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Air quality assessment in (semi) liquid food packaging environments

Pieter-Jan Loveniers [a], Frank Devlieghere [b], Imca Sampers [a,*]

[a] Research Unit VEG-i-TEC, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Campus Kortrijk, Sint-Martens-Latemlaan 2B, 8500 Kortrijk, Belgium
[b] Research Unit Food Microbiology and Food Preservation (FMFP), Department of Food Technology, Safety and Health, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

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ABSTRACT

This study aimed to assess the prevalence of airborne microorganisms within the packaging areas of (semi) liquid food product facilities, considering the impact of outdoor microbiological air, seasonal fluctuations, rural-urban differences and environmental parameters such as temperature and relative humidity. Additionally, the effectiveness of both natural ventilation and air handling units were evaluated against total viable and non-viable particulate matter.

Air samples were collected from the packaging areas of fourteen diverse (semi) liquid food producers and the outdoor environment in Flanders, Belgium, over the course of four separate days from January to September 2022. Airborne microorganisms, including total mesophilic plate count (TMPC) and yeast and mould (Y&M) count, were sampled using impaction and settle plate methods on Plate Count Agar and Rose Bengal Chloramphenicol agar, respectively. Simultaneously, a laser particle counter was used to collect and categorise particles into six size-based channels, while environmental parameters were monitored using a hygrometer.

The study revealed a wide range of concentrations of airborne microorganisms across various companies (indoor TMPC ranging from 1.35 ± 0.65 log cfu/m^3 to 3.42 ± 0.16 log cfu/m^3 and Y&M count ranging from 1.22 ± 0.65 log cfu/m^3 to 3.10 ± 0.08 log cfu/m^3), irrespective of outdoor air quality. Neither relative humidity nor temperature exhibited any influence on TMPC and Y&M count. Companies equipped with high-efficiency filter air handling unit showed decreased concentrations of airborne microorganisms, although this trend did not align with their total particle counts. Moreover, no correlation was observed between total particle count and either TMPC or Y&M count. Additionally, impaction and sedimentation methods demonstrated a strong correlation.

In conclusion, the diversity in airborne microorganism concentrations within food packaging areas is influenced by multiple factors, with air handling unit filters playing a pivotal role. High-efficiency filters were found to reduce microorganism levels, but their impact on total particle counts was less pronounced.

1. Introduction

Air has been considered in the food industry as a potential source of food contamination during processing and packaging (Aichinger et al., 2006; Brown and Wray, 2014; Kang & Frank, 1989; Masotti et al., 2019). Especially in open food processing and packaging areas, microorganisms can be transported via the air towards open products, which can be a significant cause of contamination (Zand et al., 2022). At present, food companies are dedicated to upholding a hygienic and controlled environment, while implementing efficient measures for microbial air control. This is especially critical as airborne contamination during packaging can influence the shelf life of perishable food products. Nevertheless, the precise magnitude of the overall risk posed by airborne microorganisms remains uncertain. Based on several studies, it is assumed that a risk exists, as the air in various food sectors may harbour a considerable amount of airborne microorganisms (Botondi, 2019; Holah et al., 1995; Parussolo et al., 2019; Ren & Frank, 1992; Salustiano et al., 2003; Valle Garcia et al., 2019; Zorman & Jeršek, 2008). An attempt to quantify recontamination via the air was calculated by den Aantrekker et al. (2003) through an analysis of existing literature, encompassing various product groups, different locations within the facility and using different air sampling techniques. However, establishing acceptable quantitative limits for the air quality within specific food production or packaging zones remains challenging, primarily due

* Corresponding author.
E-mail address: imca.sampers@ugent.be (I. Sampers).

