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SURVEY OF PEANUT FUNGAL CONTAMINATION AND ITS RELATIONSHIP WITH AMBIENT CONDITIONS IN THE BAZAR OF ZANJAN

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ABSTRACT

Different food products such as corn, wheat and peanut have shown high potential to be contaminated in suitable environmental conditions, such as temperature and humidity. Some fungi can produce toxins, like Aflatoxin, and some of them are carcinogen. The purpose of this research was to study fungal contamination in peanuts being sold in the BAZAR of the city of Zanjan. 20 samples of 50g roasted and salted peanuts and 16 samples of 50g unsalted peanuts (pure) were collected from Zanjan BAZAR. Ambient conditions such as light intensity, temperature and air flow of the BAZAR and also the relative humidity of peanuts were measured. Samples were analyzed for fungal colony and were identified using slide culture technique. Results showed that Mold fungi were dominant among the colonies as. *Aspergillus* flavus (39.1%), *Penicillium* (9.2%), *Rhizopus* (7.2%), *Mucor* (2.5%), *Alternaria* (1.03%) and *Nigrospora* (0.5%). Temperature and the samples relative humidity were higher than the standard range. The results indicated a significant relation between relative humidity, light intensity, temperature and peanuts' type (pure or salted) with level of fungal contamination. Also, roasting and processing reduced the relative humidity of peanuts and the level of contamination. Hence roasting, salting and provision of appropriate ambient conditions can be useful to peanut storage

Key words: Peanut, Fungi, Mold, Zanjan, Ambient conditions

INTRODUCTION

Peanut is a known product which has widespread usages in making foods and production of oil. Furthermore, in Iran it is used as a nut. Peanut is full of fat and protein; besides its super potential as a food, in suitable conditions of humidity, light, temperature and air flow for fungi growth, it can be contaminated by toxin (Mycotoxin) producer fungi. In a recent study (Khomeiri *et al*., 2008) it was shown that respectively 58.23%, 46.67% and 36.9% of collected peanut samples from Golestan, Mazandaran and Gilan provinces of Iran were contaminated by *Aspergillus* flavus. The contaminated peanuts with *Aspergillus* flavus and other fungi can threat human's health by producing Aflatoxin, Ochratoxin and other toxins (Rivka, 2008). Aflatoxicosis is the disease that is caused by Aflatoxin. Aflatoxins may cause livers acute cirrhosis and necrosis (Mortazavi and Tabatabaei, 1999). Acceptable limit of Aflatoxin by FDA standard in food products is 5 µg/kg (FDA,1997).

Many other studies have shown that various foods, fruits and corns can be contaminated by fungi. For example respectively 73% and 31.5% of tea and flour samples have had fungal contaminations higher than standard in Tabriz (Kazemi *et al*., 2008). In another study in Kermanshah 23% of waste breads were contaminated by toxic fungi (Pasdar *et al*., 2000). In many cases, the toxins were also measured along with the contaminating

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fungi. In a study in Argentina (Vaamonde *et al*., 2003), 94% of extracted *Aspergillus* flavuses had production of cyclopiazonic acid. In another study in Cordova-Argentina, all of samples had been contaminated by *Aspergillus* niger and Ochratoxin A had been extracted from them (Magnoli and Astoreca, 2007).

Peanut is used as an animal food. In a study in India, Aflatoxin and other fungal toxins in poultry food (such as peanut) was surveyed, In which 38% of the samples were contaminated by Aflatoxin (Mayo *et al*., 2002). Furthermore, some studies have shown a relationship between fungal contamination and ambient conditions. Nakai found that the growth of *Aspergillus* flavus is mainly influenced by temperature and relative humidity (Nakai *et al*., 2008). Also, the predominant genera in peanuts of São Paulo were *Fusarium* spp. and *Aspergillus* spp. These studies showed that different genera of fungi are predominant contaminants.

In this study, the fungal contaminations of Zanjan market's peanuts and some ambient conditions of the storage and selling places were studied in order to find the relationship between them. Surveying fungal contamination of peanuts, ambient conditions of storage and their relationship can be useful in controlling fungal contaminations of peanuts, decision making about its using limitations and optimization of selling and storage places.

