

Microbial and Physico-chemical contamination in the wheat flour of the twin cities of Pakistan

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Abstract

Wheat flour samples from ten different locations were selected and examined for physiochemical and microbiological. Totally 150 samples were examined, for moisture ash and some toxic metals by using Atomic adsorption spectrophotometer while TVC, coliforms, E.coli, Salmonella and Yeast&molds were selected for microbial contamination. In the Physico-chemical the results has been under the permissible limits the heavy metals were not detected in all flour samples and the differences among investigated areas were non significance ($P < 0.99$) and microbiologically in terms of TVC, E.coli, and moulds were significant ($P < 0.05$) in two cities. The results obtained show significant variations in microbes in different wheat flour at different collection point.

Key words: Physico-chemical quality, microbiological quality, wheat flour, Pakistan

Introduction

Wheat (*Triticum aestivum* L.) is largest food grain crop, produced in Pakistan and about 40% of its area was used under its cultivations. Probably 80% of farmers in Pakistan cultivate wheat and it stands within the top-10 of the world ranks for its production. The annual wheat production stood at 21.74 million tonnes during the year 2007-08 (GOP, 2008). Almost 80% of this is consumed in the form flour for making of flat breads locally known as chapattis, rotis and naan. It is a staple food and contributes 68-75% of total food intake in daily diet for the people of Pakistan (Aslam *et al.*, 1982). Wheat flour is powder, soft form of wheat which is made by grinding wheat seeds. It is one of the most important foods in European, North American, Middle Eastern and North African cultures, and is the defining ingredient in most of their styles of breads and pastries. Flour contains a high proportion of starches, which are a subset of complex carbohydrates also known as polysaccharides. The kinds of flour used in cooking include all-purpose flour, self-rising flour, and cake flour including bleached flour. The higher the protein content the harder and stronger the flour and will produce crusty or chewy breads. The lower the protein the softer the flour for better cakes, cookies, and pie crusts. Wheat is the most favourite cereals used for the

production of flour due to the high content of gluten (Anjum *et al.*, 1991). High content of gluten help in making bread or chapatti and create elastic toughness that holds its shape well once baked staple. It provides upwards of 60 percent of the protein and carbohydrate in the average diet. (Bodroža-Solarov *et al.*, 2006 and Šarić M *et al.*, 2004). Moisture in flour is very important factor and it's normally ranges from 11-14 percent. When moisture content rises above 14 percent, flour is susceptible to fungus and mold growth, flavour changes, enzyme activity, and insect infestation. Apart from containing nutrients (high level of vitamins, minerals and cellulose fibers) wheat grain and flour also contains a number of elements (Cu, Zn, Fe, Ni, Mn) vital to our biological functions, but if its concentration increases it's adversely affect the biochemical system. These metals concentration will come in food due to environmental pollution. (E.Friden., 1983; H.W Numberg., 1985).

Flour being, a food product with high nutritional content can harbour a variety of bacteria including pathogenic and non-pathogenic forms (Berghofer *et al.*, 2003). Some earlier studies showed that *Salmonella*, *Escherichia coli*, and some other micro-organisms are present in wheat and flour are causes its spoilage (Ottogalli and Galli 1979; Spicher 1986; Eyles *et al.*, 1989; Richter *et al.* 1993). Reported bacterial counts in wheat and flour vary considerably, ranging from a few hundred per gram to millions i.e., 5,000 bacterial per g in flour and 15,000/g in canned biscuits (Doty., 1961). The

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number of bacteria present will be related to the bacteriological status of the wheat but the presence of bacteria in flour depend on the type of flour either it is high grade flour or low grade. Bacteria normally originate from the outer site skin of seed kernels and mostly in low grade flour contain significant number of bacteria. The mould contamination is also important in view of the possible mycotoxin production by great number of mould species (Hussein & Brasel, 2001).