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to variations in air sampling techniques, a lack of consistent data and an abundance of speculation. Recently, EN 17141:2020 provides first-time guidance for microbial air control in the food sector, including a risk assessment approach in Annex D for monitoring total microbial concentration in ham cutting and packaging, allowing food manufacturers to establish their control levels (European Committee for Standardization, 2020). In the absence of quantitative limits, regulations, such as the regulation 852/2004/EC on the hygiene of foodstuffs and the current Good Manufacturing Practices (cGMP) in the United States, focus on the prevention of airborne contamination in food processing plants. Such preventive measures include stringent cleaning protocols, the use of air filters, the control or elimination of potential contamination sources like floor drains, the implementation of controlled airflow systems directing air from clean areas to dirty areas, and other GMP practices (Brown & Wray, 2014; Masotti et al., 2019). Air handling units are engineered to remove airborne particles and microorganisms by entrapping them in filters. Currently, air filter classifications primarily focus on particulate matter (PM), which include both viable and non-viable particles (International Organization for Standardization, 2016). While research has indicated that airborne microorganisms affect filter efficiency differently than mineral dust, with some filters boasting a 90% efficiency rating according to standard methods ISO 16980, demonstrating an efficiency exceeding 99.99% in removing airborne microorganisms (Whyte et al., 2012). This contrasts with the observations that an H11 filter, according to standard EN1822, achieved 100% collection efficiency for mineral dust particles but exhibited diminished efficiency in the case of mixed bioaerosols (Miaskiewicz-Peska & Lebkowska, 2012). To the best of the authors knowledge, such comprehensive research in the context of (semi) liquid food packaging areas is lacking. In light of this knowledge gap, the present study seeks to provide a comprehensive assessment of airborne microorganisms within these (semi) liquid food packaging environments. This investigation takes into account various factors, including outdoor air quality, seasonal variations, the rural-urban location of the production setting, and environmental parameters such as temperature and relative humidity.

The primary objectives of this research are to quantify the presence of airborne microorganisms, assess their potential impact on food contamination via the air, and explore the relationship between total airborne particles and airborne microorganisms. Furthermore, the study aims to establish guidelines for selecting optimal filter classes for air handling units operating in these specific areas. By achieving these goals, the research aspires to contribute valuable insights and recommendations to enhance the hygienic conditions and safety of (semi) liquid food processing and packaging processes.

2. Methodology

2.1. Classification of selected (semi) liquid food producers

The study was conducted across fourteen (semi) liquid food producers located in different parts of Flanders, Belgium. The classification of these companies, based on produced product type, risk for microbiological spoilage (class I: low risk to class V: high risk), urban-rural location, final air filter class, open or closed filling rooms, adopted positive air pressure, volume of the filling room and amount of operators in the room is detailed in Table 1. Natural ventilation refers to the process of bringing fresh outdoor air into a building without the use of mechanical systems. This is typically achieved through openings such as windows, doors, or vents, allowing for airflow driven by natural forces like wind (Zhivov et al., 2020).

2.2. Air sampling procedure

From January to September of 2022, samples were collected from each of the fourteen (semi) liquid food production plants on four distinct occasions. These visits were evenly distributed, with two occurring during the winter season and two during the summer season, except for companies 8, 9 and 10, which were visited once during the summer and twice during the winter, while companies 6 and 12 had two visits during the summer, and company 13 was visited once during the summer.

Sample collection involved both indoor and outdoor locations at the factory premises. Indoor sampling was conducted in the filling room at a height of 1 m above ground level and approximately 1 m away from the filling head of a packaging line. In cases where there were two filling

Table 1
Classification of the selected companies by product type, microbiological risk group (VDMA, 2007), urban-rural location and final air filter class in the filling room.

Company	Product type	Micro-biological risk group[1]	Urban-rural location	Final air filter class in filling room[2]	Positive air pressure	Closed/open room[3]	Volume (m^3)	Operators in room
1	Alternative dairy (plant-based yoghurt and drinks)	Class IV - V	Urban	ISO $ePM_{2.5}$ 65% (F7), H13	No	Closed	220–640	2–3
2	Fermented dairy (dairy-based yoghurt, fresh cheese)	Class IV - V	Suburban	ISO $ePM_{2.5}$ 65% (F7)	No	Closed	750	3
3	Fermented dairy (dairy-based desserts)	Class II - III	Nonurban	None – Natural ventilation	No	Open	80	2
4	Savory spreads (vegetables)	Class IV - V	Nonurban	None – Evaporative cooling	No	Open	450	2
5	Savory spreads (fish/meat)	Class IV - V	Suburban	ISO ePM_1 80% (F9)	Yes	Open	685	7
6	Savory spreads (fish/meat)	Class IV - V	Suburban	ISO $ePM_{2.5}$ 65% (F7)	No	Open	1755	18–20
7	Savory spreads (fish/meat)	Class IV - V	Urban	ISO $ePM_{2.5}$ 65% (F7)	No	Open	1600	9–10
8	Acidified sauces	Class II - IV	Urban	None – Evaporative cooling	No	Closed	614–167	1–2
9	Acidified sauces	Class II - IV	Nonurban	None – Natural ventilation	No	Closed	779	7–8
10	Acidified sauces	Class II - IV	Nonurban	None – Evaporative cooling	No	Open	8750	6
11	Fruit purees	Class III	Nonurban	None – Evaporative cooling	No	Closed	5280	3
12	Heat treated vegetables	Class I	Nonurban	None – Natural ventilation	No	Open	429–2002	2–3
13	Fruit purees	Class II - III	Urban	None – Natural ventilation	No	Closed	540	4–5
14	Margarines	Class III - IV	Urban	ISO $ePM_{2.5}$ 65% (F7)	No	Closed	15000	10–14

