Molds and Mycotoxins in Ration Wheat Flour in Sulaimani Provence

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ABSTRACT

Wheat flour is one of the main food sources of mycotoxin exposure, hence its safety warrants investigation. Aflatoxin B1 (AFB1) and Deoxynivalenol (DON), specifically, and their levels of contamination in wheat flour samples from ration wheat flour samples in Sulaimani City, Kurdistan, Iraq, were examined in the current study. According to the findings of the fungal study, Penicillium (35%) infected the bulk of the samples, with Aspergillus (27%) and Rhizopus (17%) coming in second and third, respectively, and Alternaria (9%), Fusarium (6%), Helminthosporium (4%), Mucor (1%), and Cladosporium (1%).In November and December, the number of molds reached intolerable heights. The findings of the enzyme-linked immunosorbent assay (ELISA) test revealed that AFB1 was present in varying amounts in 100% of the wheat flour samples. AFB1 surpassed the 2 μgkg^{-1} maximum permissible quantity in 37.5% of samples, particularly in November and December. In terms of DON, 100% of samples were contaminated with DON without going over the 750 μgkg^{-1} EU limit.

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1. Introduction

Breads, biscuits, cakes, pastries, pasta, and noodles are among the staple meals that are mostly made with wheat flour [1]. Wheat is susceptible to mycotoxin contamination by toxigenic fungi such as Aspergillus species, Fusarium species, Penicillium species, and Alternaria species, particularly in their outer layer [2,]. This can be attributed to a variety of factors, including climate variations, resistance, crop damage, insect activity, and unsuitable storage conditions, such as proper humidity and temperature [3, 4]. Existing mycotoxins in wheat grain with spatial location [5] can be redistributed in various milling fractions, including flour, grits, germ, or bran, but milling cannot eliminate or eliminate them [6-7]. One of the main sources of mycotoxins, particularly AFB1 and DON, is thought to be the eating of wheat flour. This occurs when infected crops or processed food products are directly swallowed [4,8]. Aflatoxins, are toxic secondary metabolites of some *Aspergillus* molds such as *A. Flavus*, *A. Parasiticus* [9]. In the field and during storage aflatoxins are produced on different types of grains. [8,10]. Aflatoxin B_1 is an important indicator in food safety assessment due to its highly toxic effects. According to the International Agency for Research on Cancer (IARC)aflatoxin B_1 is considered one of the most potent hepatocarcinogenic to human beings [11,12]. Due to the fact that mycotoxins are heat-stable substances, they may survive during food processing and get into the human food supply via commodities made from grain [13]. These toxins can cause a wide variety of toxic effects in both humans and animals [14]. Although Deoxynivalenol or vomitoxin is considered non-carcinogenic compound, is of importance since it heavily contaminates crops and animal feeds [15]. Deoxynivalenol production thrives in cool, wet conditions, their levels can be slightly reduced by food processing steps [16]. Maximum tolerable daily intake of DON is 1 μgkg^{-1} of body weight [17]. *Fusarium graminearum* contamination in cereal crops like wheat and barley can result in Fusarium Head Blight (FHB) disease, which can lead to eventual economic loss and health risks caused by mycotoxin (DON) buildup in products [18]. The consumption of contaminated food commodities with mixtures of mycotoxins can pose serious problems to public health. Monitoring of mycotoxins in food products plays important role in public health, because mycotoxins are stable compounds and don't completely destroy during food processing [19]. This study aimed to investigate the molds contamination of wheat flour collected from different storage sites of Sulaimania province during different months (September, October, November, and December) as well as to identify the isolated species and analyzed the occurrence of AFB_1 and DON in wheat

flours through Enzyme-linked immune-sorbent assay (ELISA) method to highlight the possible risks to community health

2. Experimental

2.1 Samples collection

During September, October, November, and December 2022, eight samples of rationed wheat flour were collected from different stores in the city of Sulaimani. All samples were collected from homogeneous flour bulks in three equal subsamples weighing approximately 1 kg each. As a consequence, three packets of each type of wheat flour were collected for laboratory analysis. Five days after collection, flour samples were stored in sterile plastic containers at 5 °C for analysis.

