

DETECTION OF AIRBORNE PSYCHROTROPHIC BACTERIA AND FUNGI IN FOOD STORAGE REFRIGERATORS

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ABSTRACT

The purpose of this study was to determine the microbiological air quality (psychrotrophic bacteria and airborne fungi) and distribution of fungi in different types of ready-to-eat (RTE) food-storage refrigerators (n=48) at selected retail stores in the city of Edirne, Turkey. Refrigerators were categorized according to the type of RTE food-storage: meat products, vegetables, desserts, or a mix of food types. Microbiological quality of air samples was evaluated by using a Mas-100 Eco Air Sampler. Four refrigerators (all containing meat products, 8.3%) produced air samples with undetectable microorganisms. The highest detected mean value of airborne psychrotrophic bacteria and fungi was 82.3 CFU/m³ and 54.6 CFU/m³, respectively and were found in mixed-food refrigerators. The dominant airborne fungal genera found were *Penicillium* (29.0%), *Aspergillus* (12.0%), *Mucor* (9%), *Cladosporium* (8%), *Botrytis* (7%), and *Acremonium* (6%). By definition, RTE food does not undergo a final treatment to ensure its safety prior to consumption. Therefore, ensuring a clean storage environment for these foods is important to prevent food-borne disease and other health risks.

Key words: Airborne fungi; psychrotrophic bacteria; ready-to-eat food; air sampler; refrigerator

INTRODUCTION

The microbiological quality of air is very important to ensure the safety and quality of food during both production and storage (5). Airborne microorganisms can arise from many sources including air-conditioning systems, raw materials, and specific food production systems (3) and commonly include the propagules of micro- and macrofungi (31) along with bacteria and their resting spores. Airborne microorganisms can be

electrostatic and have been shown to stick to and deposit on surfaces (48) and growth of these organisms can cause contamination in the air of refrigerators via aerosol formation (40). However, the extent to which airborne microorganisms inside food-storage refrigerators contribute to food contamination is largely unknown.

Temperature is an important factor in the growth of microorganisms. The practice of storing food at refrigeration temperature is common for controlling the growth of

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psychrotrophic microorganisms, some pathogens, and maintaining product quality (34). Nevertheless, fungi and psychrotrophic bacteria are commonly associated with spoilage of food at refrigerator temperatures. Fungi predominate in refrigerated food spoilage when low water activity, high acidity or packaging conditions select for their growth over bacteria in foods. Psychrotrophic fungi are also commonly isolated from refrigerated fresh animal products, fruits and vegetables (3), and ready-to-eat foods. Mould genera that have psychrotrophic species include *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Fusarium*, *Geothricum*, *Monascus*, *Mucor*, *Penicillium*, *Rhizopus*, and *Trichothecium*. Among the yeast genera involved are *Candida*, *Debaryomyces*, *Saccharomyces*, *Torulopsis* (14, 24). Therefore, determination of levels, types, and persistence of airborne fungi in food storage and processing environments is an important part of hygiene monitoring programmes (37).

Air sampling is useful for monitoring airborne biological agents and can be conducted qualitatively or quantitatively (5). Quantitative methods include active air sampling (impaction technique) and passive air sampling (sedimentation technique) on solid surfaces (3). Active air sampling typically relies upon devices that draw a fixed volume of air at a specific speed over a specific period time for the assessment of viable airborne microorganisms (1) and can collect spores of different sizes (18). Active air sampling is faster and recovers more airborne microorganisms than passive techniques. Additionally, active sampling methods are more sensitive in determining pathogenic contamination in specified areas (3, 40). The MAS-100 Eco air sampler (Staefa, Switzerland) belongs to a new generation of single stage impactor air samplers. The performance of this sampler for enumeration of viable airborne microorganisms is statistically higher compared with other portable samplers (16, 47).

The objectives of this study are to investigate the incidence of airborne fungi flora in different retail food storage refrigerators (meat products, vegetables, dessert and mixed foods) using active air sampling and to determine the

association of temperature with the population of psychrotrophic bacteria and airborne fungi.

