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Fungal Load, Coliform and Aflatoxins in Wheat Flour of Lahore Metropolitan City

Hafiz Muhammad Usman Siddiq*[1], Arfan Ahmad[1], Aftab Ahmad Anjum[1], Mamoona Chaudhary[2], Irshad Hussain[1]

[1]*Institute of Microbiology, Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan*
[2]*Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan*

Abstract

In Pakistan, wheat flour is mostly utilized for making flat breads locally called chapattis. Other uses of wheat flour are in bakery products manufacturing. It is an important constituent of daily diet of people. Shelf life of wheat flour is one of the most important factors for its quality determination. Wheat flour is often tainted with pathogenic fungal species and their toxic secondary metabolites called Mycotoxins. The present study was designed to make a comparative analysis of Microbiological quality (fungal load and total coliform count) and detection of aflatoxins in raw and branded whole wheat flour samples of Lahore Metropolitan city. Total 100 samples were collected for determination of fungal load, coliform and aflatoxins. The Standardized methods were applied to count the colony forming units of fungal species and total coliform bacteria respectively. The results indicated both branded and raw whole wheat flour groups were of good quality for human consumption. However, the microbiological quality of branded whole flour was better than raw whole wheat flour. There was a significant difference ($P<0.05$) in fungal load of raw and branded whole wheat flour. Similarly, coliform bacteria were only cultured form the samples of raw flour. In the last stage of study, aflatoxins level was analysed by using a commercially available kit. All the samples of both raw and branded whole wheat flours were negative for aflatoxins detection. The overall quality of raw and branded whole wheat flours in Lahore city is satisfactory for consumers.

Key words: Wheat flour, fungal load, coliform count, Aflatoxins

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**Corresponding Author:*
usmansiddiq923@yahoo.com

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1. INTRODUCTION

Wheat (Triticum aestivum) is one of the largest food grain crops in Pakistan. The share of wheat is 8.9 percent in value addition in agriculture and 1.6 percent in the GDP of Pakistan[1]. Almost 80% of farmers in Pakistan are connected with the cultivation of wheat crop. There is 4.4 percent decrease in wheat production and estimated wheat production is 25.5 million tons during the last year[2]. Pakistan ranks 8th largest wheat producing country, contributing about 3.17% of the world wheat production[3]. It is easily accessible for the inhabitants of the world and one of the cheapest and important sources of energy and protein. It is the principle constituent of diet in Pakistan contributing more than 60% of the total protein and calorie requirement and nearly 80% of total dietary intake[4]. More than 80% of wheat is consumed in

the form of flour which is a powder, soft and prepared by grinding the wheat grains. Wheat flour is used to make flat breads locally called chapattis and naan in Pakistan[5]. Flour contains high contents of polysaccharides like starch and gluten protein. It is the major cereal crop used for the production of flour as it contains high amount of gluten which provide elasticity during baking[6].The moisture in flour is very critical for the growth of fungus and other harmful microorganisms. It contains about 11-14 percent moisture. When the moisture level rises above 14 percent, the flour is susceptible to fungal contamination, flavour changes, enzyme activity and insect infestation[7].

There is a considerable high demand of wheat flour at national and international level. At present million tons of wheat flour is produced and stored. Flour is stored for many months and subjected to fungal load and its mycotoxins. Mycotoxins are secondary metabolites of fungi produced during spoilage of food products. Mycotoxins are one the most important contaminants of agricultural food and feedstuffs. According to a report of Food and Agriculture Organization, 25 percent of the world's crop is contaminated with mycotoxins each year which adversely affects the cereal food industry[8]. Fungal growth and mycotoxin contamination have detrimental effects on the quality of flour and ultimately plot a health hazard for the consumers. Extensive negative physicochemical changes in the flour facilitate the mycelium growth. The growth of fungi not only spoils the flours but also the primary cause for the deterioration of breads and pastries. There are chances of recontamination of these cereal products after baking due to bakery dust consisting of contaminated flour particles and may result in mycotoxin contamination[9]. The most important mycotoxins include Aflatoxin (AF), deoxynivalenol (DON) and fumonisins which cause serious illness and ultimately death in humans and animals[10]. These contaminated food and feedstuffs and highly pathogenic for animals and human, thus the Food and Agriculture Organization have established the important guidelines concerning the permissible levels of mycotoxins in food and feedstuffs. On the other hand, Aflatoxins (B1, B2, G1 and G2) are more common in wheat flour[11]. The quality assurance is very important factor for wheat flour production and its consumption by human beings. It is of prime importance to produce good quality wheat flour at commercial level. Government of Pakistan is taking appreciable decisions to enhance the export of quality wheat flour[12]. Pakistan is an agricultural country and exporting different cereal food products including wheat flour and due to the strict parameters of importing countries, the finest food is exported, and occasionally contaminated food is sold domestically. So it is necessary to monitor, and control contamination of mycotoxins in wheat flour in both domestic and international trade[13].