MATERIALS AND METHODS

Sampling

In this research, 20 samples unpackaged 50g of unpackaged salted peanuts (roasted and salted) and 16 samples of 50g unsalted peanuts (pure) were randomly collected from about 30 markets in Zanjan BAZAR. (there were only 16 markets as sellers of pure peanut). Separately, the samples were preserved for humidity analysis. All of the samples were taken in sterilized pockets.

Ambient conditions

Ambient conditions such as light intensity, temperature and air flow velocity were measured in the BAZAR of Zanjan. Light intensity was measured by TES-1339 light meter Prova; temperature was measured by thermometer and air flow was measured by means of Alnor RVA801 Digital Vane Anemometer in each peanut sampling point in the BAZAR.

Relative humidity

The samples, after being smashed and weighed , were put in 105°C for a time until their weight became approximately fixed, and the relative humidity was calculated based on the weight loss (Csuros, 1997).

Fungal contamination

In fungilogy laboratory of Zanjan University of Medical Sciences, 5 peanuts were selected randomly from each 50g sample and cultured by direct culturing in plates and then conserved at 27-30°C for a week. The applied media culture was sabouraud dextrose agar plus chloramphenicol and a blank culture plate was lodged concomitant with sample plates. The plates were monitored for fungal colony and in case of fungal growth, the genera were detected by direct observation (Well Mount method). Slide culture technique (under microscope) was applied to identify fungi genera, in the cases which diagnosis was not possible by direct observation.

Statistical analysis

Pearson correlation coefficient was used to evaluate the relationship between peanuts type (salted and pure), ambient conditions (light intensity, temperature and air flow velocity), peanuts relative humidity and fungal contamination. The T-Test analysis was applied to determine the significance of differences between peanuts types in various ambient conditions and their fungal contamination.

RESULTS

194 colonies were extracted from 20 salted and 16 pure peanut samples. *Aspergillus* niger and *Aspergillus* flavus, respectively with 38.6% and 39.1% of colonies, were the most dominant genera; no fungal growth was seen in blank culture. Contamination by *Aspergillus* flavus in pure peanut samples (93.7%) was more than salted samples (60%). While, contamination to *Aspergillus* niger in salted samples (90%) was higher than pure samples (62.5%).

9.2% of colonies belonged to *Penicillium*. Contamination by this fungus in salted samples (with 40% of samples) was higher than pure samples with (6.2%). 7.2% of colonies belonged to *Rhizopus.* Contamination by this fungus did not show significant difference between pure and salted samples. 40% of salted and 37% of pure samples were contaminated by this fungus. only 2 colonies of *Nigrospora* were extracted from one of the salted samples and 2 colonies of *Alternaria* were extracted from one of pure samples. *Mucor* included 2.5% of colonies. only one of salted samples was contaminated by *Mucor* and more contamination by this fungus was observed in pure samples. *Absidia* was seen in only one pure sample and contamination by *Aspergillus* spp. was seen in one of the salted samples. Table 1 shows the number of isolated colonies from each type of peanut.

The ambient conditions of sampling points, measured in this study are presented in Table 2.

Table 1: Number of isolated colonies in pure and salted samples

	Absidia	Asp.niger	Asp.flavus	Asp.spp	Mucor	Penicillium	Alternaria	Nigrospora	Rhaizopus
Pure	1	28	47	0	4	2	2	0	6
Salted	0	47	29	1	1	16	0	2	8

Table 2: Ambient conditions of peanuts sampling places in the BAZAR of Zanjan

	Relative humidity (%)			Temperature (°C)			Light intensity (Lux)			Air flow velocity (m/s)		
	Average	STD	Range	Average	STD	Range	Average	STD	Range	Average	STD	Range
Salted peanuts	5	1.02	3.11-6.87	29.1	1.25	27-31	757.5	410	230-1600	0.16	0.156	0-0.45
Pure peanuts	6.81	0.94	5.68-8.06	23.9	3.51	21-30	51.1	103.82	0-260	0.01	0.02	0-0.05

Most of pure peanuts' storage places were dark and without measurable light. Statistical Analysis showed that there was significant correlation (P-value≤0.05) between peanut type and contamination by *Aspergillus* flavus. Contamination by this fungus increased in pure samples. Also, T-Test analysis showed a significant difference (P-value=0.012) between pure and salted samples in contamination by *Aspergillus* flavus.