MATERIALS AND METHODS

Sampling procedure

During the period of June and August 2006, 96 petri dishes (48 petri dishes for psychrotrophic bacteria and 48 Petri dishes for airborne fungi) were collected from 48 ready-to-eat food storage refrigerators air of retail stores (restaurants, cafeterias, and buffets) in Edirne, Turkey.

Ready-to-eat food refrigerators included: meat products (salami, sausage, fermented sausage-sucuk, meatball; n=12), mixed foods (appetizers, vegetables, dairy products, meat products; n=26), vegetables (n=6), and dessert (baklava, profiterole, keshul, etc.; n=4). The ready-to-eat foods were most commonly stored unpackaged and retail stores were selected based on high regional sales. Samples were transported to the laboratory under refrigerated conditions (4-6°C).

Air from the refrigerators was sampled using a MAS-100 Eco air sampler (Merck, 1.09227). The air sampler was calibrated to sample 100 L of air per min, and Petri dishes (9 cm in diameter) were used to intercept a volume of 100 L of impacted air per sample. The air sampler's lid was first sterilized at 121 °C/ 15 minutes and sanitized with 70% ethyl alcohol, before and after each sampling. The instruction manual provided by the producer was used for the sampling process (2).

Microbial analysis

The number of psychrotrophic bacteria and airborne fungi were determined with standard plate count agar (Oxoid CM 463, Basingstoke, UK) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck 1.00466, Darmstadt, Germany), according to methods proposed by APHA (3). Psychrotrophic bacteria were enumerated after 10 days of incubation at 7°C (aerobic) and airborne fungi colonies were

enumerated after 5 days at 22-25°C (3). Colony forming units (CFU) were counted and reported as CFU/m³ of air sampled.

The average number of bacteria and fungi (CFU/m³) count was determined using the conversion formula recommended by the air sampler manufacturer: $Pr = N[1/N + 1/N - 1 + 1/N - 2 \dots 1/N - r + 1]$, where Pr = probable statistical total number (CFU/air volume); N = 400 (number of holes in perforated lid of the sampler), and r = number of CFU counted on the Petri dish (2).

Identification of fungi was determined by macro and micro-morphological characteristics according to taxonomic keys (25, 35, 41, 42).

After the enumeration of moulds, colonies were sub-cultured on malt extract agar (MEA) [Merck 1.05398 (Darmstadt, Germany)], as recommended by the Second International Workshop on Standardization of Methods for the Mycological Examination of Foods as a medium for use in identifying moulds (21). Plates were incubated at 25°C for 5–7 days.

Temperature analysis

The internal temperatures of the sampled refrigerators were measured with a digital thermometer (Testo 110, Testo AG, Lenzkirch, Germany), with an accuracy of a 0.2 °C within the range of -25 to +75°C.

Statistical analysis

One-way ANOVA and Duncan's multiple range tests were used to analyze microbial counts/m³ air and temperature. Statistical estimations were performed using the Statistical Package for the Social Sciences (44).

RESULTS AND DISCUSSION

Food-borne disease and food poisoning are common throughout the world. Both of these public health problems and the microbiological spoilage of foods can be minimized by proper storage, the careful choice of raw materials, and proper food preparation (22). Refrigerated foods can become vectors

for food-borne illness by contamination with food-borne pathogens in retail stores, processing plants, or consumers' homes (26).

Restaurants, cafeterias, and bars are the most frequently cited origins of food-borne outbreaks (10). In this study, ready-to-eat food retail stores were visited and 48 refrigerators were sampled. Of the refrigerators sampled, 42 (87.5%) tested positive for psychrotrophic bacteria or fungi. Only 4 meat-containing refrigerators (33.3%) were found to be free of microorganisms in air samples. This results can be related the good hygiene conditions (cleaning status of refrigerators) of meat containing refrigerators (n: 4). Six out of 48 petri dishes did not detect psychrotrophic bacteria (12.5%; 4 mixed-foods and 2 vegetable refrigerators) and 6 other Petri dishes did not detect airborne fungi (12.5%; 4 mixed foods and 2 dessert refrigerators). According to the researchers, psychrotrophic bacteria flora was found dominant in meat containing refrigerators (6, 13).