It has been established that moulds not only cause spoilage but sometimes it produce toxin if environmental conditions are conducive to their growth. It has been estimated that 25% of the world's food crop may be contaminated with the mycotoxin (Boutrif & Canet, 1998). the occurrence of toxins and microbes to moulds infestation is influenced by environmental factors such as temperature humidity, and harvesting conditions of the wheat. Hence, storage conditions, like, time, temperature, and material are key factor that influences the quality of flours.

The objective of the present study is there is very little or no information available on the Bacteriological and Physico-chemical quality of flour in Pakistan. This survey is intended to augment the scarce information on the bacteriological and Physico-chemical quality of flour. In under develop countries freshly milled wheaten white flours mill cannot remove all the bacteria, for these reasons, flour must have made of various mills product of flour in twin cities. The question is whether bacterial counts are relevant to flour quality for insight into these problems and to establish the micro flora of domestic wheat and commercial flours.

Material and Methods

Sample Collection. A total of 150 wheat flour samples obtained from five sites in Rawalpindi. (1)Chaklala cantt (2). Allahabad (3). Westridge (4). Satellite town (5).Murree road, and in Islamabad. (1) I-10/2 (2). I-8 (3) G-9 (4) G-11 (5) F-6/2, during the summer (June-August) .A 1000 g portion of each sample was immediately transported to the laboratory in chilled containers at 4-6 °C and subsequently analysed in terms of physico-chemical and microbial parameters. All chemical were analytical grade of Merck, (Germany) and media were obtained from Oxoid (Basingstoke, UK).

Sample Location. Pakistan is agricultural based country and wheat contributes its major part of economy. Most people of this country used wheat as in the form of flour for preparation of plane bread (Chappati) in whole country. Islamabad is the capital city of Pakistan . Located within the population of the city has grown from 100,000 in 1951 to 1.7 million in 2011.Thegreater Islamabad-Rawalpindi Metropolitan Area is the third largest conurbation in Pakistan with a population of over 4.5 million inhabitants.

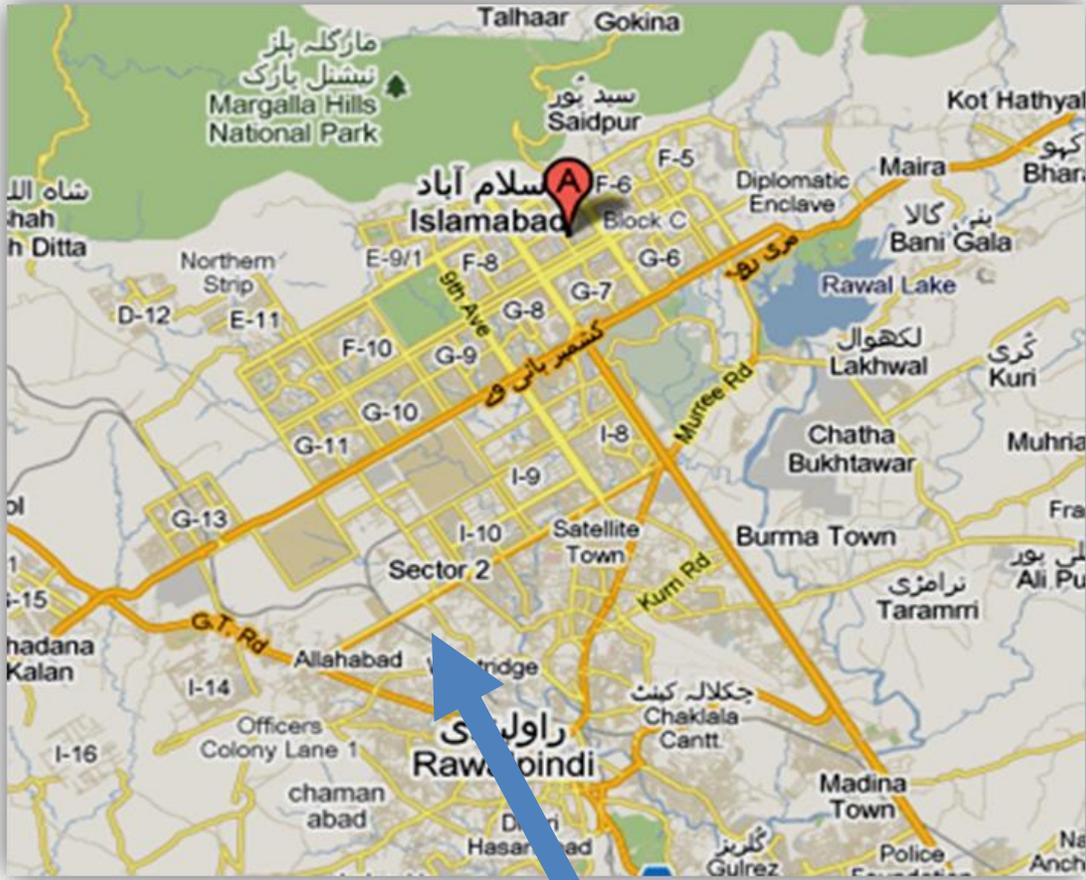
We selected the different most popular markets of both cities which are shown on Figure1.

