Mesopotamia Environmental Journal **ISSN 2410-2598**
Mesop. environ. j. 2015, Vol.1, No.2:pp. 18-25.

Mycoflora of mold contamination in wheatflour and storage wheat flour

Mahammed E. Jabbar Al-Defiery[1] **Ali Fathil Merjan[2]**

1 Local Environmental Research Center- Babylon University

2 Soil and Water Resource Department-Agriculture College, Al-Qasim Green University

Corresponding author: al_defiery2004@yahoo.com

To cite this article:

Al-Defiery, M. E. and Merjan, A. F. Mycoflora of mold contamination in wheat flour and storage wheat flour. *Mesop. Environ. J,* 2015, Vol. 1, No. 2 , pp.18-25.

Abstract

The investigation was carried out to determine the extent of molds or spores contamination on wheat flour in three types (tow locally + one imported)on Babylon province.

Molds were isolated from wheat flour samples by using PDA(Potato dextrose Agar) medium. Four replicates were inoculated with1 mL of different dilution of wheat flour into medium agar by spread plate technique and identified the mold by compound microscope with followed taxonomic keys of reference.
The major genera of molds isolated according to decreasing frequency were *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Alternaria*, *Mucor*, *Rhizoctonia*, *Trichoderma*, *Rhizopus*, *Nigrospora*, *Bipolaris*, *Macrophomina*. The coefficient correlation analysis revealed to positive correlation between moisture content and molds present(0.73).The results showed that differed species and genera association of mold in wheat flour after three months storage at two temperature(5 and 30 ºC). The results indicated that stored at temperature 5 ºC decrease the population and types of molds on wheat flour.

Keywords; wheat, mold, flour, storage

Introduction

Cereals, especially the wheat as the basic bread cereal, represent an important strategic row material for the nutrition of the nation and domestic animals, before cereals being consumed as food, go through the processes of cultivation, harvesting, drying, preparation and marketing (including storage) all under natural conditions, and therefore, often involve microbiological contamination and infection[1]. Wheat flour is a powder made from the grinding of wheat used for human consumption and all its products can be contaminated by molds at all phases of the production chain.

Molds can infect wheat during all growth stages but the most susceptible and economically important developmental stage is at flowering. Many of those molds are toxic being potential producers of various mycotoxins with harmful consequences for the health of the population. Great importance for the wheat flour/dough quality besides the content and quality of gluten proteins, also have starch and the enzymatic activity and worse the bread making quality[2]. Conditioning wheat to increase the moisture content to a level suitable for milling can also increase the counts of bacteria, yeasts and molds [3]. Twenty-four taxa of *Fusarium* were identified in wheat and barley grains. *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*,

F. sporotrichioides and *F. sambucinum* were most frequently isolated from grain surface, while *F. poae*, *F. sporotrichioides*, *F. avenaceum*, *F. graminearum* and *F. culmorum* dominated in the internal tissues with a decreasing prevalence [4].

Muthomi and Mutitu [5] reported that the wheat samples were collected from 5 wheat growing on districts of Kenya were contaminated by different fungi, the major genera of fungi isolated according to decreasing frequency were *Epicoccum* (52.8%), *Alternaria* (34%), *Fusarium*(6%), *Aspergillus* (2.3%) and *Penicillium* (1.8%). The frequently isolated *Epicoccum* species was identified as *E. purpurascenes*. *Cladosporium* and *Rhizopus* spp. were also isolated at very low frequencies. Concerning mold contamination, no difference was observed between the samples from Poland and East Slovakia, the highest incidence was observed of *Fusarium*, *Aspergillus*, and *Penicillium* genera and most of the investigated samples of wheat, contained less than 10^4 CFU/g [6]. Fakhrunnisa *et al*.[7] resulted a significant contamination with fungal genera was detected in analyzed 19 wheat samples they found 12 genera and 21 species of fungi, this is the first report of *Chaetomium globosum* and *D. hawaiiensis* on wheat and not appear to be any previous report of *Absidia* sp., *Aspergillus sulphureus*, *Fusarium subglutinans* and *Rhizoctonia solani* on wheat in Pakistan.