2.2 Determination of molds Count

Mold contaminants were isolated from flour samples by plating them on potato dextrose agar (PDA) plates using the surface spread method. Three 11-gram subsamples were taken from each sample and suspended in 99 mL of sterile normal saline. Each suspension was serially diluted up to a 1:1000 ratio in sterile physiological saline solution. Then, aseptically pipet 0.1 ml of each dilution in duplicates on pre- poured, solidified PDA plates amended with ampicillin and streptomycin (0.25 mg/L) to prevent bacterial growth and spread inoculum with a sterile, bent glass rod. Under aerobic conditions, the plates were incubated for 5 days at 25C°. The amount of molds was counted and expressed as colony forming units per gram (cfu/g). The percentage of common genera of fungi found in wheat flour samples was calculated as follows:

% of genus =Number of samples contaminated with a genus × 100/Total number of samples

2.3 Isolation, Purification and Identification of Fungi

Molds was purified using a malt extract agar media. Using a sterilized needle, a little portion of the fungus growing on PDA Agar was aseptically removed and placed on petri plates with malt extract agar. The dishes were incubated at 25^{o}C for 3–5 days. The purified fungi were identified according to their morphological and microscopical characteristics according to Pitt and Hocking [20] identification keys.

2.4 insect detection in wheat flour samples

The samples of wheat flour were tested for the presence of live insects or insect body parts using the following procedures: A sample of 500 grams of wheat flour was passed through a set of sieves, and residual materials that remained on the sieves were analyzed visually and under a light microscope.

2.5 Detection of Mycotoxins

2.5.1 Aflatoxin B_1 extraction

Wheat flour samples (50 g) were mixed with NaCl (10 g) and methanol: water (80:20) (200 mL) in a blender jar and homogenized at high speed for 1 minute to extract the AFB_1. The extract was passed through filter paper, and (20 mL) of water was added to the (10 mL) of filtered extract, then well mixed. Through a glass microfiber filter, the diluted extract was filtered. The diluted filtered extract (2.0 mL) was passed through the immunoaffinity column (AflaTest, Vicam, Milford, MA 01757, USA) at a rate of around 1 drop/second. Purified water (1.0 mL) was added twice to wash the column at a rate of 1-2 drops/second to remove any sample residues. HPLC grade methanol (1.0 mL) was used to elute the sample [21].

2.5.2 Deoxynivalenol extraction

Five grams of wheat flour samples were put into a 50 mL PTFE centrifugal tube, along with 25 mL of 84:16 (v/v) solvent mixture, acetonitrile, and water. The mixture was agitated for an hour. The mixture was centrifuged for 5 minutes at 5 °C at 4500 rpm, and the supernatant phase was filtered through paper. A centrifugal evaporator (Savant) was used to evaporate five milliliters of the filtrate. Dry extract was then thoroughly mixed with 1 mL of a CH3OH/H2O solution (70:30, v/v), vortexed, and then filtered through a 0.22 µm syringe filter (Chemtek Analytica, Bologna, Italy) in preparation for ELISA analysis [22].

2.5.3 AFB_1 and DON determination by Enzyme-linked immune-sorbent assay (ELISA)

The final extracts were analyzed for AFB_1 and DON using ELISA kits equipped from Shenzhes Lvshiyuan Biotechnology CO., Ltd following manufacturer instructions. ELISA 96 well plate reader was used to measure the optical density at 450 nm, wherein all standards and samples solutions were analyzed at least in duplicate. Standard-curve realized in same conditions was used to calculate samples values. After testing samples, the toxins were compared with standard values of the European Commission established for mycotoxins in cereals [21,23], 2 μgkg^{-1} for AFB_1 for all cereals and cereals products and 750 μgkg^{-1} for DON for cereal flours.

3. Results and Discussion

3.1 Fungal count and identification

The counts of fungus in the wheat flour samples ranged from 3 to 136 CFU/g, as shown in Table 1. Molds can contaminate wheat flour during the growing, postharvest handling, and milling processes [23]. The same Table indicates that the number of molds has significantly increased,

particularly in November and December. Over these two months, and especially in December, there were more molds than were permitted. The rise in relative humidity in unapproved storage locations during the rainy months could be the cause of the mold growth. Because grain production is seasonal and demand is constant, grain storage facilities are essential. As a result, unsuitable grain storage locations and heavy rains tend to coincide, increasing grain moisture and, in turn, fungal development and mycotoxin production [24].After analyzing wheat flour samples, Penicillium was determined to be the most common species of molds, followed by Aspergillus, Rhizopus, Alternaria, Fusarium, Helminthosporium, Mucor, and Cladosporium (Fig. 1).

Table 1: The means of total counts of molds CFU /g in wheat flour samples from different stores during different months.

