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# Detection of Fungi Spectrum in Industrial and Home Bakeries and Determinated Fungal Allergy with Skin Prick Test

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**SUMMARY** Airborne fungal pathogens such as *Penicillium*, *Aspergillus*, *Cladosporium*, *Trichophyton*, and *Alternaria* may cause health problems. In this research, the fungal flora at different bakeries and their potential allergenic effects on the workers were investigated. We investigated 148 workers at 17 industrial type bakeries and 62 workers at 17 home type bakeries in Afyon. Our study was performed in two different seasons and climates, between January 2004 and June 2004. Fungal flora was detected by using Petri-dish method. In the winter, *Penicillium* was the dominant genus, while *Cladosporium* was the dominant genus during the summer, in both types of bakeries. The allergenic properties of dominant culturable fungi on workers involved in the bakeries were determined with the skin-prick test. It was found that with workers in the industrial type bakeries, the most common skin test positivity was caused by *Penicillium*. In the other hand, the skin test positivity, performed on workers in the home type bakeries, was equally caused by *Penicillium*, *Trichophyton* and *Aspergillus*.

Fungal allergen exposure is generally considered to arise from outdoor environment. However, *Penicillium* and *Aspergillus* species are seen at greater rates in the indoor air rather than the outdoor air.[1] Fungi in the air have been proposed as a cause of increasing adverse health effects. Many fungi have been reported to cause several types of human health problems, such as infections, allergies, atopic dermatitis as well as toxic effects.[2,3] More than 80 genera of the fungi have been associated with respiratory tract disorders.[4] Several studies have demonstrated that there is a relationship between increased spore counts and fungal antigen levels with the presence of allergic symptoms.[5,6] In order to systematically evaluate the relationship between airborne fungi and adverse health effects, the fungal types and their relative frequencies in both indoor and outdoor air need to be known. Epidemiological, environmental, and clinical researches were focused on relevant species, like *Alternaria, Aspergillus, Cladosporium*, and *Penicillum*.[7] It has been demonstrated that fungal spores are present in high concentration in the air of

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working places including bakeries.[8-10] In the past it has generally been assumed that all work-related symptoms in bakeries have an allergic origin.[11] Platt *et al.*[12] showed that occupants of wet, moldy buildings had more subjective complaints. There have been many studies of moist and mold-damaged buildings. Damp buildings often have a moldy smell or obvious mold growth. Some molds are human allergens and may therefore give rise to adverse health effects.[13]

Many kinds of fungi are commonly found in indoor environments, especially those where organic material is handled and where relative humidity is at least locally elevated.[14] Species of *Cladosporium*, *Penicillium*, *Aspergillus*, and *Alternaria* (among others) have been identified as readily able to grow and sporulate in sites such as food storage containers, humidifier reservoirs, and other damp indoor environments.[15]

The objective of the present study was to investigate the airborne fungal concentrations in industrial and home type bakeries, as well as the allergenicity of dominant culturable fungi on the people who are occupationally involved in the bakeries. Since many fungi are potential allergens, this study has provided us with the basis for future work on health effects. It is anticipated that this study may assist in the protection of workers from some aspects of occupational allergic diseases.

## MATERIALS AND METHODS

### Study design

We investigated 17 industrial bakeries and 17 home bakeries in Afyon. One hundred and forty-eight subjects were working in industrial bakeries and 62 were in home bakeries (Table 1). This study was carried out over two different seasons (winter and summer). The selected bakeries were, in similar working conditions; situated in the center of the city, hence made it easier for the people to shop.

Each worker in the bakeries studied was given a questionnaire asking questions about their personal details and if they had any problems in their respiratory system. Those who took any medication in a week period for flu, allergic problems, immunity and other problems such as diabetes mellitus, chronic renal failure, malignancy, AIDS, etc., were excluded from this study.

Local Ethical Committee of the University of Afyon Kocatepe has approved the study and all the participants gave an informed consent.