The most toxic fungi belong to the genera *Fusarium* and *Alternaria*, from wheat of organic farms 22 while from wheat of conventional farms 12 fungal species were isolated, barley from organic farms 22 fungal species and from conventional farms 20 species[8]. Mold can develop/ increase during storage period, five samples were taken at 30 days intervals during the six months of storage period that assessing fungus. The results showed that in all 35 samples analyzed many mycotoxins such as aflatoxins, ochratoxin A, zearalenone, deoxinivalenol and fumonisin FB2 were detected and the fumonisin FB1 were present in 11 samples (31.4%) with levels of 36.3 to 2,891 mg/g [9]. Mycotoxins prevention is very important as, once developed, they become stable at environment temperature and very resistant to thermal changes [10].

In order quality and safety of wheat flour and wheat products to be maintained and prevention from contamination, the objectives of the investigation were to examine molds presence or contamination of wheat flour as well as to identify the isolated species and define the effect storage of wheat flour on presence molds.

Materials and methods

Sample collection

One kilogram wheat flour samples were collected from market of districts of Babylon in sterile plastic bags as it took four random samples each of tow locally types wheat flour(A,B)which contain some bran and wheat flour (C) (Turkey imported and white with approximately free bran). To detect effect temperature storage on molds on wheat flour, the samples of A, B and C were incubatedat5 and 30 ºC for three months for molds examine to purpose of studying the effect of storage on the types of fungi attached in flour.

Isolation and identification of fungi

For isolation fungi were carried out using PDA(Potato dextrose Agar) medium .Four replicates were inoculated with1 mL of the dilution 10 to 10^9 of wheat flour onto medium agar by spread plate

technique. Petri dishes were incubated at temperature of25 for5 days. The number of molds in 1 g of the analyzed sample was calculated on the basis of the colony numbers grown on the plates from particular dilution. It was calculated colony forming units (CFU).

The individual genera of molds were determined on the basis of their macroscopic and microscopic morphology. The identification of the isolated mold was done both macroscopically and microscopically and followed taxonomic keys[11] [12][13][14].

The relative isolation frequency (Fq) of each genus was calculated as follows [15].

$$Fq\ (\%) = \frac{\text{Number of isolates of genus}}{\text{Total number of molds genus}} \times 100$$

Determination of moisture content

In order to determine the moisture content in wheat flour they were weighing 100grams of each samples on four replications and transferred to the electric oven at a temperature of100ºC for 5 hours, the samples weight and calculated the percent of moisture content. The percentage moisture content was then calculated as:

$$\%\ \text{Moisture Content} = \frac{\text{Original weight} - \text{Final weight}}{\text{Original weight}} \times 100$$

Correlation coefficient was estimation between moisture content and population density of mold in samples of wheat flour.

Result and Dissection:

Cereal products, which provide income of important nutritional substance for human health[16].The quality and safety of agricultural product are of major concern due to increasing occurrence of chronic diseases associated with consumption of contaminated [17].Wheat, represent a strategic raw material from the aspect of human nutrition. The results of mold contamination investigated on all wheat flour samples showed that abundance and varies significant of molds species and genus, mold can grow on different foods included wheat flour. According to the results (Figures 1,2 and 3) they have noticed a very wheat flour samples were dominant with genera mold *Penicillium*, *Aspergillus* and *Rhizopus*, whiles wheat flour samples exhibited other genera of mold such as *Bipolaris* and *Ulocladium*.

Many studies emphases that wheat flour associated with mold, Weidenbörner *et al.* [18]were investigated whole wheat flour and a white wheat flour (type 405) for their total qualitative as well as quantitative mycobiota, total fungal counts of the whole wheat flour amounted to 1833 molds while the white wheat flour contained 1730 CFU 2 g^{-1}. The mycobiota of both flours was dominated by *Aspergillus* spp. accounting for 84% and 77.3% of the isolations, respectively. Fungi of the genus *Penicillium* spp. occurred only to a minor degree: 8% of the isolations in whole wheat flour and 15% in white wheat flour. *Aspergillus candidus* was the most frequently encountered mold and *Penicillium aurantiogriseum*, *Cladosporium cladosporioides*, *A. flavus*, *Eurotium herbariorum*, *P. griseofulvum*, *P. brevicompactum* and *P. viridicatum* were isolated to a lesser degree. The mycological tests indicated that wheat from organic farms was contaminated with fungi by 70.5% ($p < 0.05$) more and barley by 24.8% ($p > 0.05$) less as

compared with wheat from conventional farms and grains taken from both types of farms were contaminated with fungi from the *Fusarium*, *Alternaria*, *Cladosporium*, *Penicillium*, *Aspergillus* and other genera [8]. However, cereals and cereal products can be contaminated with molds in any phase of a processing cycle: in fields, during harvest, storage, processing, transport, and over a period between production and consummation [19].