Physico-Chemical Analysis. Wheat flour samples have been analysed for ash, moisture, crude fiber, protein and fat gluten according (AOAC, 2002), respectively. Metals content have been determined using acid digestion method on Atomic Adsorption Spectrophotometer (AAS Vario 6 Analytical Jena).

Isolation and Enumeration of Bacteria. The determination of the total microbial contamination of the flour samples was performed fortnightly by the method by outlined in compendium of methods for the microbiological examination of foods (AMPH, 2001).For the determination of total viable count pour plate method were applied as describe by Morton, 2001. For coliforms and fecal coliforms MPN procedure was applied as describes by Kornacki and Johnson, 2001. For the detection of *Salmonella* spp., 25 g of wheat flour samples were placed in sterile blender homogenized for 2 min in 225 mL of sterile Lactose broth (LB) and pre-enriched for 24 h at 35-37 °C. Subsequently, 1 mL of the pre-enriched culture was transferred to Selenite Cystine Broth (SC) and incubated for 18-24 h at 35-37 °C. In parallel, 0.1 mL of the pre-enriched culture was transferred into 10 mL of Heketonc enteric agar (HE) plates and incubated at 35°C H.Wallace et al., 2001.

Isolation and Enumeration of Fungal Flora. Mould counts were determined on Dichloran Rose Bengal Chloramphenicol agar plates (DRBC). Plates were inoculated by spreading 0.1 mL of sample dilution on the surface of the agar and incubation was in the dark for 5-7 days at 25 °C(Beuchat and Cousin,2001). According to the colony morphology and gram staining fungal were identified for the further conformation and organism were subcultured to the PDA(potato dextrose agar) and were classified according to Bergey,s manual(Bergey,1984).

Statistical analysis. Physico-chemical results have been statically analyzed for mean standard deviation using Excel Microsoft 2007. Data on the concentration of the food-borne pathogens was entered into Excel and transformed into log₁₀ Colony-Forming Units per gram (CFU/g) of food sample. All statically calculation has been done according to Caulcutt and Boddy 1983.



Sampling location indicated =

City location =

Rawalpindi. (1).Chaklala cantt (2).Allahabad (3). Westridge (4). Satellite town (5).Murree Road
Islamabad. (1).I-10/2, (2). I-8 (3). G-9 (4) G-11 (5). F-6/2.

Figure 1. Location of study areas and wheat flour sampling point in twin city, Pakistan.

Results and Discussion

Physiochemical composition. The physiochemical composition of flour samples collected from Rawalpindi and Islamabad are shown in Figure 2.