Months / Sample no.	September	October	November	December	LSD value
S1	5.00	12.00	23.00	57.00	7.32 *
S2	5.00	14.00	31.00	33.00	7.05 *
S3	6.00	8.00	23.00	60.00	9.14 *
S4	6.00	9.00	133.00	40.00	6.98 *
S5	7.00	10.00	39.00	74.00	9.47 *
S6	7.00	13.00	21.00	136.00	21.08 *
S7	3.00	13.00	23.00	51.00	7.51 *
S8	4.00	12.00	20.00	100.00	8.97 *
LSD value	4.38 NS	5.41 NS	18.92 *	17.66 *	---
* ($P \leq 0.05$).					

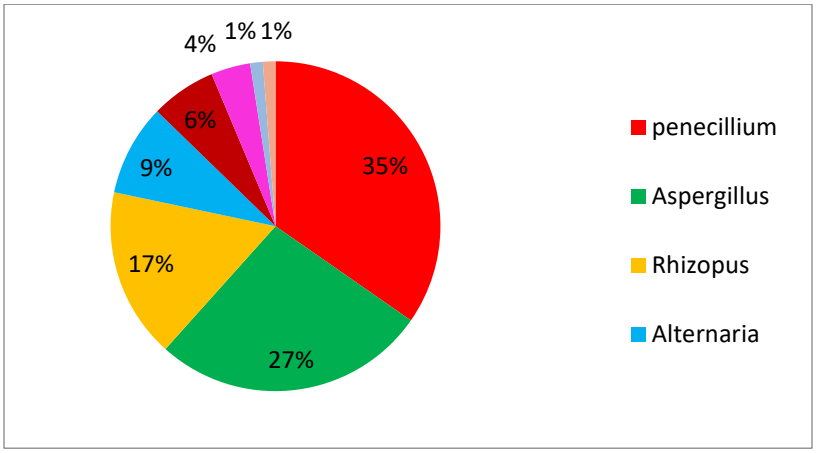

Figure 1: The percentage of common genera of fungi found in wheat flour samples

3.2 Insect detection in wheat flour samples

Adult insects were found in 7 wheat flour samples (30.4%) especially in the samples that carry highest number of molds. This suggests a correlation between fungal levels and insect infestation. Infestation of stored wheat flour with insects can be serious problem. Insects can carry fungus spores in their body that increase fungal count. A population of insects in stored wheat flour, if not controlled, may generate heat and water that lead to accelerate fungal growth, with subsequent wheat flour deterioration and mycotoxins production [25].

3.3 Mycotoxins in wheat flour samples

Occurrence data of mycotoxins in wheat flour samples are summarized in Table (2 &3). AFB_1 and DON were detected in 100% and 91.93% of the total number of samples, respectively.

3.3.1 Aflatoxin B1

In general, statistical difference was seen for AFB_1 in relation to months of sampling as showed in table (2), Mean concentrations from 0.1 to 13.0 μg kg−1 were found in samples collected in Sulaimania City. Comparing the different months, and according to European Commission, there is 22% and 33% of wheat flour samples exceeded the maximum allowable concentration of AFB_1 during September and October months respectively. While during November and December, the exceeded concentration of AFB_1 increased significantly to reach to 44% and 78%, respectively. The increase of AFs formation was registered with an increase in the storage period in environment with high relative humidity [26,27]. Tirado et al. [28] reported that the AFs are increased in countries that suffer more from climate change.

3.3.2 Deoxynivalenol

DON is the most studied trichothecene in cereals [29]. and wheat is the predominant source of exposure of this mycotoxin in most diets [30]. DON is associated with acute gastrointestinal adverse effects such as vomiting in both animals and humans. Long-term dietary exposure of animals to DON will lead to weight loss, loss of appetite, and fluctuation of nutritional efficiency [16]. All samples do not exceed the DON maximum level of 750 μgkg^{-1}, showing satisfactory results with regard to European Commission legislation. In 2002 the Scientific Committee for Food (SCF) limit the tolerable daily intake (TDI) for DON at 1 μgkg^{-1} body weight per day. The Tolerable Daily Intake (TDI) of DON, acetyl derivatives (3-acetyl-DON (3-Ac-DON), and 15-acetyl-DON (15-Ac-DON) was revised by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2010, and an Acute Reference Dose (ARfD) of 8 μgkg^{-1} body weight was additionally recommended. The national studies concluded that high consumers and young children were exposed to DON at levels close to or even higher than the TDI [31]. In general bread followed by pasta and bakery goods is the main contributor to chronic exposure to mycotoxins. Because different processing procedures lower DON levels in the finished product from those present in the original raw wheat, the FDA prefers to state the maximum permissible concentration for DON on wheat products rather than the original raw wheat. [16, 31]. The mycotoxins present in wheat flour may not be necessarily due to wheat contamination, but also related to prevalence of high humidity in storage places [32].

