





Fungal and mycotoxin burden in Portuguese bakeries

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1. Introduction

There are more than 3300 bakeries registered in Portugal.

This is a major industrial activity in Portugal and directly relates with the fact that Portuguese bread is a well-known product that is appreciated both nationally and internationally.

(Guiné et al. 2016; Carbas et al. 2016)

Although without exact numbers, this implies a considerable work force involving many workers in Portugal.







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Flour is a complex organic dust covering one or a mixture of different cereal grains that have been processed or grounded by milling.

(Meo and AL-Dress 2005)

Flour may contain several contaminants, such as fungi and mycotoxins being the raw materials entering the facilities the principal contamination sources for this occupational environment.
(Milanowski et al. 2002; Karpinski 2003; Viegas et al.

2016, 2018)









- Several studies report respiratory health effects in workers exposed both in small and large-scale industries.
- Respiratory system symptoms and diseases have frequently been reported to be induced by occupational dust being influenced by the type of dust, dose, duration of exposure and genetic factors.

(Milanowski et al. 2002; Patouchas et al. 2009; Subbarao et al. 2009).







- Different sampling methods should be applied to ensure a more detailed occupational exposure assessment to fungal burden, since each method has unique advantages and disadvantages.
- A multi-approach in the sampling methods will enrich data findings, enabling a more accurate risk characterization.

(Reponen 2017; Viegas et al. 2018).









Assess the exposure to fungal burden (fungi and mycotoxins) on 13 Portuguese artisanal bakeries applying active and passive methods as sampling strategy.







2. Materials and methods

13 Portuguese bakeries located in the Lisbon district

Financial support from the Portuguese Authority for Working Conditions

Three different areas:

- Production—where kneading machines and ovens were located and where dough shaping was performed;
- Raw material warehouse—where workers have to go several times to collected the raw materials for dough preparation;
- Store—where final product is sold (bread or pastry).



| | | Sampling approaches (samples number) | | | | |
|-----------------------|---------------------|---|--|-------------------|-------------------|----------------|
| Bakery | Facilities | Indoor air sampling impaction MEA and DG18 | Indoors air sampling impinger | Surfaces swabs | Settled Dust # | EDC* |
| 1 (22-11) | Enlarged company | 3 | 3 | 3 | - | 2 |
| 2 (6-12) | Enlarged company | 5 | 5 | 5 | - | 3 |
| 3 (10-01) | Enlarged company | 4 | 4 | 4 | 1 | 2 |
| 4 (19-01) | Enlarged company | 4 | 4 | 4 | 1 | 2 |
| 5 (24-01) | Enlarged company | 5 | 5 | 5 | 1 | 3 |
| 6 (31-01) | Enlarged company | 4+ | 4 | 4 | 1 | 3 |
| 7 (8-2) | Enlarged company | 5 | 5 | 5 | 1 | 3 |
| 8 (15-2) | Enlarged company | 4 | 4 | 4 | 1 | 3 |
| 9(12-4) | Supermarket | 4 | 4 | 4 | 1 | 3 |
| 10(26-4) | Supermarket | 4 | 4 | 5 | 1 | 3 |
| 11(18-5) | Supermarket | 4 | 4 | 4 | 1 | 3 |
| 12(23-5) | Supermarket | 3 | 3 | 3 | 1 | 3 |
| 13 (7-6) To | Supermarket | <u>4</u> 53 | 4 53 | 4 58 | 1 11 | 3 36 |



canteens and vending machines.

5 bakeries - supermarket facilities and belonged to the supermarket holder









Multi-approach sampling strategy – Active methods

- Air samples of 100 liters (impaction method) (N= 53)
- 600 liters (impinger method) (N= 53)







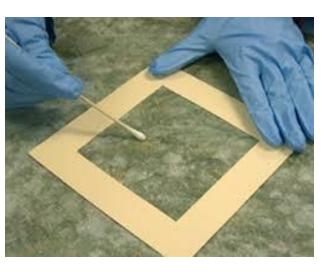






Multi-approach sampling strategy – Passive methods

- Surface samples (N=58)
- Settled dust samples (N= 12)
- Electrostatic dust cloths (N= 36)













Multi-approach analyses strategy



- Quantification and morphological identification by culture-based methods
- MEA and DG18
- Molecular detection of the toxigenic
 Aspergillus sections Flavi, Fumigati,
 Circumdati and Versicolores.
- 36 Mycotoxins in the air and settled dust samples were analyzed by LC-MS/MS system.





