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A SURVEY OF FUNGAL FLORA OF WHEAT FLOUR IN PAKISTAN

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Isolation of fungi infecting wheat flour collected from different parts of the country yielded mainly *Aspergillus* and *Penicillium*, spp., regardless of source and type of flour mill. The total fungal count varied from 200 to 29,000 per gram of flour. Out of the two culture media, malt salt agar gave higher number of colony count as compared to malt extract agar. Period of storage had a marked effect on microbial activity and pH of the flour. Samples stored for about 7 months had a low pH of 4.5 to that of fresh samples with pH 5.5 to 6.3.

INTRODUCTION

Like all other foods and feeds wheat is also infected by various micro-organisms. There is, indeed, a host of evidence available elsewhere which shows conclusively that a sizeable amount of microflora is associated with the milling fractions at the time of milling and during storage (Christensen, 1946, 1947; James and Smith, 1948; Gattani, 1951; Thatcher *et al.* 1953; Hesseltine and Graves, 1966; Hesseltine, 1968; Mehrotra and Basu, 1975) but for Pakistan there is no available information for the number, kinds and distribution of different fungi in different flour samples.

The fungi attacking the grain and the flour, adversely affect their quality through various biochemical changes. The role of certain molds in producing mycotoxins has also been established (Hiscocks, 1965). Therefore, not only because of the loss of produce due to infection and infestation but also from the stand point of human and animal health it is essential that factors responsible for fungal infestation be investigated. A preliminary survey was, therefore, made to determine the number and kinds of molds present in flours stored under various conditions so as to explore the possible sources of contamination.

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MATERIALS AND METHODS

Wheat flour samples were collected from different sources (flour mills, ration depots and private houses) located at Quetta, Lyallpur, Sheikhupura, Gujranawala, Sialkot, Multan, Rawalpindi and Lahore. Samples from flour mills were obtained by inserting a small sterile polythene bag into the spout of the mill to get a desired quantity (12-16 oz) of flour; the bag was sealed just after collection. Samples were then screened for their fungal content within a week of their collection.

Isolations from the flour samples were made on malt extract agar and malt salt agar (with 7.5 per cent sodium chloride) so as to know the frequency and kind of the fungi.

In order to determine fungal population, dilution plate method of Christensen and Cohen (1950) was used; Penicillin-Streptomycin at a concentration of 1:25,00 was added to the media to avoid bacterial contamination. The petri plates were kept at 30±1°C and the fungal colonies were counted with the help of a colony counter after 3–4 days of incubation. Colonies growing quite slowly were recounted to get accurate data of fungal population.

To know pH value of the samples, flour suspensions were made with 15 g flour by adding slowly 60 ml double distilled water and keeping the mixture continously stirred with a spatula to make a homogenous paste. The pH value of the different stored flour samples was determined by direct pH meter.

RESULTS AND DISCUSSION

Isolations of fungal colonies from the flour samples collected from different places showed that 22 different genera of fungi were associated with the flour samples. Except for a few Fungi Imperfecti and Mucorales, the majority of the isolates belonged to the genera *Aspergillus* and *Penicillium* Out of Aspergilli, *A. candidus* was more frequent on malt salt agar whereas, *A. ochraceus* was a common inhabiting species on both culture media (Table 1). Graves and Hesseltine (1966) reported similar results. According to them, in both flour and dough, field fungi (*Alternaria, Fusarium, cadosoorium, Diplodia* and a few other general were encounted infrequently and the storage fungi (primarily species of *Aspergillus* and *Penicillium*) were quite common.

Table 1. *Colony counts and frequency of fungi on two culture media*

Culture media	Fungal colonies/ gram of flour (range)	Frequently occurring fungi (in order of prevalence)
Malt extract agar	500—22.900	*Aspergillus ochraceus, A. nidulans, A. fumigatus, A. versicolor* and *Penicillium corylophilum.*
Malt salt agar	200—2.900	*Aspergillus ochraceus, A. candidus, A. nidulans, A. niger, A. versicolor, A. fumigatus* and *Penicillium Islandicum.*

The total fungal counts varied from 200 to 29,000 per gram of flour. The number of prevalent aspergilli varied between 80 to 90 per cent of the total count regardless of the location and type of mill. The samples from Quetta, however, gave the least number of colonies indicating a significant effect of temperature on the prevalence of these fungi. Species of *Penicillium* were not common in samples collected from these places (Table 2). Christensen in 1947 examined more than 100 flour samples from mills located in different parts of the United States. He found that the total fungal counts in the flour samples varied from 200 to 5,000 colonies per gram, with the higher count in flour milled in more humid regions. *Aspergillus glaucus* and A. *candidus* made up 60 to 90 per cent of the count in majority of the samples.