According to Joint Expert Committee for Food Additives[14], the chances of liver cancer can be reduced to 300 case/year/billion people by adopting the strict aflatoxins standard permissible limit from 20 ppb to 10 ppb in 25 percent population diagnosed with Hepatitis B Virus (HBV). Many bakery products are prepared by using wheat flour dough. The frozen dough is at risk of mold contamination during the period of storage in refrigerators. Sometimes the wheat flour stored in houses get contaminated with spoilage causing species of fungi i.e. Aspergillus, Fusarium, Alternaria and Penicillium. The dough prepared from contaminated flour is not suitable for human consumption. It is a serious health issue for humans because the toxins of fungi are carcinogenic, teratogenic and mutagenic in nature[15]. The dough of wheat flour is used in different bakery products like cookies, pastries, pizza and cakes. The cookie dough is assumed to be potential source of Salmonella due to common use of raw eggs as a part of ingredient. Similarly *E.coli* strain O121, is also responsible for abdominal cramps, bloody diarrhoea and kidney damage after consuming the uncooked flour based products[16]. The adulteration in wheat flour should be condemned. There is a dire need to standardize the level of fungal contamination, aflatoxins and coliform bacteria in keeping quality of wheat flour.

2. MATERIALS AND METHODS

2.1 Studied Area

The current study was carried out in district Lahore and samples were collected from five different towns of Lahore including Aziz Bhatti town, Data Gunj Bakhs town, Gulberg, Samanabad, and Iqbal town (fig.1).

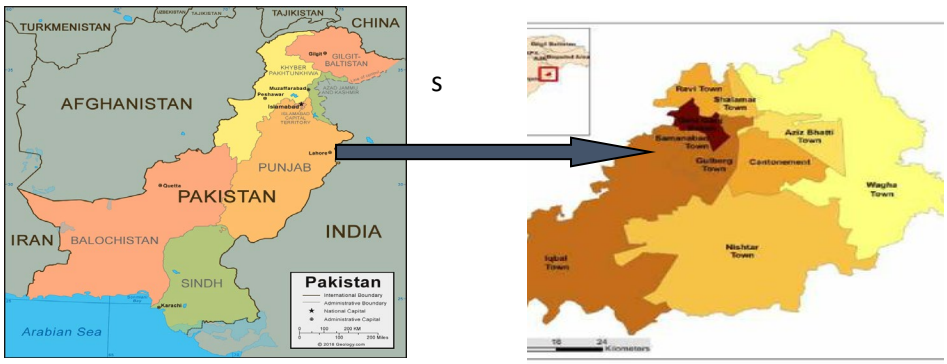

Fig. 1. Sample Collection area

2.2 Samples collection

Wheat flour samples were divided into two groups; raw flour and branded flour. Flour samples were evaluated for fungal load as well as coliform bacteria. Level of total aflatoxins was determined quantitatively by using a commercially available kit with the name called CHARM kit. A total of 100 samples were collected in properly labelled sterile polythene bags from local shops, chakkies and commercial markets. A total of 250 grams of each wheat flour sample was transported to Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan and used for mycological and bacteriological examination.