Temperature is one of the major controlling factors of food quality and food safety because of its influence on microbial growth rates. Despite the fact that low temperature can reduce the growth rate of many species of microorganisms, it has been reported that psychrotrophic microorganisms can grow at normal refrigeration temperatures (30). In cooler climates, psychrotrophic bacteria constitute a higher percentage of the microflora than in warmer climates (6, 15). Most psychrotrophic bacteria found in food are species of *Acinetobacter*, *Aeromonas*, *Bacillus*, *Clostridium*, *Klebsiella*, *Esherichia*, *Lactobacillus*, *Listeria*, *Morexella*, *Pseudomonas*, *Serratia*, *Streptococcus* etc. (3).

In this study, refrigerators containing a mix of foods had the highest mean value of psychrotrophic bacteria (82.3 CFU/m³) and the lowest mean value was found in dessert-containing refrigerators (Table 1). In a different study of domestic refrigerators, airborne psychrotrophic bacteria were found in the following distribution: < 200 CFU/m³ (17.2%), 200-500 CFU/m³ (31.0%), 500-1000 CFU/m³ (34.6%), and >1000 CFU/m³ (17.2%) (48). However, in our study we found

<200 CFU/m³ of psychrotrophic bacteria in all the air sampled.

Refrigerators containing mixed foods also had the highest mean value of airborne fungi (54.6 CFU/m³) (Table 1). This result is consistent with Zickrick *et al.* (48) that found that 66.5% of domestic refrigerators in Germany contained < 100 CFU/m³. Yeast and mould counts determined in air samples from food processing areas are higher than those found in this study (5, 28, 40, 43).

In this study we found the average mean value of all psychrotrophic bacteria counts were higher than the average mean value of airborne fungi. Conversely, Col (13) found the distribution of yeast and mould higher than aerobic flora (309 CFU/m³ and 229 CFU/m³, respectively) in air samples of food cold-storage areas in

Turkey.

As shown in Table 2, the moulds isolated from air samples were broadly represented by 17 genera. In total, 172 mould and 4 yeast (*Candida krusei*) isolates were obtained from 48 samples. *Penicillium italicum* was the most frequently detected species, found in 12.6% of 86 air samples, followed by *Botrytis cinerea* (5.8%), *Mucor rasemosus* (5.8%) and *Rhizopus oryzae* (5.8%). Several researchers have reported the frequency of *Penicillium*, *Aspergillus*, *Mucor* and *Cladosporium* in food processing air samples (5, 28, 40, 43). Additionally, Asefa *et al.* (5) and Sørensen *et al.* (43) reported *Penicillium* spp. as one of most predominant moulds in food processing areas.

Table 1. Mean value of psychrotrophic bacteria and airborne fungi counts and temperature of food storage refrigerators (n = 48)

Type of refrigerators	Psychrotrophic bacteria x±Sx (cfu/m ³)	Airborne fungi x±Sx (cfu/m ³)	Temperature x±Sx (°C)
Meat (n=12)	48.3 ± 15.5 ^a	23.3 ± 6.4 ^a	5.3 ± 0.2 ^a
Vegetable (n=6)	70.0 ± 35.2 ^a	40.0 ± 9.67 ^a	6.1 ± 0.3 ^a
Mixed-Food (n=26)	82.3 ± 14.3 ^a	54.6 ± 6.8 ^a	5.9 ± 0.2 ^a
Dessert (n=4)	40.0 ± 11.6 ^a	35.0 ± 20.2 ^a	5.2 ± 0.2 ^a

*Means in a column with the same letters are significantly (P > 0.05) different from one another.

Table 2. Frequency of fungal isolates from different food storage refrigerators' air

