Evaluation of fungal contaminations and humidity percent of consumed flour in the bakeries of Tabriz city

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ABSTRACT

Foods contaminations with Mycotoxin producing fungi is the common problem in production and maintenance of foods leads to production and presence of many types of Mycotoxins with extensive clinical effect on human called Mycotoxicosis. The aim of this study is to determine humidity percent and fungal contaminations of flour which is used in bakeries of Tabriz city. From 89 bakeries that were baking every kind of wheat bread, flour samples were collected .At first, humidity percent of samples was measured with standard method; Then experimented with standards of Iran(997 and 2393):10 gram flour sample were dissolved in 90cc of ¼ sterile Ringer solution; then10^{-2} ,10^{-3} ,10^{-4},10^{-5}and10^{-6} dilutions were prepared. From each dilution, 1cc were spilled in different sterile plates; afterward, 15-20cc of sterile YCGA culture medium were spilled on the plates and they were shacked slowly; hereafter, they were left until the culture mediums were coagulated. Thereupon, the plates were incubated in 25 ^{o}c for 5 days. Finally, fungi colonies were identified and counted. From 89 samples, 28 samples (31,5%)contain fungi contaminations more than 104colonies/gram of flour(allowable limit) [$p<0.05$]. Results showed the most fungi were Aspergillus niger and Acromonium. Mean of humidity percent in samples was12.8±0.76 which was in standard level (≤14%) ($p=0.000$). Findings of this study are according to results of other studies. With respect to wheat is a staple food of Iranians, attention and follow up in all steps of production, distribution and maintenance of wheat and flour with the aim of minimizing fungi contaminations is necessary.

Key words: Fungi; Flour; Contamination; Bakeries; Iran.

INTRODUCTION

Despite enhancing people's health awareness and development of the technology in science and food health as well as decreasing the infections and food poisoning resulting from bacteria and their poisons, fungi contaminations of food and their effects on health are increasing. Lack of information of farmers and food producers about the growth and development of microorganisms and fungi leads to considerable economic damages and irretrievable diseases[1].

Environmental factors, including temperature, humidity, air compositions and general situation of the seeds are effective in spoilage of cereals [2]. Besides spoilage of flours, molds are the primary cause of deterioration of breads and pastries. Recontamination of these cereal products usually occurs after baking, mainly due to the bakery`s dust, consisting of flour particles [3]. Fungal contamination of wheat and flour has a detrimental effects on the quality of flour and it leads to proteolytic, lipolytic, and saccharolytic effects, to produce aroma decreasing metabolites and to decrease the content of gluten and eventually effecting rheological characteristics of dough [4,5]. In addition, some fungi produce secondary metabolites, which are named mycotoxins. Mycotoxins are the derivative products of acetate or amino acids, which are mainly produced by Aspergillus, Penicillium, Fusarium, Claviceps, Alternaria genus. [4]. The data of Food and Agriculture Organization (FAO) represents the fact that about 25% of foodstuffs produced worldwide are

contaminated with mycotoxins [6]. Mycotoxins are relatively heat resistant and they are not disrupted in baking process. Constant consumption of contaminated food with mycotoxins, results in serious risk of the respiratory, digestive, nervous, and other organism systems disorders and also it increases the risk of carcinogenesis, mutagenesis and teratogenesis [4, 7]. Most of the mycotoxin, producing fungi, grows in warm and wet situations [8]. Fungal contamination of flour has been subject of different investigations. Current studies suggest that major fungi species, which were found in wheat grains and wheat flour, are Aspergillus [9-12], Penicillium [12], Fusarium[9, 12, 13], Cladosporium[12, 14] and Alternaria [12]. Distribution of these kinds of fungi in nature highly depends on geographical situations. In Iran, because of various climate situations, probability of presence of extended type of these fungi in the environment is high [1]. Considering the importance of wheat in Iranian food, the aim of this study is determination of fungi contaminations and humidity percentage of flour, used in bakeries in the city of Tabriz.

MATERIALS AND METHODS

Selection and Description of Samples

Flour samples were collected with simple random method from 89 bakeries, baking different kinds of wheat bread. Five gunny-bags were selected randomly in each bakery and flour samples were collected from the middle of each gunny-bag, with a special device used for flour sampling.