The increasing moisture content might result in increased molds, the high, total mold counting were resulted when percent of moisture was 14.17 % as given on Table 2. The water activity level in food is of practical importance as it controls the onset and severity of mold spoilage. It is commonly observed that foods most likely to show rapid deterioration due to biological and chemical changes are usually those with high water content [1]. Egbebi and Aboloma [20] were concluded that high moisture content encourages growth of fungi in gari samples. It was verified that there is a correlation 0.73 between the accounting of molds and moisture content in wheat flour samples, mold like all living organisms, require the essential factors for life such as water.

Further investigations should be also under taken on the storage temperature, storage conditions, especially temperature and humidity, play a very important role mold growth. It was observed the wheat flour samples after three months of storage period abundance of mold *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. Joshaghani [21] reported that the most common molds isolated on wheat were *Alternaria* spp. 26.7%, *Aspergillus niger* 21.4%, *Fusarium* spp. 17.8%, *Aspergillus. flavus* 10.7%, *Cladosporium* spp.10.7%, *Penicillium* spp.8.9% and *Rhizopus*spp.3.5%.

The results were obvious differed in their species and genera association of molds in wheat flour at two temperature storage (Figures 4,5,6,7,8 and 9).At temperature 30 ºC showed *Trichoderma harzianum*, *Aspergillus niger*, *Cladosporium* sp, *Penicillium* sp, *Mucor* sp, *Fusarium solani*, *Rhizoctonia solani*, *Alternaria alternate* on wheat flour samples, whiles at temperature 5 ºC showed *Aspergillus flavus*, *Cladosporium* sp, *Penicillium* sp, *Fusarium* sp on wheat flour samples. However, wheat flour samples A and B were contaminated with molds more than sample C. The results therefore indicated that stored at temperature 5 ºC decrease the population and types of molds on wheat flour. The most important factors in theindoor environment are temperature and the type of wheat flour and for each species of fungi there is an optimal temperature range for growth to occur. Conversely, not all molds that grow in stored. It is important to prevent their development by reducing the temperature and moisture content[9]. Molds development during storage can be controlled or prevented by ensuring that grain is adequately dry at intake, further protection can be provided by preventing the development of temperature and moisture gradients by cooling and/or aeration of the grain. Protection from insect infestation will also help prevent mold development in stored grains, both in bulk or bag storages [22].After 50 days of storage, the number of molds of the grains was 80×10^5 cell/g and storage fungi were isolated from the wheat grains, which were colonized by *Aspergillus*, *Eurotium* spp. and *Penicillium* spp., the total fungal populations of wheat grains were increased significantly by increasing the storage period [23].Jonathan et al. [24] was observed that the longer the storage time, the higher the concentration of aflatoxin produced. Moreover, if these food products will be kept for a long time, the storage in humid environment must be discouraged. These materials must be placed in dry environment (with very low relative humidity) in order to limit the tendency of the growth of aflatoxigenic fungi which could produce mycotoxins after a long storage period. Standard blotter and deep freezing methods were used to study the seed-borne mycoflora of 19 samples of wheat, 27 samples of sorghum and 14 samples of barley, a significant contamination with fungal genera was detected in analyzed samples and fungi most frequently isolated and identified were *Absidia* sp., *Alternaria alternata*, *Aspergillus* sp., *A. candidus*, *A. flavus*, *A. niger*, *A. sulphureus*, *Cephalosprium* sp., *Chaetomium globosum*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera dematioidea*, *D. halodes*, *D. hawaiiensis*, *D. tetramera*, *Fusarium moniliforme*, *F. oxysporum*, *F. pallidoroseum*, *F. subglutinans*,

Nigrospora oryzae, *Penicillium* spp., *Piptocephalis* sp., *Rhizoctonia solani*, *Rhizopus* sp., *Stemphylium* sp., *Syncephalastrum racemosum*, *Trichoderma hamatum*, *Trichothecium roseum* and *Ulocladium* sp.[7].