### Description of the selected bakeries and associated characteristics

There are two different types of bakeries in Afyon. The first types work by electricity and are

**Table 1** Demographic characteristics of the subjects

| Predictors | Industrial type bakery | | Home type bakery | |
|---|---|---|---|---|
| | N | % | N | % |
| **Gender** | | | | |
| Male | 144 | 97.3 | 16 | 25.8 |
| Female | 4 | 2.7 | 46 | 74.2 |
| **Age distribution** (years) | | | | |
| < 30 | 80 | 54.1 | 30 | 48.4 |
| > 30 | 68 | 45.9 | 32 | 51.6 |
| **Education distribution** | | | | |
| Secondary and university | 82 | 55.5 | 24 | 38.8 |
| Primary | 66 | 44.6 | 38 | 61.3 |
| **Smoking** | | | | |
| Never | 46 | 31.1 | 28 | 45.2 |
| Former | 12 | 8.1 | 2 | 3.2 |
| Current | 90 | 60.8 | 32 | 51.6 |

called industrial type bakeries. The second types are known as the 'home type' bakeries, where cooking and baking are made at home using firewood.

**Evaluation of participant's health**

The aim was to collect the information about the participant's respiratory and allergic symptoms.[16] In this article, we specifically examined the workers both upper and lower respiratory systems. Workers were questioned for their symptoms such as cough, phlegm, wheezing anamnesis, diagnosed asthma, and allergic rhinitis.

**Skin-prick test**

The skin-prick test was performed on the ventral side of the forearm. After cleaning the forearm with alcohol, 0.01 ml to 0.02 ml of antigens (1:50 dilution) were placed in a parallel array to the axis of each sterile forearm at a distance of two centimeters apart. The Stallerpoints device was used to perform the prick test. Sites were then pricked with No. 26 hypodermic needles. The five solutions of *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Trichophyton* were used according to a blind protocol. An allergenic extract is considered to have a value of 100 index of concentration (IC)/ml when the manufacturing parameters result in the same mean dilution ratio as that of standardized extracts from the same group with a value of 100 IR/ml and which are used as reference standards. For groups not having a standardized reference extract, a value of 100 IC/ml corresponds to an extract for which the dilution ratio has been established by clinical experience (Stallergenes SA, Antony, France). Prick tests were also done with a positive control to confirm the state of reactivity of skin. Histamine diphosphate (1 mg/ml) and buffered saline diluent were used as positive and negative controls, respectively. The results were recorded after 20 minutes and allergic reactions were graded as '+', '++', '+++', '+ + + +', and '-' on the basis of intensity of reactions, erythema or wheal formation.[17]

**Sampling procedures**

The Petri Gravitational Settling method was employed for the isolation of airborne fungi because of its practical usage and low cost. Malt Extract Agar (MEA) (Sigma M8927, Germany), Potato Dextrose Agar (PDA) (Sigma P2182, Germany), and Rose Bengal Agar (RBA) (Fluka 17211, Basingstake, Hampshire, England) media in petri dishes were exposed to the air at the height of 1.5 meters above the ground for 15 minutes. The specimens were collected 6 times, from January to June. Cultures were incubated at room temperature (27 ± 2°C). Plates were firstly inspected after 4 days of incubation and it continued up to 14 days.[18,19] Mould colonies were transported to tubes containing MEA and PDA. Fungi were identified to genus level using the set of dichotomous keys and a set of picture keys.[20]

**Measurement of Moisture**

Moisture in the bakeries were measured by Barigo-% Normal hygrometer A.

**Statistical analysis**

Data were analyzed with SPSS 10.0 software. The results of allergic reactions were analyzed with $\chi^2$ test and continuous variables by Student's t-test where appropriate. A *p*-value less than 0.05 was considered as statistically significant.

## RESULTS

There were 144 male and 4 female workers in the industrial type bakeries, whereas there were 16 (25.8%) male, and 46 (74.2%) female workers in the home type bakeries. Most of the subjects graduated from primary school and had similar smoking anamnesis in both groups. Symptoms were found statistically not to be different in the two types of bakeries (Table 2). The mean age of the workers in the industrial type and home type bakeries were 19.22 ± 16.79 and 37.61 ± 27.13, respectively. The mean working duration was 8.36 ± 9.47 years in industrial and 8.58 ± 8.58 years in the home type bakeries (Table 3). No correlation was found between symptoms, skin test positivity and fungal concentration in air in both groups.