2.3 Enumeration of fungal colonies

Fungal load was determined from each sample by using Dilution plate method[17]. Fungal load was counted in one gram of each flour sample. Tenfold serial dilutions of wheat flour were seeded on Sabouraud's Dextrose agar and number of colonies were counted as colony forming units in per gram of original sample. One ml (standard volume) was inoculated from each sample on Sabouraud's dextrose agar media plates in the centre by using micro pipette and spread using L- shaped bent glass rod[18]. Sterilized glass petri plates containing inoculated sample were placed at 25±3˚C for 3 to 4 days.

2.4 Enumeration of coliform colonies

Serial dilutions of wheat flour were seeded on MacConkey Agar and number of colonies were counted from growth by using spread plate counting method. Three plates with highest dilution were selected for counting colony forming units of bacteria. The normal range for counting colony forming units in case of bacteria is 30-300 CFU. The petri plates were incubated for 24-48 hours in incubator[19].

2.5 Estimation of aflatoxins

Total Aflatoxins were estimated from those samples with high number of colonies forming units per gram by aflatoxin detection kit. Commercially available CHARM kit was used for this purpose. The CHARM® ROSA WET-S5 Aflatoxin Quantitative Test is an immunoreceptor assay which uses ROSA (Rapid One Step Assay) lateral flow technology and Water Extraction Technology (WET). The sample took 5 minutes to complete the evaluation. The numbers displayed were the concentration of aflatoxins (ppb) in the sample. The sensitivity range of this kit was from 0 to 150 ppb.

2.6 Statistical analysis

Independent samples T test was used. The probability level was 95 percent using Statistical Package of Social Sciences (SPSS) version 21. Data on the concentration of the fungal and bacterial pathogens was entered into Excel and transformed into log10 Colony-Forming Units per gram (CFU/g) of wheat flour sample.

3. RESULTS AND DISCUSSIONS

3.1 Fungal colonies

Fungal load was determined in raw flour (chakki atta, n=50) collected from shops located in various towns of Lahore. Similarly, same number of branded flour (Branded flour, n=50) were also collected and analysed. All the raw flour samples were found positive for fungus however, the colonies were found in the acceptable range (Table. 1). The fungal load in raw flour was found in the range of $1.0x10^2$ to $4.5x10^3$ cfu/g. In this study, all the branded flour samples were also declared positive for fungal load however, the load was less than in the raw flour samples. The range of fungal load in branded flour samples was ranged from $1.0x10^2$ to $2.3x10^3$ cfu/g (Table. 1).

Table 1. Fungal counts (CFU/g) on both raw and branded wheat flour samples collected from retail outlets in Lahore.

Wheat Flour	Total No. of Sample	Fungal Load CFU/g (10^2–10^4CFU/g acceptable)	Mean ± S.D	P Value
Raw Flour	50	1.0 x 10^2 to 4.5 x 10^3	2.75±0.42	0.007*
Branded Flour	50	1.0 x 10^2 to 2.3 x 10^3	2.53±0.35	

The comparison showed that statistical mean fungal load was higher (2.75±0.42**)** in raw flour as compared to branded flour with statistical mean fungal load of 2.53±0.35. Fungal load of raw flour was significantly different from branded flour ($p<0.05$). The represented plates of media containing fungal colonies were photographed using Samsung galaxy mobile camera (fig. 2).

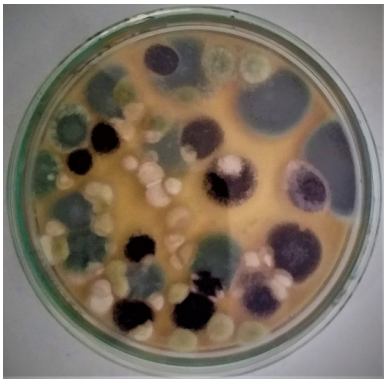

Fig. 2. Fungal colonies on Sabouraud's dextrose agar

3.2 Coliform colonies and aflatoxins

In our study, all the samples of branded wheat flour (n=50) were negative for the total coliform count however among raw flour 12 samples were found positive for coliform bacteria. Pink to pinkish red colonies were observed (fig. 2). The bacterial colonies in the range of 30-300 cfu/ml were considered for counting as per standard protocol. The data of the coliform count and aflatoxins detection has been shown in table 2. Aflatoxins were detected quantitatively through a commercially available kit. All samples were found negative (table. 2).