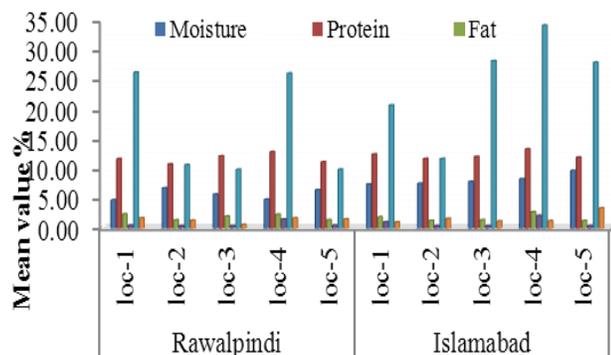


Figure 2. Physiochemical quality parameters of wheat flour samples (n=150) obtained from twin city

Moisture is an important parameter when considering flour quality because it significantly effect the shelf life and growth of the microbes (ICMSF,1998).The moisture in wheat flour were lies 13.5% and 15.5% according to the Codex Alimentarius Standards 1995.Our study showed the moisture content in Rawalpindi flour samples range from 4.76±0.05,to 6.76±0.27,and in Islamabad 7.40±0.03, to 9.68±0.03,which were under the approximately range of international limits it showed significance between two areas ($p < 0.05$). Maliha et al., 2010 find the 12% moisture content in different wheat grain samples in different district of Pakistan. Our results are closely related to some previous studies carried out by Muhammad et al., 2009 who had found the moisture content in wheat grain and flour in the range of 9.11%-9.79%. These low moisture low levels might be the reason for the low yeast content. Although water activity of wheat flour samples is too low to support the growth of molds and may it leads to the toxin production. According to some earlier studies the low moisture content from 1% to 2% leads to the molds growth and toxin contamination also observed. (Eyles et al., 1989). Moisture depends on the genetic makeup of wheat varieties and it largely influenced by the agronomic and climatic conditions (Mahmood, 2004).Ash is composed of inorganic matter naturally present in wheat kernels. It includes iron, copper, potassium, sodium, and zinc. Besides providing needed minerals to the diet, ash increases yeast fermentation by providing minerals to yeast. In milled white flour is relatively low in ash (less than 0.6 percent) .In this study the Rawalpindi region wheat flour samples have ash with mean value of 0.63±0.05 to 1.72±0.02, while in Islamabad the mean ash value observed was 1.04±0.01 to 3.39±0.02 which is lies with the limits of Pakistan standards

but its high as compared to the international codex standards. the present findings show disagreement with the previous studies who had reported the ash content in wheat flour samples were 0.54% to 0.71% and 0.57%-0.64% (Aydin et al., 2009 Ekinici *et al.*, 2002).on the other side these result agreed with the Alp et al., (2006) findings which observe ash content in wheat were 1.36%.The high level of ash are generally associated with the addition of bran in the wheat (Ekinici and Unal 2002). While crude fiber were found in, 0.39±0.03 to 1.52±0.08, in Rawalpindi and in Islamabad it was found in the range of 0.39±0.01 to 2.13±0.02. The protein content found in the range of 10.77±0.21 to 12.83±0.24, in Rawalpindi and in Islamabad location it was lies between the range of 11.15±0.10 to 13.32±0.03.Our results revealed high value as compared to the standards which was 7.0%.this studies closely agreed with the earlier studies which reported the protein content in the range of 11.19% to 12.78%.The protein content is very important content to check the quality of wheat. It is key factor determine the stability of wheat for different wheat product. The fat content found in flour samples in Rawalpindi was 1.38±0.02 to 2.39±0.04 and in Islamabad it was 1.29±0.01 to 2.74±0.02.The dry gluten found in Rawalpindi region was lies between the range of 9.88± 0.13 to 26.21±0.07 and in islambad11.70±0.01 to 34.12±0.02.on the hand regarding gluten the differences between areas was statically non significance ($P \leq 0.99$).It showed higher gluten in both cities as compared to the international and national standards. These findings show similarities with the recently studies by Muhammad et al., 2009. The difference of gluten level in all locations is due to the difference in the genetic makeup of the wheat varities, and climate conditions, growth conditions. (Randhawa et al., 2002).Changes in the Physico-chemical properties of flour during storage has been widely documented (Sur et al., 1993; Hruskova and Machova, 2002).