Significant correlation between relative humidity and contamination by *Aspergillus* flavus was observed and with increasing of relative humidity, contamination by *Aspergillus* flavus increased.

There was a positive significant correlation between contamination by *Aspergillus* flavus and temperature in salted peanuts.

A significant correlation between type of peanut and contamination by *Penicillium* showed that contamination by *Penicillium* increased in salted peanuts. The difference between two types of peanuts was significant (T-Test P-value=0.023).

There was a positive significant correlation between contamination by *Rhizapus* and temperature in salted peanuts.

The results showed a positive significant correlation between air flow velocity, contamination by *Aspergillus* spp and *Nigrospora*.

In most cases there was negative correlation between light intensity and fungal contaminations, but they were not in significant range of $\alpha<0.05$.

Figs.1 and 2 show the percentage of salted and pure peanuts samples that had been contaminated by each fungus.

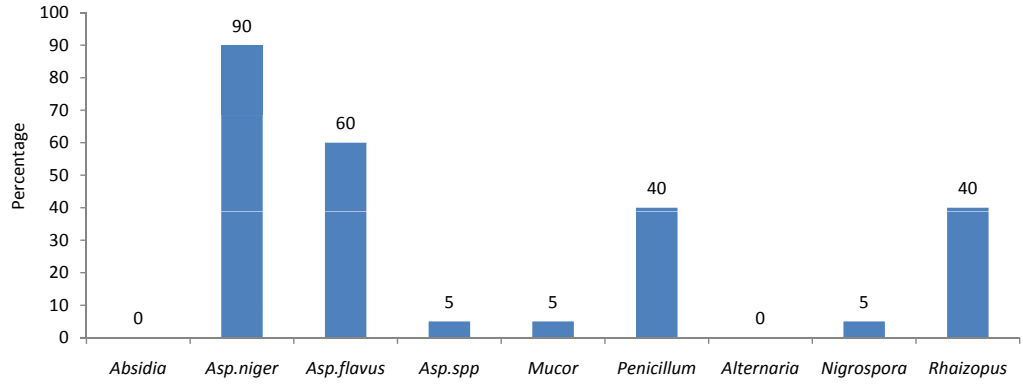

Fig. 1: Percentage of fungal contaminations in salted peanut samples

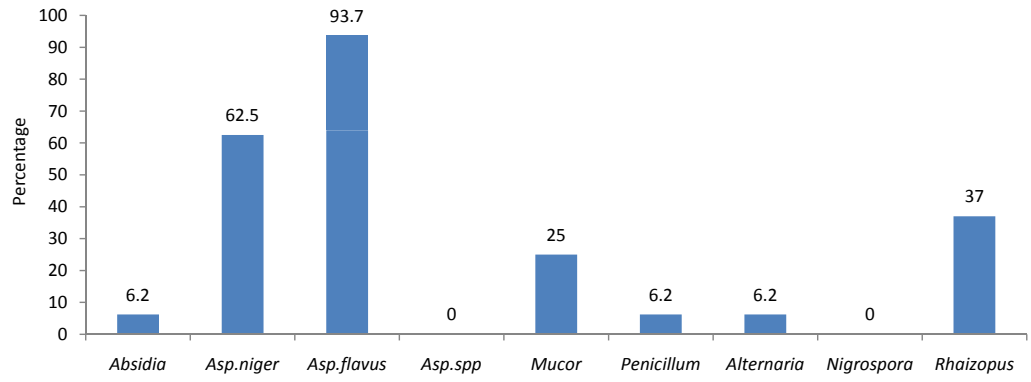

Fig. 2: Percentage of fungal contaminations in pure peanut samples

DISCUSSION

The obtained results show that the dominant fungal contamination of peanuts was *Aspergillus* flavus, which include 60% of salted peanut samples and 93.7% of pure samples. Contamination by this fungus in pure samples was closer to the peanut productin Thailand (Pitt *et al*., 1993) with 95% of contamination by this fungus. Contamination by *Aspergillus* flavus in Argentina (Vaamonde *et al*., 2003) with 69%, was more than salted and less than pure peanut samples' contamination in Zanjan.

The results of another study on stored peanuts fungal contamination in Sao Paulo (Nakai *et al*., 2008), are shown in Table 3 in comparison with the BAZAR of Zanjan.

The Figs. in this Table show the percent of samples that had been contaminated by each fungus.