Table 2. Results of total coliform count and aflatoxins in raw and branded wheat flour samples.

Wheat Flour	Total No. of Sample	Coliform load CFU/g (10^2–10^4CFU/g acceptable)	Aflatoxins in Wheat Flour	Mean±S.D
Raw Flour	50	$1.3x10^3$-$1.2x10^4$	0.000 ppb	3.43±0.26 (12 Samples)
Branded Flour	50	Nill	0.000 ppb	Nill

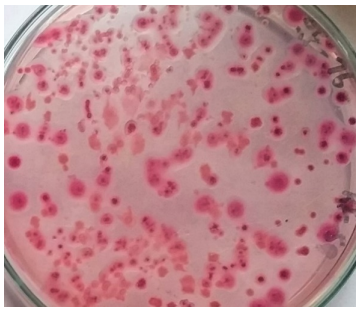

Fig. 3. Pink to pinkish red coloured colonies on MacConkey Agar

The moisture content of wheat flour is very crucial regarding its shelf life. As the moisture content crosses its safe limit, certain microorganism get chance to grow and deteriorate the wheat flour[20]. Wheat flour is vulnerable to mold contamination during pre as well as post-harvest conditions. Aflatoxins B1, B2, G1, and G2 are very important mycotoxins of wheat flour which are a health hazard for humans and livestock. These toxins also damage the economy of international trade of food and animal feed over the entire world. Important species of Aflatoxins production are Aspergillus flavus and *Aspergillus parasiticus* and Aspergillus nomius[21]. This study was designed to estimate the fungal load in wheat flour of Lahore. Moreover, Aflatoxins and total coliform count was also evaluated to check the quality standard of wheat flour. The results of the presented research were analysed statistically, and results were also compared with other related researches. In a previous study in Pakistan, reported the fungal count with the range of $\log_{10}$ 4.41-5.27cfu/g [7]. In our finding, the range was within $\log_{10}$ 2-3.65cfu/g in raw flour and $\log_{10}$ 2-3.36cfu/g in branded flour respectively. Similarly, in another study conducted on wheat flour in Malaysia, all wheat flour samples were positive for fungal colonies with the range of 180-13230 cfu/g samples with the mode values 360 and 450 cfu/g[22]. In our study, the fungal colonies ranged from 100-2300 cfu/g samples in branded flour and 100-4500 cfu/g samples (mode = 200 cfu/g for branded and 250 cfu/g for raw flour). As per the International Commission on Microbiological Specifications for Foods (ICMSF) the fungal load in the range from 10^2–10^4 per gram is permissible. Rejection of wheat flour with >10^5 fungi per gram[23].

Total coliform count is very important for overall quality of food products. Total coliform count was measured in wheat flour and it was shown that the branded wheat flour samples were negative while only 12 raw wheat flour samples were positive for coliform count. Coliform count in previous studies of wheat flour showed mean coliform count 10^2 cfu/g coliform count. Similarly, these results were also reported by Ottogalli and Galli 1979[24]. On the other hand, our findings were different from the previous studies. In our research we find the coliform count with the mean value of 10^3 cfu/g. The contamination of coliform in wheat flour is usually occur during inappropriate cutting step of milling process. Level of toxins were significantly different from the study conducted by scientists in Malaysia[22], which showed 21.7% samples of

wheat flour were contaminated with aflatoxins while in the present study every sample contained nil amount of aflatoxins.

4. CONCLUSIONS

Both the raw and branded wheat flour were found positive for fungal load and coliform bacteria. Fungal load was within recommended range however, the coliform bacteria need to be addressed. The underdeveloped countries like Pakistan, India, Bangladesh and Sri Lanka, the people in these countries are not aware of the standards of cutting the cereal and their storage condition. By adopting the principle methods of wheat flour handling and storage conditions, we can prevent the wheat flour from fungal contamination and its toxins.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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