The workers who underwent the skin test in the industrial type bakeries, showed positive results with *Penicillium* having the highest frequency, followed by *Cladosporium* and *Trichophyton*. The fourth and the fifth were *Alternaria* and *Aspergillus* which were apparent especially in the industrial type

**Table 2** The percentages of the symptoms in the groups

| | Industrial type bakery | | Home type bakery | |
|---|---|---|---|---|
| | N | % | N | % |
| **Cough** | 28 | 18.9 | 12 | 19.4 |
| **Phlegm** | 12 | 8.1 | 4 | 6.5 |
| **Wheezing** | 24 | 16.2 | 8 | 12.9 |
| **Allergic rhinitis** | 24 | 16.2 | 14 | 22.6 |
| **Asthma** | 8 | 5.4 | 4 | 6.5 |

**Table 3** Characteristics of the buildings

| | Winter | | Summer | |
|---|---|---|---|---|
| | Industrial type bakery (winter) | Home type bakery (winter) | Industrial type bakery (summer) | Home type bakery (summer) |
| **Humidity** | 52% | 40% | 68% | 50% |
| **Temperature (°C)** | 28 | 22 | 35 | 30 |
| **Age of building** (years) | 19.22 ± 16.79 | 37.61 ± 27.13 | | |
| **Working years** | 8.36 ± 9.47 | 8.58 ± 8.58 | | |

**Table 4** Number of patients and their percentages showing skin test positivity

| Fungus | Industrial type bakery | | Home type bakery | |
|---|---|---|---|---|
| | N | % | N | % |
| ***Penicillium*** | 16 | 10.8 | 4 | 6.5 |
| ***Cladosporium*** | 12 | 8.1 | 2 | 3.2 |
| ***Trichophyton*** | 12 | 8.1 | 4 | 6.5 |
| ***Alternaria*** | 6 | 4.1 | 2 | 3.2 |
| ***Aspergillus*** | 2 | 1.4 | 4 | 6.5 |

bakeries (Table 4). In the home type bakeries, *Penicillium*, *Trichophyton* and *Aspergillus* were seen in same frequency. They were followed by *Cladosporium* and *Alternaria*.

In this study, 972 colonies of fungi were isolated from 34 different bakeries' indoor air (17 in industrial type bakeries and 17 in home type bakeries) between January and June 2004. In the study, *Moniliales* has been seen in most of the fungi. Excess colony numbers were *Penicillium*, *Cladosporium* and *Aspergillus*, respectively. *Penicillium* was the most occurring organism found in both bakeries' air, followed by *Cladosporium*, *Aspergillus* during the winter (Table 5). However, during the summer, *Cladosporium* was found in highest concentrations. Statistical analysis revealed no significant differences among the groups ($p > 0.05$).

## DISCUSSION

In occupational environments, including bakeries, workers may be exposed to a high concentration of airborne fungal spores.[17] Health effects are associated with exposure to fungal spores and considered to be genus-specific. While *Penicillium* is associated with occupational asthma at food processing plants, *Alternaria* and *Aspergillus* are associated

**Table 5** Rates of occurrence of fungal spores isolated from bakeries

| | Winter | | | | Summer | | | |
|---|---|---|---|---|---|---|---|---|
| | Industrial type bakery | | Home type bakery | | Industrial type bakery | | Home type bakery | |
| | N | % | N | % | N | % | N | % |
| ***Penicillium*** | 107 | 31.2 | 96 | 52.2 | 75 | 25.7 | 56 | 28.0 |
| ***Cladosporium*** | 65 | 21.6 | 50 | 27.2 | 86 | 29.5 | 75 | 37.5 |
| ***Aspergillus*** | 26 | 8.8 | 18 | 9.8 | 39 | 13.4 | 20 | 10.0 |
| ***Alternaria*** | 16 | 5.4 | 2 | 1.1 | 17 | 5.8 | 7 | 3.5 |
| ***Geotricum*** | 15 | 5.1 | 2 | 1.1 | 10 | 3.4 | 5 | 2.5 |
| ***Trichophyton*** | 12 | 4.1 | 2 | 1.1 | 13 | 4.5 | 2 | 1.0 |
| ***Mucor*** | 17 | 5.7 | 3 | 1.6 | 11 | 3.8 | 12 | 6.0 |
| ***Fusarium*** | 6 | 2.0 | 6 | 3.3 | 3 | 1.0 | 2 | 1.0 |
| **Others** | 32 | 10.8 | 5 | 2.7 | 38 | 13.0 | 21 | 10.5 |
| **Total** | 296 | | 184 | | 292 | | 200 | |

with the same problem in flour mills, bakeries as well as food processing plants. Moreover, many common fungi are associated with hypersensitivity pneumonitis.[14]