Metals Toxicology. Trace metal play important role in the health maintance of human body if its amount increases or decrease in traces. Some metals like iron zinc are play vital role in the biological activity of man, but some toxic metals like cadmium arsenic ,mercury are hazardous for human health. According to our result no traces of toxic metals were observed in all flour samples, only iron was detected in Rawalpindi in the range of 14.94±0.03 to 25.23±0.01ppm, while in Islamabad it was observed 22.33±0.09 to 25.49±0.09 ppm. Difference between locations were significant in terms of metals ($P < 0.01$).Some previously studied the different concentration of metals in flour samples (G.Q et al. 2002).

Microbial Contamination. Cereal or cereal products, especially flour constitute large part of the daily Pakistani diet in the form of chapatti(plane bread).Studies on the physiochemical composition and microbial contamination of the Pakistani flour are very valuable from the view point

of risk assessment. The microbes present in flour generally consist of Yeast & molds, thermophilic bacteria lactic acid bacteria and pathogenic bacteria like *E.coli* and, *Salmonella spp.* Although cereals ,milled product have very few cases that they have been implicated in food born diseases (Deibel et al.,2001).Flour generally produced by wheat that have been significantly expose the microorganism that promoted the retrieval of data on frequency of pathogens bacteria and microorganism that would render the food unfit for the consumer.

Total Viable count or total plate count is used as an indicator of bacterial populations on a sample. It is widely used to gain the opinion about the hygienic quality and microbiological load of foodstuffs .Many of earlier studies Many of earlier studies on wheat flour showed the TVC in the order of 10^4 cfu/ml.(Potous and Suchet 1989; Spicher 1986;Richter et al.,1993 Berghofer et al.,2003).Our finding show that the range of total viable count found in Rawalpindi locations lie between the of \log_{10} 3.40-5.13 cfu/g and in Islamabad it is between the range of \log_{10} 3.27-4.01cfu/g which is shown in Figure,3.

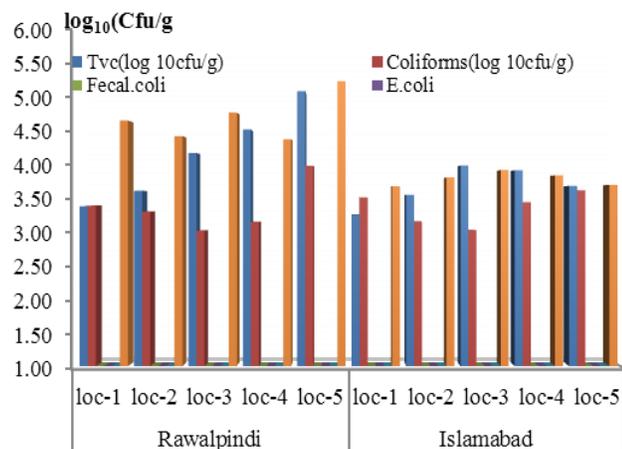


Figure 3. Microbial counts of wheat flour samples (n=150) obtained different location of twin city

Our results show's similarities with the previously studies done in Australia in wheat flour who had found the total viable count in the range of 10^5 and 10^2 cfu/g (Berghofer et al.,2003 ; Aydin et al.,2009).The presence of TVC generally shows that food processor information regarding raw materials, processing conditions, storage conditions and handling product. It also gives the information regarding shelf life or organoleptic changes in foods, and it indicates poor sanitations or problems with process control or ingredients. Coliforms in foods products have importance because it give the all over quality of food. The differences between the two cities were statically significant ($P < 0.01$). In present result the coliforms are present in Rawalpindi flour sample is ranging from \log_{10} 3.02-4.01cfu/g, while in Islamabad it is between the range of \log_{10} 3.04-3.64cfu/g which is higher than the recommended value of Codex

