms of bacteria and fungi accompanying the study models and also conduct a mycotoxin examination using the technique high- performance liquid chromatography, as well as arranging the results within sequential tables, writing research on the subject and preparing them for publication purposes within international journals, As for researcher No. 2, he has prepared a special laboratory to conduct isolation and diagnosis of bacteria and fungi and to prepare scientific sources related to research, while researcher No. 3 has provided scientific sources for research.

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