Table 2: Concentrations of AFB_1 (μgkg^{-1})in wheat flour samples collected from different stores during different months

Month / Sample No.	September	October	November	December	LSD- value
S1	1.00	0.10	1.50	1.40	0.503 NS
S2	1.50	0.20	2.00	4.00	1.17 *
S3	2.70	1.20	2.00	13.00	2.74 *
S4	0.40	2.40	1.80	2.00	1.08 *
S5	1.40	2.40	1.80	5.40	1.42 *
S6	1.80	3.20	3.00	2.00	1.14 *
S7	0.30	2.00	4.00	3.00	1.52 *
S8	2.00	1.80	5.40	3.00	1.49 *
LSD value	1.06 *	0.993 *	1.48 *	1.19 *	---
* ($P \leq 0.05$).					

Table 3: The mean of DON toxin μgkg^{-1} in wheat flour samples from different stores during different months

Month / Sample no.	September	October	November	December	LSD value
S1	10.00	4.00	2.00	8.00	2.38 *
S2	2.00	10.00	14.00	10.00	2.55 *
S3	5.50	8.00	4.00	20.00	4.81 *
S4	16.00	10.00	4.00	8.00	2.19 *
S5	0.00	4.00	12.00	20.00	4.62 *
S6	4.00	0.00	22.00	20.00	5.03 *
S7	20.00	4.00	10.00	4.00	3.46 *
S8	8.00	18.00	26.00	6.00	4.74 *
LSD value	5.69 *	3.98 *	5.07 *	4.21 *	---
* ($P \leq 0.05$).					

4.Conclusions

The current study, conducted in Sulaimania, Kurdistan, added to the body of knowledge regarding the presence of DON and AFB1 in wheat flour. While about 37 samples of wheat exceeded the maximum amount of AFB1 allowed by the European Commission and no sample above the maximum amount of DON allowed by the European Commission, all of the wheat samples were contaminated with both AFB1 and DON. November and December in particular had the highest concentration of mycotoxins in wheat flour; this could be because of the relatively high humidity and changing environment, which encourage the growth of fungi and the generation of mycotoxins. In general, a number of interrelated factors, such as poor storage and transportation conditions, work together to build the foundation for the development of fungal growths and mycotoxins. As a result, we should be mindful of grain storage facilities as they are critical to maintaining grain quality and managing pests with the use of biopreservatives. Annually conduct routine monitoring, potentially with additional sample. Lastly, the flour sample quality is compared to EU allowed limits.

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الاعفان والسموم الفطرية في دقيق قمح الحنطة التموينية في محافظة السليمانية

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المستخلص

أحد المصادر الغذائية الرئيسية للتعرض للسموم الفطرية هو دقيق القمح ، وبالتالي فإن سلامته تستحق النظر. في هذه الدراسة ، تم الكشف عن وجود ومستويات تلوث العفن والسموم الفطرية على وجه التحديد الأفلاتوكسين (AFB1) و Deoxynivalenol (DON) في عينات دقيق القمح من عينات دقيق القمح التموينية في مدينة السليمانية ، كردستان العراق. بالنسبة للتحليل الفطري ، أشارت النتائج إلى أن غالبية العينات كانت ملوثة Penicillium (35٪) ، Aspergillus (27٪) ، Rhizopus (17٪) ، Alternaria (9٪) ، Fusarium (6٪) ، Helminthosporium (4 ٪) ، Mucor (1 ٪) ، و Cladosporium (1 ٪). ارتفع عدد الاعفان إلى مستويات غير مقبولة في تشرين الثاني وكانون الاول.. أظهرت نتائج استخدام اختبار مقايسة الممتز المناعي المرتبط بالإنزيم ((ELISA) ,أن 100٪ من عينات دقيق القمح كانت ملوثة بتركيزات مختلفة من AFB1. في 37.5٪ من العينات ، تجاوز AFB1 التركيزات القصوى المسموح بها ، 2 ميكروغرام / كجم خاصة في تشرين الثاني وكانون الاول. بالنسبة ل DON ، كانت 100٪ من العينات ملوثة ب DON دون تجاوز التركيزات الموصى بها من قبل الاتحاد الأوروبي ، 750 ميكروغرام / كجم.