Alimentarius standards. Spicher (1986) found the mean coliforms count in flour was 10^2 cfu/g, similar finding were also reported by Cicognani *et al.*, 1975; Ottogalli and Galli 1979, while according to our finding no single colony of fecal coliforms and *E.coli* .our results were showed diversity with the some previous studies done by (Aydin et al., 2009., Richter et al., 1993 and Berghofer et al., 2003).There results showed the presence of E.coli in flour samples with different percentages. The coliforms contamination in flour is due to the inappropriate cutting process especially during process in milling when cutting washing and drying will done. In under developed countries like Pakistan, India, Bangladesh the peoples are not well known about the standards of cutting the cereals in feed and also they did not measure the condition when storage the grains. According to some early study done by (Ambreen and Samina, 2009) also found the similar findings in different bakery products. Differences between Rawalpindi and Islamabad in terms of coliforms, E.coli counts were significant ($P < 0.005$).

Yeasts and Molds constitute a large and divergent group of microorganism consisting of several thousand species. It is very important factor when quality of food is considered because many food born diseases occur due to it. Food decomposition occurs due to the presence of mold (Deak, T., 1995; Pitt, J., 1997). Numerous molds can produced mycotoxin (Bullerman.,1997) and some yeast and molds are responsible for human infections.(Birmingham.,1995).In our studies the yeast and molds have been identified in Rawalpindi region with the range of \log_{10} 3.70-3.74 cfu/g and in Islamabad it has been found within the range of \log_{10} 4.41-5.27cfu/g. Regarding mould numbers, the differences between areas are significant ($P < 0.005$). In present investigation we isolated different fungi species from flour samples. The frequency isolation of these are as in Rawalpindi region it is *Aspergillus niger* \geq *Aspergillus flavaus* , \leq *Rizopus oryzae* , \leq *Pencillium frequentus* , \leq *Mucor spp.* *Aspergillus niger* was found with highest frequency with 32.11% in Rawalpindi and 48.04% in Islamabad, while \leq *Mucor spp* has been found with lowest frequency with 1.38% in Rawalpindi and 3.92% in Islamabad(Figure,4 and 5) as compared to the others.

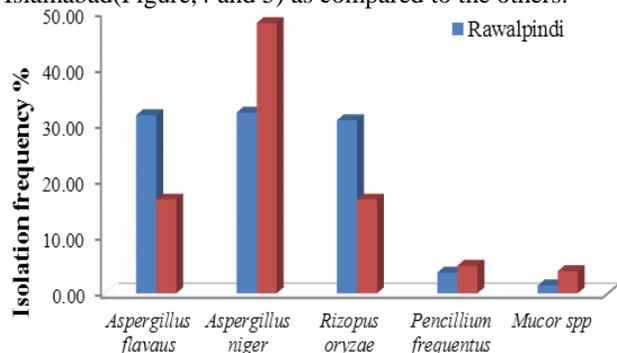


Figure 4. Frequency isolation of different fungi spp in wheat flour obtained from twin city.