Technical Information

Defining Humidity Percent: After mixing the 5 samples from each bakery, the humidity percentages of samples were assessed immediately after sampling, in accordance to the following method: First Petri dishes were put in the oven at 105 degrees Celsius for 30 minutes. After cooling, they were put in a desiccator which contained moisture absorbent. Then each Petri dish was weighed ($W_{A)}$, and 5 grams of flour measured in Petri dish to the nearest 1 milligrams (W_B). The prepared samples were put in the oven again at 105 degrees Celsius for 5 hours. They were then put in the desiccators, which contained moisture absorbent. After cooling, Petri dishes containing dried samples were weighed to the nearest 1 milligram ($W_{C)}$. Humidity percentage of the samples was calculated by standard formula [15].

Sample Culturing

Flour samples were experimented with international standard-ISO 7954-1987 General guidance for enumeration of yeasts and moulds colony count technique at 25°C. In the first step, a ¼ sterile Ringer solution was prepared and was put at 105 Degree Celsius for 5 hours. In step two, 10 grams of flour sample was dissolved in 90cc of ¼ sterile Ringer solution; then10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}and10^{-6} dilutions were prepared for each sample. In the third step, YCGA medium, prepared in accordance to its label instruction, was used for fungal culturing. Step four: 1cc of each 10^{-4}, 10^{-5} and 10^{-6} dilutions were poured in separate plates. YCGA medium was then poured on each plate. They were then shaken slowly in the form of figure 8. After coagulation of medium, plates were incubated in inverse position at 25 Degree Celsius for maximum 3 weeks. Eventually, fungi colonies were identified by standard method [16-18].

Statistics

Data analysis was carried out using the statistical package for the social sciences for windows version 11.5(SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (x ± SD). P-values of less than 0.05 were considered to indicate statistical significance. Pearson correlation analysis was used to compare the relationship between mean humidity percentage of samples and fungi contaminations percentage.

RESULTS

From 89 samples, 28 samples (31,5%) contained fungal contaminations more than 10^4 colony forming units/gram (cfu/g) of flour (allowable limit)[$p<0.05$].The fungi species identified, included Aspergillus (A.niger, A.fumigatus, A.flavus, A.glaucus, A.triticum), Acremonium, Alternaria, Fusarium, Mucor, Penicillium and cladosporium spp. But the most common fungi in cultured samples were as follows, respectively: A. niger, Fusarium, Acremonium and A. fumigatus (Figure 1).

From total fungal contamination the myco-biota of flour samples was dominated by Aspergillus spp., accounting for 50% of the

isolations. Also the mean humidity percentage of samples was12.8±0.76 which was within the standard level (≤ 13%) [p=0.00].

