



Managing raw materials of vegetable origin increases fungal indoor concentration in food companies

Mariana Elizondo-Zertuche¹ · Karen Martínez-Carranza² · Nydia Orue² · Rogelio de Jesús Treviño-Rangel¹ · Efrén Robledo-Leal²

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Abstract Fungi in indoor environments is a known cause of disease and food spoilage. However, there is currently no legislation or normativity establishing limits for fungal densities in correlation with these. Moreover, there is little knowledge of the diversity of fungi in indoor environments for industrial areas and in food-related companies in particular, a study has never been performed to evaluate the concentration and diversity of fungi in this type of places. We evaluated the fungal density of 20 food companies. We sampled 100 L of air onto rose bengal-malt extract-agar plates, using an Air Test Omega[®] sampler. After incubation, CFUs were counted and identified. *Penicillium*, *Cladosporium* and *Aspergillus* were the most commonly isolated genus, with *Penicillium* being the only genus to be present in every area sampled. Neither the companies' location nor their room temperature influenced the fungal densities significantly, however, companies using vegetable raw materials had a significantly greater concentration of fungi than the rest of the companies. While all concentrations were within previously suggested levels from a health-related point of view, more information is needed that correlates fungal concentration with food spoilage in order to suggest a range of concentrations focused for food companies' product preservation.

Keywords Indoor · Air · Fungi · Food · Contamination · Quality

Introduction

Air is a great disperser of fungal propagules, allowing the dispersion of fungi even intercontinentally (Gregory 1973). Fungal spores in air include those from mushrooms, plant pathogenic fungi and fungi that can potentially cause disease in humans and other animals. At high concentrations, fungi may cause allergic reactions, meaning that fungi can affect humans by contact alone, even without causing infections (Asano et al. 2018; Barac et al. 2018; Fernández-Soto et al. 2018; Kritas et al. 2018; Nogueira et al. 2018; Soumagne et al. 2019). In the case of closed environments, fungi also represent a threat to mostly everything, from deteriorating books and paintings to causing food spoilage for both prepared and stored foods (Dagnas and Membré 2013; Garnier et al. 2017; Martinović et al. 2016; Moss 2008).

To this day, a great amount of information has been published as a result of extensive research worldwide on the subject of indoor fungi and its implications in human health and food spoilage (Dagnas and Membré 2013; Méheust et al. 2014; Nevalainen et al. 2015; Martinović et al. 2016; Perrone and Susca 2017; Chen et al. 2017; Wigmann et al. 2018; Mendell et al. 2018; Tomczyk et al. 2018; Prencipe et al. 2018). However, despite the availability of information, there is currently no official standards, government guidelines or any normativity in order to regulate the presence of fungal spores on indoor air, mostly due to the lack of correlation between precise levels of fungal concentrations and health effects or food spoilage. Food-related companies, whether processing or packing it,

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✉ Efrén Robledo-Leal
efren.robledoll@uanl.edu.mx

¹ Department of Microbiology, School of Medicine, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, Mexico

² Department of Microbiology and Immunology, School of Biological Sciences, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, Mexico

must deal with the issue of product contamination, which affects shelf life and generates economic losses. Although quality control programs are almost always performed in this type of companies, we found that misinformation and ignorance on how to approach the microbiological assessment regarding to fungi is widely displayed. Since policies don't exist to regulate this, sanitation audit focus almost exclusively to bacteria and other microbial organisms which are contained in the normativity and fungi become relevant only when problems already occurred with the quality of the food products. In the present study, we evaluated for the first time, 20 food-related companies belonging to the Agroalimentos company cluster in Nuevo León, México, aiming for a better understanding of the indoor fungal presence in this type of buildings and generating advice for each company's staff afterwards, both related to the product's safety and the health of the workers.

Materials and methods

Area of study

Twenty food-related companies in the metropolitan area of Monterrey, México were included in the study. These companies voluntarily gave access to their buildings with the only condition of keeping their identities confidential. In each company, different areas were sampled in order to cover the whole manufacturing sites. Temperature was categorized in 2 groups: areas with temperatures below 22 °C and areas with temperatures above 23 °C. The study was performed in the course of 2 months and each company was given an alphabetical letter code.