Genera	Moulds species	Meat	Vegetable	Mixed	Dessert	Total (%) (n=172)
<i>Acremonium</i>	<i>Acremonium</i> spp.	2				2 (1.2%)
	<i>A. charticola</i>		2	2		4 (2.3%)
	<i>A. strictum</i>	2				2 (1.2%)
	<i>A. butyric</i>			2		2 (1.2%)
<i>Aspergillus</i>	<i>A. candidus</i>		2	2	2	6 (3.4%)
	<i>A. flavus</i>	2				2 (1.2%)
	<i>A. melleus</i>			2		2 (1.2%)
	<i>A. niger</i>			2		2 (1.2%)
	<i>A. tamarii</i>			2		2 (1.2%)
	<i>A. terreus</i>			2		2 (1.2%)
	<i>A. ochraceus</i>			4		4 (2.3%)
	<i>Botrytis</i>	<i>B. aclada</i>	2			
<i>Cladosporium</i>	<i>B. cinerea</i>		2	8		10 (5.8%)
	<i>Cladosporium</i> spp.	4		2	2	8 (4.7%)
	<i>C. herbarum</i>	4				4 (2.3%)
<i>Mucor</i>	<i>C. macrocarpum</i>			2		2 (1.2%)
	<i>M. racemosus</i>			10		10 (5.8%)
	<i>M. plumbeus</i>			6		6 (3.4%)
<i>Penicillium</i>	<i>Penicillium</i> spp.	2	2	4	2	10 (5.8%)
	<i>P. digitatum</i>			4		4 (2.3%)
	<i>P. expansum</i>	2		4		6 (3.4%)
	<i>P. italicum</i>	4		18		22 (12.6%)
	<i>P. rugulosum</i>			2		2 (1.2%)
	<i>P. verruculosum</i>	2		2	2	6 (3.4%)

Table 2. Continuation

	<i>Alternaria alternata</i>	4	4	8 (4.7%)
	<i>Byssoschlamys nivea</i>	2		2 (1.2%)
	<i>Epicoccum nigrum</i>	2	2	4 (2.3%)
	<i>Eurotium herbariorum</i>		2	2 (1.2%)
	<i>Geotrichum candidum</i>		2	2 (1.2%)
	<i>Moniliella acetoabutens</i>		2	2 (1.2%)
	<i>Paecilomyces niveus</i>		2	2 (1.2%)
	<i>Rhizopus oryzae</i>	6	4	10 (5.8%)
	<i>Scopulariopsis candida</i>	2	6	8 (4.7%)
Others	<i>Syncephalastrum racemosum</i>	4		4 (2.3%)
	<i>Trichothecium rasemosum</i>		2	2 (2.3%)
	<i>Trichothecium roseum</i>		2	2 (1.2%)
	Total number of mould isolates	34	20	108
	(%)	(19.8%)	(11.6%)	(62.8%)
	Yeast			10
	<i>Candida crusei</i>	2	2	4 (5.8%)
				172

In our study, the dominant airborne fungal genera was *Penicillium* (29.0%), *Aspergillus* (12.0%), *Mucor* (9%), *Cladosporium* (8%), *Botrytis* (7%), and *Acremonium* (6%). Other significant fungi identified were *Rhizopus*, *Alternaria* and *Scopulariopsis* (Figure 1). Other researchers have also reported (4, 8) *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* as predominant genera in airborne fungi in the city of Edirne.

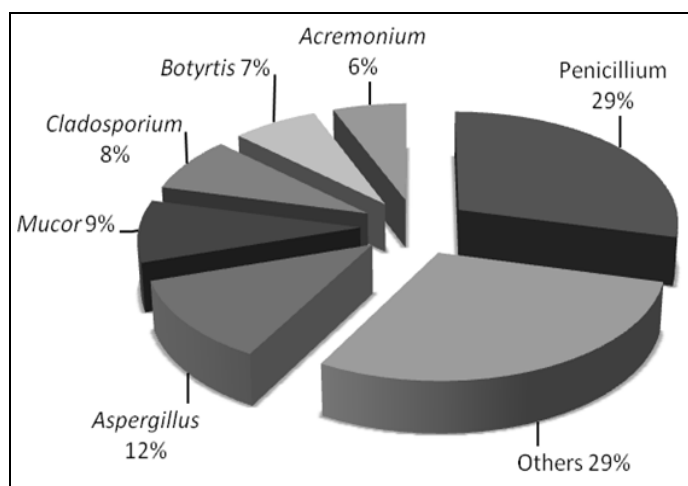


Figure 1. Distribution of mould genera captured by MAS 100 ECO air sampler

Penicillium is isolated frequently from both air and surfaces in food processing areas and was the most frequent

genus found in meat and dairy products (40, 43). In this study, *Penicillium* species was detected most commonly in refrigerators containing mixed products (31.4%) and meat products (29.4%). In addition, the dominant species, *P. italicum*, was isolated in mixed-food (81.8%) and meat refrigerators (18.2%). Blue mould, caused by *P. italicum* is among the most economically important postharvest spoilage organisms, which affects fresh vegetables and other foods worldwide (29).