Molds showed difference in samples collected, they were obtained that *Aspergillus* was growth at all wheat flour samples and it is the dominant genera on wheat flour samples tested. The most frequently isolated of molds on wheat flour samples were *Aspergillus*, *Penicillium* and *Fusarium* with frequency percentage 21.3%, 15.84% and 12.23% respectively.

Wheat flour is one of the main important food of people in most regions of the world. Differences of species and genera association of mold in wheat flour were observed at present study. Molds/spores can associated wheat flour either harvest or during storage, transport, processing, handling and at all phases of the production chain.

Table 1. Correlation coefficient of moisture content in wheat flour samples with mold counting

Sample	Molds Counting	Moisture Content%	Correlation Factor
A	15.65	9.91	0.73
B	23.62	17.07	
C	23.39	11.03	

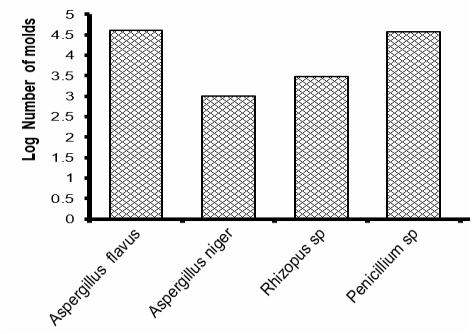

Figure 1. Molds contamination (CFU/g) on wheat flour sample (A)

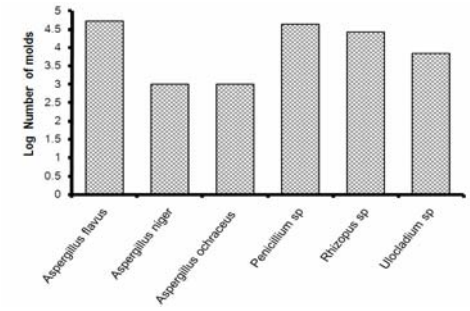

Figure 2. Molds contamination (CFU /g) on wheat flour sample (B)

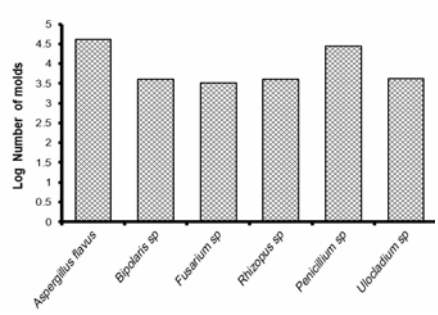

Figure 3. Molds contamination(CFU/g) on wheat flour sample (C) flour sample

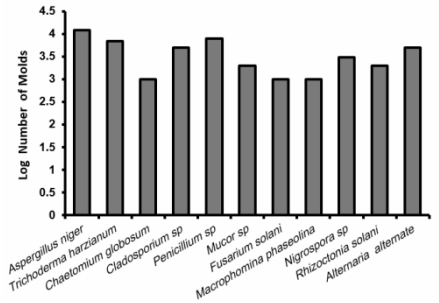

Figure 4. Molds contamination (CFU /g) on wheat (A) after three months storage at 30 ºC

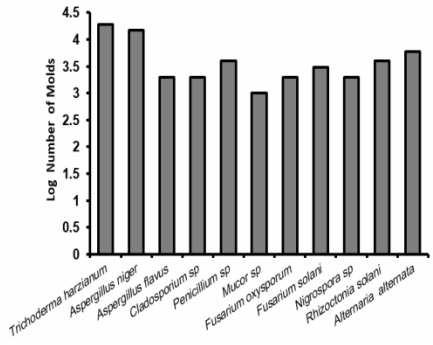

Figure 5. Molds contamination (CFU /g) on wheat Flour sample (B) after three months storage at 30 ºC