Sampling

Petri dishes with Rose bengal-malt extract-agar (RBME; BD, USA) were used for the fungal propagules collection. Volumes of 100 L of air were impacted in each plate using an Air Test Omega® sampler (LCB, France) set up at 1.5 m above the ground. All areas were sampled with 3 replicates and always between 9:00 and 12:00 h, for protocol uniformity. Between each plate, the sampler cap was thoroughly cleaned with disinfecting wipes. Plates were then transported back to the laboratory in a cooler and incubated at 28 °C for 10 days.

Colony counting and identification

After 5 days of incubation, colony forming units (CFUs) were counted and mounted for microscopic observation using the cellophane tape technique (Larone 2011). Fungal

structures were identified based on morphological features (Watanabe 2010; Guarro 2012). Fungi that didn't show sporulation were subcultured in order to isolate them and let them grow for a longer period of time and allow them to sporulate. Fungi that didn't sporulate after 30 days of isolated growth were reported as sterile mycelium. Yeasts were reported without identification nor color categorization. CFU counts were adjusted to concentration per liter of air using the manufacturer's supplementary charts.

Statistical analysis

We compared fungal concentrations using Duncan's multiple range test and Student's T test ($p < 0.05$). All analyses were done in SPSS ver. 23.0.0.0 (Armonk, NY, USA.)

Results

The global qualitative and quantitative findings of our study are shown in Table 1. Fungal colonies grown in culture were quantified according to the device manufacturer's correspondence chart and then each colony was identified as mentioned above. As expected, and in concordance with numerous reports on indoor presence of fungi (Flannigan et al. 2011), the most frequent genera of indoor air fungi included *Aspergillus*, *Cladosporium* and *Penicillium*. In this survey, it was *Penicillium* and not *Cladosporium*, the most frequently isolated genera; however, 8 companies sampled exhibited notoriously high concentrations of *Penicillium* CFU and when all data from those companies is excluded from the overall counts, *Cladosporium* becomes the most frequent genus. Even so, *Penicillium* was the only genus present in every area included in the sampling, showing the widespread distribution of these molds in indoor environments and highlighting the importance of its control due to its allergenic potential (Fernández-Soto et al. 2018) and its role as a food spoilage microorganism (Moss 2008).

The two companies with the highest fungal densities were Q and E, which grouped separately when Duncan's multiple range test was used. Among the areas sampled, some of them had a lower room temperature due to the type of activity performed there (e.g. food processing, cold packaging, etc.). A comparison of fungal density between non-cooled areas and those with temperatures below 20 °C, showed no statistical difference. This can be explained by the fact that while molds are mesophilic, they retain viability and even a slow but active metabolism at temperatures below 4 °C (Baxter and Illston 1980).

On the other hand, when all companies that used vegetable raw materials (which included Q and E) were compared to the rest using Student's T test, a significant difference was found.