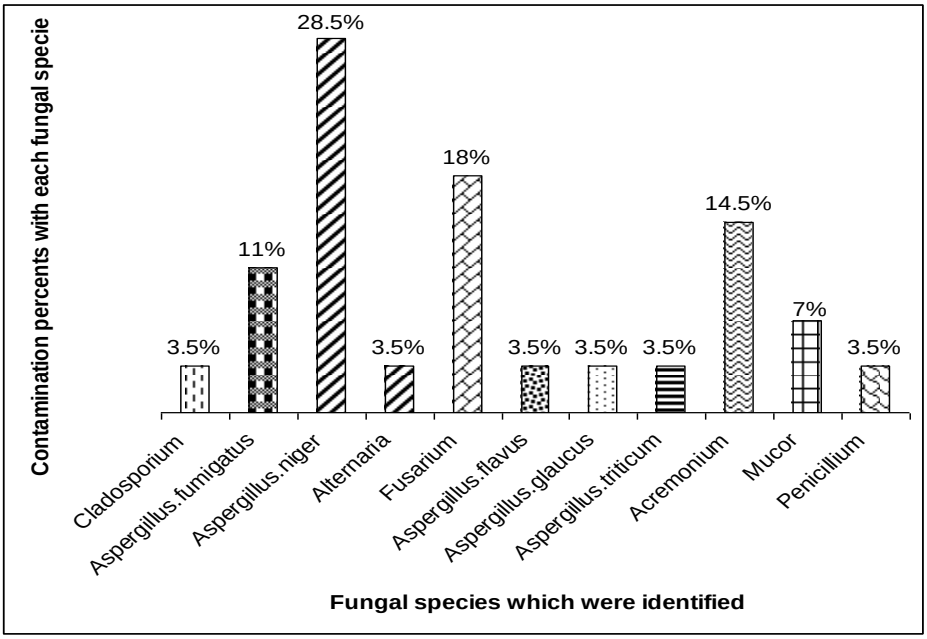

Figure1. Contamination percent of different types of fungi in flours

DISSCUSSION

Findings of this study suggest that fungal contamination of wheat flour was considerable (31.5%) and major fungal genera was Aspergillus (especially A.niger and A.fumigatus spp.), Fusarium and Acremonium. Mean humidity percent of flour samples was 12.8±0.76 which was within the standard range. Surveys suggest that inhabitant effect of cereal seeds` humidity on microbial activities is ≤ 13% [19]. On the other hand, the correlation between mean of humidity percentage and fungal contaminations percentage of samples was not significant (P>0.05). Hence, It seems that in this study other undesirable situations such as high temperature, contamination of the bakeries` flour stocks or silos of industries, microbial spoilage of seeds or contamination in production process may be effective on increasing the fungal contaminations of wheat flour and those factors may finally lead to the production of mycotoxins [1].On the other hand, 75% Micromycetes isolated from flour samples are mycotoxin producing fungi. With respect to bread, which is one of the staple foods of Iranian people, consumption of these flour samples may lead to extensive clinical effect on health as pointed out before.

The result of this study seems to be consistent with other related studies. In a study performed in Egypt [10], the most isolated fungi species were Aspergillus species. Also Okhovat & Zakeri [12], assessed the contamination of wheat varieties which was imported into Iran. In this study, imported wheat varieties used for backing bread was evaluated for the presence of fungal diseases in the silos. The results showed the presence of pathogenic fungi such as Ulocladium, Cladosporium, Alternaria, Rhizopus nigricans, Penicillium and Trichothecium, Mucor, R. nigricans, Penicillium and Aspergillus species. In the other study, Aspergillus and Fusarium species were seen in flour samples of India [9]; also, Sholenberg et al [11], investigated that the major fungal contaminations of flour were Aspergillus species. Another survey was undertaken to determine the microbiological status of Australian wheat and the distribution of microorganisms in the flour milling sections. The most common isolated molds were Aspergillus, Penicillium, Cladosporium and Eurotium species [14]. In another research, whole wheat flour and white wheat flour (type 405) were investigated for their total qualitative as well as quantitative mycobiota. The

mycobiota of both types of flours was dominated by Aspergillus species on a major degree and fungi of the genus Penicillium species occurred only to a minor degree, Aspergillus candidus was the most frequently encountered mold. Penicillium aurantiogriseum, Cladosporium cladosporioides, A. flavus, Eurotium herbariorum, P. griseofulvum, P. brevicompactum and P. viridicatum were isolated to a lesser degree. 93.3% species belong to the group of toxigenic molds [3]. Another investigation was undertaken by Mashinini and Dutton [13] to survey the fungal and mycotoxin contamination of South African wheat ranging from that of growing in the field to the processed wheat products. The major fungal contaminants were Fusarium spp. Comparison of our study's result with these studies suggest that fungal contamination of flour samples are relatively similar.

CONCLUSION

Base on the results of the current study and considering the fact that wheat is main staple food of Iranians, attention and follow up in all steps of production, distribution and maintenance of wheat and flour with the aim of minimizing fungi contaminations is necessary.
Some advices that can be effective on prevention of flour and bakery products from microbial contamination are including as following:

1.precise sanitary control in all steps of production, transportation, packaging, personnel sanitation and preservation of wheat and flour in silos, Temperature of silos must be kept under 25 ºC, because the temperature range of 25–30ºC is favorable for the development of micromycetes [7].
2.Relative humidity of the stock, the flour in silos or in bakeries, should be less than 65% [1, 2]. Changes in moisture contentsof 1 or 2% may be sufficient for growth and mycotoxin contamination [3].
3.Decreasing spoilages of flour caused by diseases, damage of wheat grains, rodents weed seeds [1, 2]. Mold spores present in flour survive for several years; therefore care should be taken in the storage of flour [3].
4.In production of certain foodstuffs and preparations of foodstuffs, usage of mill products with low microbial germ content is necessary [20].

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