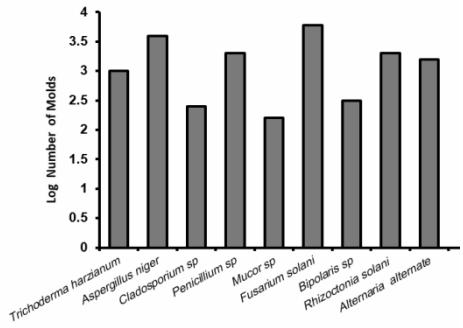

Figure 6. Molds contamination (CFU /g) on wheat flour sample (C) after three months storage at 30 ºC

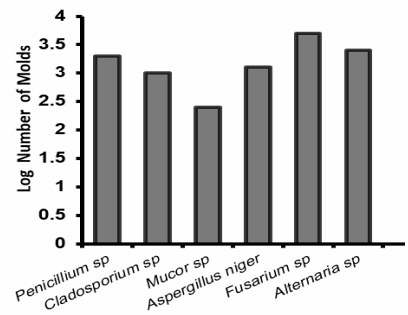

Figure 7. Molds contamination (CFU /g) on wheat flour sample (A) after three months storage at 5 ºC

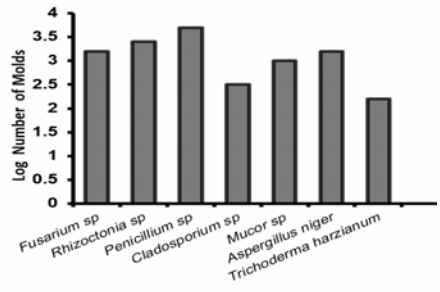

Figure 8. Molds contamination (CFU /g) on wheat flour sample (B) after three months storage at 5 ºC

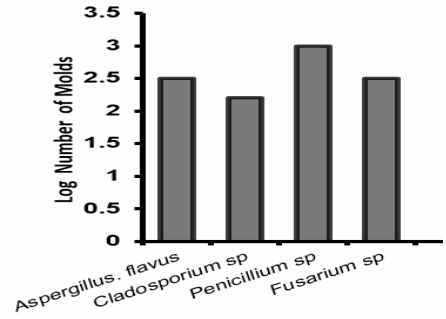

Figure 9. Molds contamination (CFU /g) on wheat flour sample (C) after three months storage at 5 ºC

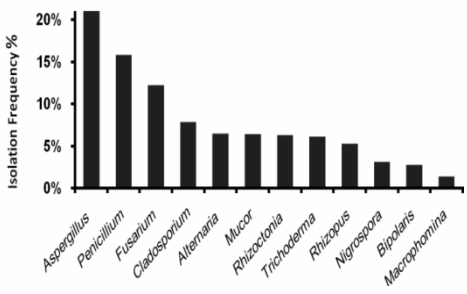

Figure 10. The isolation frequency percentage of dominant genera from wheat flour samples.

References

[1] **Abdullah, N., Nawawi, A. and Othman, I**. Fungal spoilage of starch-based foods in relation to its water activity (aw). Journal of Stored Products Research. Vol. 36,p.p. 47-54. 2000.

[2] **Menkovska, M., Sharic, M., Mateva, N., Djorgovska, N., Levkov, V. and Nikolova, N.** Changes of wheat quality caused by mold contamination. Journal of Hygienic Engineering and Design. Vol.4, pp. 65-68. 2013.

[3] **Berghofer, L.K., Hocking, A.D., Miskelly, D. and Jansson, E.** Microbiology of wheat and flour milling in Australia. International Journal of Food Microbiology. Vol.85, pp.137–149. 2003.

[4] **Mackinaite, R., Kacergius, A., Lugauskas, A. and Repeckiene, J.** Contamination of cereal grain by *Fusarium micromycetes* and their mycotoxins under Lithuanian climatic conditions. Ekologija. Vol 3, pp. 71–79. 2006.

[5] **Muthomi, J. W. and Mutitu, E. W.** Occurrence of mycotoxin producing *Fusarium* species and other fungi on wheat kernels harvested in selected districts of Kenya. African Crop Science Conference Proceedings. Vol.6, pp. 335-339. 2003.

[6] **Conkova, E., Laciakova, A., Styriak, I., Czerwiecki, L. and Wilczinska, G.** Fungal contamination and the levels of mycotoxins (DON and OTA) in cereal samples from Poland and East Slovakia. Czech J. Food Sci. Vol. 24, No1, pp. 33–40. 2006.