Table 1 Total CFU^a per company and for each fungal identity recovered

Fungal ID	Company																				Total
	A ^b	B	C ^b	D	E ^b	F	G	H	I ^b	J	K	L ^b	M	N	O ^b	P ^b	Q ^b	R	S	T	
<i>Alternaria</i> spp.	94	67	114		30			20								362	115		236	132	1170
<i>Aspergillus</i> spp.	165	188	193		1634	153	132	232	361	30	54	168	56	10	1083	60	30	7	141	3	4700
<i>Bipolaris</i> spp.	17						116	61		60						20					274
<i>Cladosporium</i> spp.	354	145	833	27		17			527	419	434	348		168		911	668	299	214	928	6292
<i>Curvularia</i> spp.	3						3													3	9
<i>Exserohilum</i> spp.	3					7															10
<i>Fusarium</i> spp.	44		7		23		23		23			17				35	16			27	215
<i>Mucor</i> spp.			13		13													30	82	3	111
<i>Neurospora</i> spp.	17				37																84
<i>Paecilomyces</i> spp.	7	13			44	57						27	71			47			75	76	417
<i>Penicillium</i> spp.	151	97	100	222	2457	778	53	33	1280	296	172	97	109	116	73	811	5083	1791	877	379	14,975
<i>Pestalotiopsis</i> spp.			7																		7
<i>Rhizopus</i> spp.	121	10	175		162	80		47	211	195					115	30	13	149	397		1705
<i>Rhodotorula</i> spp.	10	7			83																100
<i>Stemphyllum</i> spp.												102									102
<i>Syncephalastrum</i> spp.			3											5		68		130			3
<i>Trichoderma</i> spp.				67	34	145	7			114											570
<i>Trichosporon</i> spp.																		3			3
<i>Ulocladium</i> spp.	23	7					50	101		219					245		30			33	708
Yeasts	54	13	13	23	178	50			54		57	23		3		38	13	70	40	23	639
Sterile/unidentified	202	91	91	74	47	17	299	110	138	257	1330	1109	277	184		605	43	58	521	111	5473
Total	969	823	1556	413	4742	1304	683	604	2594	1590	2047	1891	518	481	1516	2987	6011	2537	2583	1718	37,567

^aCFU per liter of air^bUses vegetables as raw materials

Discussion

Cladosporium is reported as the most prevalent fungal genus on indoor environments around the world (Flannigan et al. 2011); however in our overall data, *Penicillium* showed the highest concentration. Although this is still not surprising, the notoriously high concentration of this genus in 8 companies sampled, suggests a probable endogenous production of inocula at those sites, promoted by accumulation of organic matter and increased humidity, among other causes. This hypothesis would require more evidence in order to be confirmed but those companies were anyway informed about it as a preventive measure.

Fungi, particularly asexual ascomycetes (usually referred as molds), have a wide range of temperature at which they are able to grow but since they are transmitted mainly through air, they are subject to variables besides temperature such as pollutants and relative humidity. In this report, the type of food and/or raw materials processed and the main product of each company did generate a statistical difference with those managing vegetable materials such as grains, cereals and crops. This may be explained by the nature of those materials and their natural interaction with fungi, implying that companies working with vegetable materials could benefit from reinforcing their sanitary procedures, both for the sake of their workers' health and their final product's preservation, while companies processing meat derivatives, sugary beverages among others, would not need to modify their current controls, according to our data.

Currently, there is no legislation or official guideline establishing specific fungal concentrations as a direct cause for a pathology or food contamination in particular, mostly due to the great challenge posed by the vast amount of variables that exist in trying to define this. However, Górný et al. (2011) provided an excellent analysis of the ranges of concentration values for indoor biological aerosols proposed as threshold limits within the last 140 years and offered an updated proposal which for fungi is stated as follows: 1.0×10^1 – 1.0×10^4 cfu/m³ for residential rooms and non-industrial workplaces and $< 1.0 \times 10^2$ – 1.0×10^7 cfu/m³ for productive and industrial premises; for the pathogenic microorganisms, there is no safety level in the above mentioned premises (the threshold limit should be 0 cfu/m³). Using this as a reference and considering those values for mixed genera values, all of the companies sampled in this study are within acceptable values for fungal CFU in air. Nevertheless, since foods differ greatly in their susceptibility to fungal contamination because of their intrinsic properties (i.e. moisture content, physical state, carbohydrate content, etc.), special considerations and even a subset of proposals should be offered to

food companies in relation to their products, in order to reduce spoilage and financial losses.

Conclusion

It is virtually impossible for indoor air to be without the presence of fungal propagules and variables such as the weather and anthropogenic activity generate variations on their density. In the present study, the fungal presence in the indoor air from 20 food companies was analyzed, showing acceptable quantities according to proposals previously made by experts. However, being this the first published study in Mexico evaluating the indoor fungal air quality of food companies, more research is needed that includes correlations with product losses, in order to create a proposal of threshold values focused on food products safety. While this may seem complicated and expensive, it may become extremely important as a measure for reducing food waste and increasing its availability not only in this country but in the worldwide commercial supply chain.

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