3. Results - Fungi

Fungal load in indoor air ranged from 0 to 2590 CFU.m⁻³ on MEA

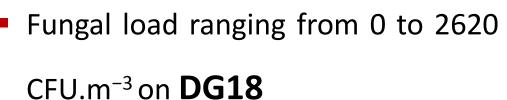
 65.3% (32 out of 49) showed higher fungal load than the limits imposed by the WHO (maximum value of 150 CFU.m⁻³)

(WHO, 2009)

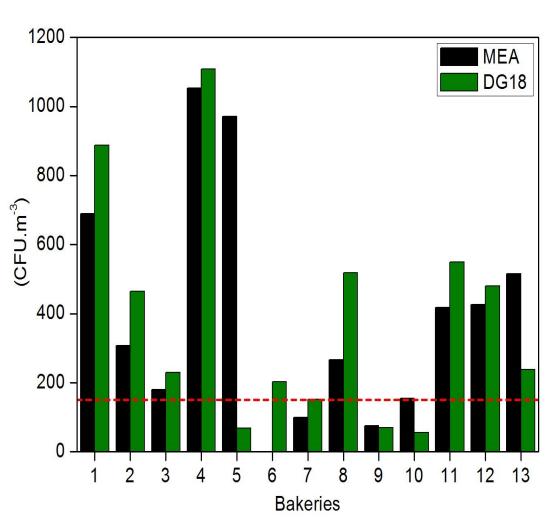
 30 out of the 49 (61.2%) air samples collected presented higher fungal load when compared to the outdoor sampling.







- 58.5% with fungal load exceeding the WHO limit.
- **43.4%** presented higher indoor fungal load when compared to the outdoor sampling.





| man | | | | | |
|--|---|--|---|--|--|
| Charling and an and a contract of the second | MEA | | DG18 | | |
| exposure | Air | (CFU·m⁻³) (%; n) | Air | (CFU·m⁻³) (%; n) | |
| And and a second | Acremonium sp. Chrysonilia sitophila Cladosporium sp. Mucorales order Penicillium sp. Others | (17.0; 3960) (17.2; 4000) (29.7; 6920) (2.2; 520) (22.3; 5190) (11.7; 2730) | Acremonium sp. Chrysonilia sitophila Cladosporium sp. Geotrichum sp. Penicillium sp. Others | (2.8; 670) (10.3; 2500) (48.7; 11860) (2.5; 620) (30.5; 7420) (5.2; 1270) | |
| | Surfaces | (CFU·m⁻²) (%; n) | Surfaces | (CFU·m⁻²) (%; n) | |
| | Acremonium sp. Chrysonilia sitophila Cladosporium sp. Penicillium sp. Others | (7.7; 2040000) (75.8;20000000) (10.7; 2820000) (4.0; 1050000) (1.8; 480500) | Acremonium sp. Chrysonilia sitophila Cladosporium sp. Mucorales order Penicillium sp. Others | (4.7; 1370000) (51.6;15020000) (10.5; 3070000) (17.5; 5110000) (12; 3500000) (3.6; 1060000) | |
| | EDC | (CFU∙∙m⁻²) (%; n) | EDC | (CFU∙∙m⁻²) (%; n) | |
| | Chrysonilia sitophila Cladosporium sp. Penicillium sp. Others | (91.9; 124403) (2.5; 3434) (5.0; 6718) (0.6; 746) | Aspergillus sp. Chrysosporium sp. Cladosporium sp. Penicillium sp. Others | (2.3; 597) (3.1; 796) (55.7; 14480) (38.3; 9952) (0.6; 149) | |







- Cladosporium sp. was the most prevalent species in indoor air samples in both media (29.7% MEA; 48.7% DG18), followed by *Penicillium* sp. (22.3% MEA; 30.5% DG18).
- Aspergillus spp. was observed on air samples on MEA and DG18 (0.3 and 1.2%, respectively).
- Among Aspergillus genera, section Candidi was the most prevalent (62.5%) on MEA followed by Nigri (25%), whereas sections Candidi and Circumdati (37.9%) were more prevalent on DG18.
- Aspergillus section Fumigati was possible to detect in 22.4% on air, 27.8% on surface swabs and in 7.4% on EDC samples; section Versicolores was detected in one air sample through molecular tools.
- Increased Aspergillus species identification on DG18





3. Results - Mycotoxins

- Regarding settled dust samples, all samples showed contamination with 6 to 8 mycotoxins in each sample.
- The mycotoxins detected are the ones reported in the table 6, namely: D3G, DON, ZEA, 15-ADON, MAS, DAS, FB1, FB2, GRIS, HT2, OTA, OTB and MPA.
- DON was clearly the mycotoxin measured in higher amounts since all the samples showed quantifiable results.
- None of the thirty-six mycotoxins were detected in air samples.