[7] **Fakhrunnisa, M., H. Hashmi, H. and Ghaffar, A.** Seed-borne Mycoflora of wheat, sorghum and barley. Pak. J. Bot. Vol. 38, No.1, pp. 185-192. 2006.

[8] **Bakutis, B., Baliukoniene, V. and Lugauskas, A.** Factors predetermining the abundance of fungi and mycotoxins in grain from organic and conventional farms. Ekologija. Vol.3, pp. 122–127. 2006.

[9] **Birck, N.M., Lorini, I. and Scussel, V.M.** Fungus and mycotoxins in wheat grain at post harvest9th International Working Conference on Stored Product Protection. pp.198-205. 2006.

[10] **Scudamore, K.A.** Identifying mycotoxins is Paramount in the fight against their spread. Word Grain Vol.23, pp.36-39. 2005.

[11] **Ellis M. B.** More *Dematiaceous Hyphomycetes*. Kew, Surrey. 1976.

[12] **Pitt, J. I. and Hocking, A. D.** Fungi and Food Spoilage. Academic Press, Sydney. 1985.

[13] **Domsch K. H., W. Gams and Anderson, T. H**. Compendium of Soil Fungi. Vol. 1, Academic Press, London. 1981.

[14] **Booth, C.** The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, England. 1971.

[15] **Gonzalez, H. H. L., Martinez, E. J., Pacin, A. and Resnik, S. L.** Relationship between *Fusarium* and *Alternaria alternate* contamination and deoxynivalenol occurrence on Argentinian durum. Mycopathologia Vol.144, pp.97-102. 1999.

[16] **Rakicka, M, E. and Sturdik, A. M.** Health-promoting substances contained in cereals (review)Acta Chimica Slovaca, Vol. 6, No. 2, pp. 256-268. 2013.

[17] **Houssou, A. P. F., Kpodo, K., Fandohan, P., Ahohuendo, B. C., and Hounhouigan, D. J.** Susceptibility of steeped and heat dried cowpea flour to fungal growth and aflatoxins production.

African Journal of Food Science. Vol.5, No. 4, pp. 227 – 231. 2011.

[18] Weidenbörner, M., Wieczorek, C., Appel, S. and Kunz, B. Whole wheat and white wheat flour- the mycobiota and potential mycotoxins. Food Microbiology. Vol.17, No1, pp.103-107. 2000.

[19] Saric, M. D., Stojanovic, T. V., Skrinjar, M. M. and Menkovska, M. B. Effects of moulds on the safety and processing quality of *Triticum aestivum*. Proc. Nat. Sci, Matica Srpska Novi Sad. Vol.114, pp.105-114. 2008.

[20] Egbebi, A. O. and Aboloma, R. I. Fungi and Moisture content of Gari sold in some locations in Southwestern Nigeria Archives of Applied Science Research, Vol.4, No 6, pp.2361-2364. 2012.

[21] Joshaghani, H., Namjoo, M., Rostami, M., Kohsar, F. and Niknejad, F. Mycoflora of fungal contamination in wheat storage (silos) in Golestan Province, North of Iran. Jundishapur Journal of Microbiology. Vol.6,pp.e6334(4). 2013.

[22] Hocking, A. D. Microbiological facts and fictions in grain storage. From: E.J. Wright, M.C. Webb and E. Highley, ed., Stored grain in Australia 2003 Proceedings of the Australian Postharvest Technical Conference, Canberra, 25–27 June 2003. CSIRO Stored Grain Research Laboratory, Canberra. pp.55-58. 2003.

[23] Shahin, A.M., Mahrous, S.R., Aziz, N. H. and El- Zeany, S. M. Relationship between fungal contamination and ergosterol content and control of wheat grain spoilage by gamma rays. Isotope & Rad. Res., Vol.35, No.3, pp. 569-580. 2003.

[24] Jonathan, G., Ajayi, I. and Omitade, Y. Nutritional compositions, fungi and aflatoxins detection in stored 'gbodo' (fermented *Dioscorea rotundata*) and 'eluboogede' (fermented *Musa parasidiaca*) from South western Nigeria. African Journal of Food Science. Vol. 5, No.2, pp.105 - 110. 2011.