Table 2. *Number and kind of fungi isolated from flour collected from different places.*

Location	Fungal colonies/ gram of flour (range)	Moisture (%) (range)	Period of storage (week)	Frequently occurring fungi (in order of prevalence)
Faisalabad	1600—15,400	11.3—17.3	1—6	*Aspergillus ochraceus, A. niger, A. candidus, A. versicolor, A. fumigatus,* and *Penicillium islandicum*

Sheikhupura	11,500—29,000	11.5—16.0	4—5	*Aspergillus A. nidulans, A. niger, A. candidus, Rhizopus oryzae, and Penicillium corylophilum,*
Quetta	200—2000	10.0—12.0	4—6	*A. fumigatus, A. nidulans, A. niger,* and *Penicillium corylophilum*
Rawalpindi	2400—24,000	14.8—15.5	2—4	*A. nidulans, A. versicolor, A. ochraceus,* and *Penicillium corylophilum*
Lahore	3,700—23,700	11.9—16.5	4—8	*A. ochraceus, A. nidulans, A. versicolor, A. candidus,* and *Penicillium funiculosum*
Sialkot	1000—16,200	7.5—10.3	2—4	*Aspergilus ochraceus, A. versicolor, A. nidulans, A. niger, A. candidus,* and *Penicillium oxalicum*
Multan	6,000—7,600	10.0—10.5	4—6	*A. ochraceus, A. versicolor, A. nidulans, A. candidus, A. niger,* and *Penicillium cyclopium*

Data presented in Table 2 indicated that the most widely distributed species of *Aspergillus* were *A ochraceus, A. niger, A. candidus, A. versicolor, A. nidulans,* and *A. fumigatus* and these accounted for about 90 per cent of the isolates whereas species of *Penicillium* were of infrequent occurrence. A few other fungi such as species of *Alternaia, Curvularia, Septonema Sclerotium, Paecilomyces, Graphium, Epicoccum, Helminthosporium, Phoma, Memnoniella, Candida, Cladosporium, Verticillium, Hormodendrum* and *Fusarium* in fungi Imperfecti, and species of *Mucor, Rhizopus, Absidia, Circinella* and *Syncephalastrum* among Phycomycetes were recorded, but their frequency was considerably low.

The data presented in Table 3 indicate that flour from flour mills and ration depots have quite an abundance of microflora and are generally infested with different viable mold spores. However, it varies with the geographical location of the storage places (Table 2.) It was also observed that the sanitary conditions of the mills also play an important role in the multiplication of the inoculum. In these samples storage fungi predominated and frequency of field fungi as compared to storage fungi was very low. Isolations from these flours also showed that there was no significant difference in the colony count of samples collected from private flour mill and ration depots. However, the counts were considerably low in samples collected from private houses (Table 3). It was also observed that out of these collected samples even some freshly milled flour samples had a considerable number of microbes which reflected the sanitary condition of our flour mills.

Table 3. *Comparison of wheat flour collected from different sources*

Source of sample	Colonies/gm of flour (range)	Frequently occurring fungi (in order of prevalence)
Flour Mill	200—29,000	*Aspergillus ochraceus, A. candidus, A. nidulans, A. versicolor, A. niger, A. fumigatus, Penicillium cyclopium* and *P. corylophilum.*
Ration Depot	3,700—22,000	*A. ochraceus, A. nidulans, A. versicolor, A. niger, Rhizopus oryzae* and *Penicillium islandicum.*
Private House	1600—11,800	*A. niger, A. nidulans, A. fumigatus, Alternaria tenuis, Penicillium funiculosum, Helminthosporium sativum,* and *Cladosporium cladosporioides.*

Effect of storage period showed some effect on pH of the flour samples due to the microbial development in the flour. Fresh samples had a pH 5.5 to 6.3, whereas the pH of the samples stored for about 7 months was 4.5 (Table 4). A negative correlation was observed between the pH. value and the storage period of wheat flour. The changes in pH. may be due to the activity of micro-organisms.

Table 4. *Relationship of different storage periods with pH of wheat flour.*

Storage period (months)	pH value
½ to 1	5.5—6.3
1 to 2	5.5—6.0
2 to 3	5.3—6.1
3 to 4	5.3—6.0
4 to 5	5.2—5.6
5 to 7	4.5—5.0

A change in colour and odour due to growth of the fungus was also observed and it was suspected to be due to certain metabolites produced by certain species of *Aspergillus* and *Penicillium.*

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