Aspergillus ochraceus, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus* were the most common species from the *Aspergillus* genera found in mixed-food refrigerators. These moulds may cause invasive aspergillosis in immunocompromised individuals (16). Additionally, *Aspergillus ochraceus* is associated with Balkan Endemic Nephropathy (8).

In this study, *Mucor racemosus* (62.5%) and *Mucor plumbeus* (37.5%) were the dominant species of *Mucor* genera and are associated with spoilage of cold-stored vegetables, cheese, and meat products (36). We also found *Botrytis cinerea*, a common spoiler of fruits and vegetables.

Many species of *Cladosporium* are distributed world-wide and commonly found in air and indoor environments including food processing area (16, 41). In our study, *Cladosporium* species were found in a total of 14 air samples, mainly in refrigerators containing meat (57.1%) and mixed-foods

(28.6%). *Acremonium* was also commonly found in meat storage refrigerators (40.0%). Airborne *Cladosporium* and *Acremonium* have been isolated from meat processing areas (33, 43), indoors (9), and other food processing areas (23).

Health effects caused by exposure to fungi and the role of fungal metabolites have been the focus of increased attention (38). Various species including *Aspergillus* and *Penicillium* genera are often associated with allergic symptoms of the respiratory system (17). Concerns have been raised about exposure to mycotoxin-producing fungi (*Aspergillus flavus*, *A. ochraceus*, *Aspergillus terreus* etc.) in indoor environments, food production area, and food-storage areas. In addition to these genera and species, *Alternaria alternata* isolated in the present study (n=8) is a potential mycotoxin producer. Mycotoxin producing fungi commonly found in the air of refrigerators include species of *Aspergillus*, *Penicillium*, and *Alternaria* (4, 8). All of these genera were found in our study. *Byssoschlamys nivea*, also found in this study, can produce patulin and has been reported in foods from Turkey (7). Mycotoxin-producing fungi can easily become airborne and mobile and their spores are hazardous to humans and/or animals (32).

Temperature control is important in restraining the growth of psychrotrophic bacteria and pathogenic microorganisms (*Listeria monocytogenes*, *Salmonella* sp.) in foods (39). Generally, microbial growth is related with increasing temperature. (45). According to several studies on refrigeration temperatures, a wide percentage of retail and home refrigerators show temperatures above 4°C, a temperature that prevents most microbial growth and is recommended by many ready-to-eat food producers (9, 11, 12, 20). Similarly, WHO (46) recommended the storage temperature of foods at a maximum of 5 °C in refrigerator. We also found that the mean temperature of the sampled refrigerators (Meat= 5.3 ± 0.2, vegetable= 6.1 ± 0.3, mixed foods= 5.9 ± 0.2, and dessert= 5.2 ± 0.2) was above the recommended temperature. Garrido *et al.* (19) has reported that a drop in temperature is important to retard the growth of pathogens and other microorganisms

within the shelf life of product, especially once the product is purchased and under the responsibility of food producers or consumers.

Ready-to-eat foods are products that do not undergo a final treatment to reduce microbial load before consumption. Therefore, these foods should be stored at the recommended temperature ($\leq 4^{\circ}\text{C}$). Even at proper storage temperatures, some spoilage-causing or pathogenic microorganisms grow at low temperatures and lead to reduction of shelf life or affect consumer's health (27, 45). In this study, we found that the mean value of psychrotrophic bacteria and airborne fungi was relatively low in air samples taken from refrigerators containing ready-to-eat foods. Even so, many potential allergenic, pathogenic and mycotoxin-producing airborne fungi were found.

Finally, consumers' awareness regarding the microbiological safety of the food is important (11). Many consumers believe that food safety is the responsibility of food manufacturers. This belief has significant implications for public health initiatives to educate and inform the public in matters relating to procuring, storing and preparing food in a microbiologically safe manner.

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