Bread is produced in home and industrial type bakeries in Afyon. Both of the bakeries have high heat and moisture which are suitable environments for growing fungal spores. The study presented here examined, simultaneously, the prevalence of SPT positivity to fungi, allergic symptomatology and the presence of mold spores in bakeries. In both types of bakeries, *Moniliales* (*Penicillium*, *Aspergillus* and *Cladosporium*) has been observed to be the most occurring mold fungi. *Penicillium* was the most occuring organism in both types of bakeries, and followed by *Cladosporium* and *Aspergillus* during the winter. In contrast, *Cladosporium* has the highest concentration during the summer in both bakeries. Levy has reported similar results consistent with our findings. *Penicillium* was the dominant genus in winter, while the dominant genus was *Cladosporium* during the summer.[21] The incidence of allergenically significant fungal aerosol in a rural bakery of west Bengal, India, was studied by Atin.[17] It was found that the dominant fungus species, both culturable and non culturable, were *Aspergillus*, *Penicillium*, and *Cladosporium* spp.[17] Temperature and relative humidity played an important role in fungal spore generation, by releasing and dispersing them in both indoor and outdoor environments.[22-26] Although the age of a building in home type bakeries was higher than the industrial type bakeries, the fungal concentrations in the air were found not to be higher than that of industrial type. The reason was that, in industrial bakeries, cooking is carried out by using steam and electrical systems, hence the air inside is hot and moist. *Aspergillus* was found to be in third rank in all the fungi collected during summer and winter in both bakeries. Spores of *Cladosporium* spp. occurred more abundantly worldwide than any other spore types and were the dominant airborne spores in many areas, especially in hot climates.[4] Our study has shown that *Cladosporium* was the most frequent fungus in the summer in both bakeries.

The rate of positively skin test was 20% in industrial type bakeries and 12% in home type bakeries. Worldwide skin test results suggest that at least 3% to 10% of adults and children are affected by fungal allergy.[27] Approximately 3% of the patients in Portugal and 20% of the patients in Spain had positive skin test reaction to either *Alternaria* or *Cladosporium* species.[28] In our research, it was observed that *Penicillium* caused the most frequent skin test positivity, followed by *Cladosporium*, *Trichophyton*, *Alternaria*, *Aspergillus*, respectively. In home type bakeries, *Aspergillus*, *Penicillium* and *Trichophyton* were the most allergens. Singh *et al*.[29] showed that different species of *Aspergillus* are of potential allergenic significance in bakery workers and the general atopic population. Although the

mechanisms involved are not totally understood, they include allergic, infectious and toxic reactions.[4] Gergen *et al.*[30] showed that 3.6% of the population was sensitized to the fungus *Alternaria*, after performing a general population epidemiological survey in the United States.[30] Our study showed that the rate of *Alternaria* skin test positivity was 4.1% in industrial type bakeries and 3.2% in home type bakeries. This result reinforces the well-known observation that the results of SPT to moulds require interpretation. Improved knowledge of the epidemiology and mechanism behind fungal induced human disease will hopefully establish this causal link and suggest methods for reducing morbidity.[31] Since extrinsic agents cause allergic diseases, environmental control measures are often recommended to reduce exposure to the offending allergens.[32] The number of fungal spores were found to be high in industrial type bakeries in relation with the rate of moisture, while there was no effect on asthma and related symptoms.

This study demonstrated that bakeries contain significant amounts of viable moulds of the genera known to be allergenic. Fungal allergens differed in the seasons of winter and summer in both industrial and home type bakeries. However, there was no difference between the two types of bakeries in the detection of fungal spectrum and fungal allergy. This conclusion has important implications both for regulatory authorities, who are interested in setting exposure limits and also for bakery management with respect to the future targeting of control measaures to prevent the development of sensitisation in bakery employees.

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