| Mycotoxins | Number of samples with | Concentration range | | |
|------------|---------------------------|----------------------------------|--|--|
| | detectable values | | | |
| DON | 12 (100%) | 15.95 - 211 | | |
| D3G | 9 (75%) | <loq 25.77<="" th="" –=""></loq> | | |
| ZEA | 12 (100%) | <loq -="" 1.60<="" th=""></loq> | | |
| 15-ADON | 3 (25%) | 5.01-5.22 | | |
| MAS | 6 (50%) | 0.40-1.6 | | |
| DAS | 1 (8.3%) | < LOQ | | |
| FB1 | 3 (25%) | 5.23 - 7.36 | | |
| FB2 | 4 (33.3%) | 3.73-5.49 | | |
| GRIS | 1 (8.3%) | 5.90 | | |
| HT2 | 2 (16.6%) | 0.99-1.93 | | |
| ΟΤΑ | 10 (83.3%) | < LOQ-0.64 | | |
| ОТВ | 1 (8.3%) | 0.54 | | |
| MPA | 7 (58.3%) | < LOQ - 3.04 | | |

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4. Main findings discussion

 76.9% (10 out of 13) of the assessed bakeries surpassed the WHO guideline

Additionally, fungal species with toxigenic potential were identified in all bakeries in the different environmental matrices collected, such as *Penicillium* sp., *Fusarium* sp. and *Aspergillus* sections.

(Varga et al. 2015)

Mycotoxins detection only on settled dust

Assessing mycotoxins through passive sampling methods, such as settled dust or other environmental samples that collect contamination instead of load, seems to be the trend for the mycotoxins contamination assessment in occupational environments .

(Krysinska-Traczyk et al. 2001; Nordby et al. 2004; Halstensen et al. 2006; Mo et al. 2014; Lai et al. 2014; Straumfors et al. 2014).







It was not possible to observe or detect fungal growth in settled dust but several mycotoxins were present.

Mycotoxins can persist in an occupational environment even in fungi absence since they resist more to adverse abiotic factors, such as temperature and humidity and this is the reason why the absence of fungal growth cannot be a surrogate for the absence of mycotoxins' contamination.

(Alborch et al. 2011; Halstensen 2008; Viegas et al. 2015; Mayer 2015; Viegas et al. 2016).



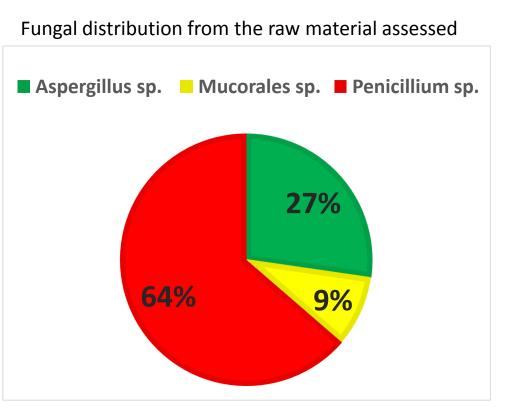
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- Raw materials can be contaminated with fungi and multiple mycotoxins
- In 5 bakeries from the 13 assessed was collected bread raw material (N= 39)
- In **4 bakeries** was verified fungal growth in different raw materials.
- In 22 samples (56.4%) of raw material was observed quantifiable results of DON and was clearly the mycotoxin measured in higher amounts followed by OTA and FB1 and FB2.









Particulate matter can boost exposure to mycotoxins

Mycotoxins are commonly present in airborne dust and in fungal spores or fragments of microbial growth and both can act as mycotoxin carriers to the workers respiratory system, since in this occupational setting exposure to organic dust is commonly observed enhancing exposure to mycotoxins by inhalation.

(Croft et al. 1986; Flannigan 1987; Burstyn et al. 1997; Roberge et al. 2012; Viegas et al. 2017; Huttunen and Korkalainen, 2017).









5. Conclusions

- The multi-approach on sampling methods and laboratory assays enriched data findings
- Co-exposure to more than one risk factor fungi and metabolites (mycotoxins) – should be considered.
- To assess occupational exposure to mycotoxins through the use of biomonitoring tools is crucial. This will allow recognizing if workplace exposure adds significantly to the exposure resulting from ingestion of mycotoxin-contaminated food.











Thank you for your attention

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