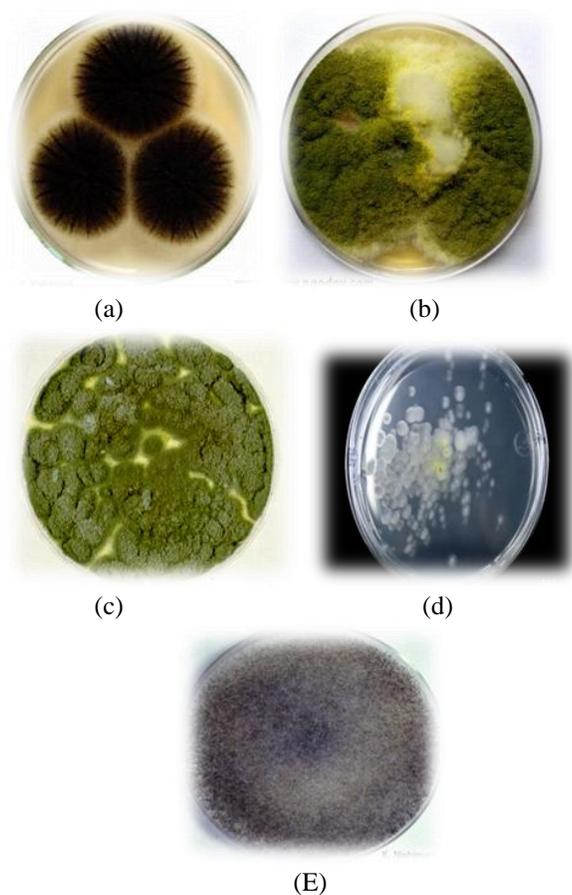


Figure 5. Figures of different fungi species.
(A) Aspergillus niger (B) Aspergillus Flavous
(C) Pencillum (D) Mucor (E) Rizopus

Some earlier studies showed that *Aspergillus*, *Pencillum*, *Mucor* is found in wheat and flour (T.D., 2010). *Aspergillus flavaus* is the main source of mycotoxin productions in the world's food supplies, (Busby WF., 1981), while *Pencillum* were recorded the second point of research in grain mostly it will come from Mechanical cleaning of grains but they are not pathogenic. Moisture content in cereal or flour is the most important factor that causes the molds and yeast growth. Mould contamination on cereals, which can exist at the farm or at the site of storage, affects the yield, quality, and nutritional value of the products (Aran and Eke., 1987). When the water content exceeds the alarm level for wheat flour (13%-15%) moulds start growing (Jay, 1996). This situation illustrates how some chemical parameters could affect microbiological parameters. Fungal contamination of flour has been the subject of various investigations (Farhat and Masood 1978; Aran and Eke 1987; Beuchat, 1992; Weidenbörner et al. 2000). There are different sources for moulds present in flour for example fungi prevailing in the grain, the mill machinery and lower quality of sanitary control (Berghofer et al., 2003), while generally moulds in the seeds and as present as mycelium in

the outer layer on the grain and other factor is that the climate condition of sub continent like India and Pakistan are hot and humid and are favourable for the growth of moulds in cereal products during storage. As some recently studies done by Saeed et al., 2008, who found that the storage condition play important role for the growth of moulds and microbes in cereals.

Conclusion

In conclusion, this study has found that flour produced from wheat in the twin cities of (Rawalpindi & Islamabad) is contaminated with food pathogens, especially fungi. Flour is the main food ingredient used in daily routine in throughout the country and although food is cooked at a high temperature which is enough to inactivate pathogens, post-contamination and cross-contamination that is being promoted by unhygienic food handling, and incorrect storage practices are causing this "safely prepared food" to be unsafe. One of the high risks in domestic kitchens is storage of leftover food (Beumer and Kusumaningram, 2003). Microbial contamination occur in flour is widely depending on a number of factors such as initial counts in the grain from crop conditions, milling practices, post-milling handling, moisture content of flour and storage conditions (Sperber, W.H. 2003). So this study conclude that the occurrence of these pathogens requires attention of the Ministry of Health, Environmental Health organization, and they should arranged food safety education campaign and quality measure should be implemented by the mill owner and government agencies should implemented the standards in flour miller and distributors. Present results recommend that similar surveys for other food products and stored conditions should be carried out to make a clear picture for quality assurance and quality of food and also in the development of advanced quality system by advanced techniques in agriculture of Pakistan.

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