Table 3: Fungal contamination of Sao Paulo and Zanjan BAZAR(%)

Sao Paulo		Zanjan's BAZAR	
Fungal contaminant		Pure	Salted
Aspergillus flavus	21.8	93.7	60
Fuzarum	25.8	-	-
Penicillum	1.6	6.2	40
Rhizopus	4.8	37	40
Aspergillus niger	0.6	62.5	90
Drechslera	0.05	-	-
Thericoderma	0.9	-	-

Drechslera, *Fuzarum* and *Thericoderma* were not seen in this study and other fungal contaminations of Sao Paulo are less than the BAZAR of Zanjan peanuts. This can be a result of suitable conditions for growth of fungi in the BAZAR of Zanjan such as high temperature (especially in salted peanuts), high relative humidity (especially in pure peanuts), low light intensity in pure peanuts

storage and adequate air flow for traveling the fungi and growth of aerobic fungi. Also these results show that, contamination by *Aspergillus* flavus in Golestan, Mazandaran and Gilan respectively whit 58.23%, 46.67% and 36.9%, were less than the BAZAR of Zanjan.

Contamination by *Aspergillus* flavus in pure and roasted peanut samples respectively, were 19% and 51% for the three provinces (Khomeiri *et al*., 2008), which are different from our results, because in Khomeirie's study the pure peanuts have had hull which was not the case in our study. Also Chun that had shown 10.6% of peanut samples contained Aflatoxin and many of of them were roasted (Chun *et al*., 2007).

It seems that the hull may act as a shelter from fungal contamination. As the hull is not comestible for man and occupies space in storage. it is often the hull removed after pickup of peanuts. The results showed a positive significant correlation between ambient temperature and contamination by *Aspergillus* flavus in salted peanuts. This relationship states that probability of contamination by *Aspergillus* flavus in salted peanuts, which had high storage ambient temperature, was more than those with lower storage ambient temperatures. Also there was a positive correlation between relative humidity and contamination by *Aspergillus* flavus; this relationship was also observed in Sao Paulo peanuts (Nakai *et al*., 2008).

The significant difference between contamination by this fungus in pure and salted peanuts showed that contamination by *Aspergillus* flavus in pure peanuts was more than salted ones, which had higher ambient temperature than pure ones. Therefore it seems that reducing relative humidity, especially in pure peanuts, many result in decrease of contamination to this mold fungus, and to reduct contamination by this fungus, keeping lower relative humidity has priority to ambient temperature.

Contamination by *Aspergillus* niger for Thailand's peanuts has been 86% (Pitt *et al*., 1993) which is less than Zanjan's salted peanuts with 90% contamination and more than Zanjan's pure peanuts with 62.5% contamination.

Peanuts contamination by *Penicillium* in Thailand's study (Pitt *et al*., 1993), with 46%, was more than pure and salted peanuts' contamination by this fungus in Zanjan BAZAR's samples, respectively with 6.2% and 40%.

Results of this study showed a significant correlation between salted peanuts and increasing of contamination by *Penicillium*. This fungus can produce Patulin and Penicillic acid. Patulin can cause toxicosis in embryo, but it can not cause malformation in embryo. Researches have shown that oral consumption of Petulin can cause cancer more than injection (Mortazavi and Tabatabaei, 1999). Penicillic acid that often contaminates flour and corn is carcinogenic and can make mutation in concentrations higher than 10 M/L(Egmond and Jonker, 2008).

The average storage temperature of peanuts in this study was higher than standard (18-20 ^{o}C) and this is suitable for fungi growth (Khajepur, 1995).

There was a significant correlation between contamination by *Rhizopuse* and ambient temperature.

The average relative humidity of pure peanuts samples was 6.8% and for salted peanuts' samples was 5%; this relative humidity particularly in pure peanuts is over than standard range of 5%, (Khajepur, 1995) and fungi can grow in these condition. According to the results, roasting and salting the peanuts reduced some contaminations and also reduced the relative humidity of peanuts, providing better condition for peanuts storage. Also roasting of peanut is profitable for inhibition of its oil oxidation (Radfar *et al*., 2003). These high levels of contamination by *Aspergillus* flavus have the risk of Aflatoxin production, which is very important because of its carcinogenic risk, hence more control is needed for storage and selling of peanut.

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