[1] Based on VDMA hygiene classes for filling machines for (semi) liquid food products in VDMA Doc. No. 2.
[2] Filter class in accordance with ISO 16890–1:2016 (until June 2018 according to EN 779:2012) and EN 1822–1:2009.
[3] Open: packaging room communicating with processing area.

rooms, samples were obtained from each room. Air sampling was carried out during operational hours to ensure representation of typical work activities. The density of operators per unit volume remained uniform throughout sampling. Post-sanitation or Clean-in-Place (CIP) procedures were conducted prior to sampling. In cases where small batches were packaged during the day (companies 3 and 5), sanitation was performed between batches using high-pressure hosing. The companies did not employ air disinfection or fumigation methods. To ensure the reliability of the air sampling results, the Quality Manager and/or Technical Department of each company confirmed that regular maintenance of the air handling units, typically conducted 1–2 times a year, was in place. For outdoor sampling, samples were taken at the entrance gate or door through which raw materials entered the factory.

During each visit, microbial sampling was carried out using both the settle plate technique (passive) and the impaction method (active), each conducted in triplicate (Fig. 1). For the settle plate technique, open Petri dishes with a respective medium were exposed to the air for approximately 1 h, closed and incubated (Pasquarella, Pitzurra, & Savino, 2000). Petri dishes contained Plate Count Agar (PCA, Biokar Diagnostics, BK144HA), or Rose Bengal Chloramphenicol Agar (RBC, Biokar Diagnostics, BK151). For the impaction technique, two air samplers (Spin air V2, IUL instruments, 90005500) were chosen for microbial collection on Petri dish agar. The air sampler's lid was sanitized with 0.5% umonium solution before each sampling. The sampler draws air through a sieve plate at a preset flow rate of 100 liters/min). The rotating Petri dish of the Spin Air allows to have a real count avoiding the use of colony count correction tables.

Throughout the sampling process, environmental parameters, such as temperature and relative humidity, were measured with a digital hygrometer (VWR Traceable®, 620–2273). Additionally, a Laser particle counter (Extech, VPC300) was used to determine total particle numbers in the air. The flow rate of the particle counter is 2.83 L/min. Samples were taken in the differential mode over a 21 s interval (i.e. 0.9992 L), yielding values for six distinct particle size categories (0.3–0.5 μm, 0.5–1 μm, 1–2.5 μm, 2.5–5 μm, 5–10 μm, >10 μm). The differential mode includes all particles that are greater than or equal to the selected channel but less than the next channel.

2.3. Preliminary study

To optimize the sampling process with the air sampler, experiments were conducted to determine the appropriate duration and volume of air needed to achieve countable Petri dishes and obtain representative samples, which is defined as samples with less than 300 cfu/plate). During the initial visit to each company, sampling times of 30 s, one, two-and-a-half, and 5 min were tested, corresponding to flow rates of 50, 100, 250, and 500 liters/min, respectively. For most companies and outdoor sampling locations, a sampling duration of 1 min (or equivalently, a volume of 100 L at 100 liters/min flow rate) was sufficient to achieve countable microorganisms on the Petri dishes. However, companies 1, 7 and 14 required a larger air volume of 500 L to obtain countable microorganisms on the Petri dishes, indicating lower microbial concentrations in the air. In contrast, for company 3, a lower volume of 50 L was already sufficient to yield countable microorganisms on the Petri dishes for both media types.

2.4. Incubation and enumeration procedures

Yeast and mould (on RBC) and total mesophilic microorganisms (on PCA) were incubated for 5 days at 25 °C and 3 days at 30 °C, respectively. The data are expressed as cfu per cubic meter of air for the impaction technique, calculated using the formula:

$$C_{air} = \frac{cfu}{m^3} = \frac{\left[number\ of\ cfu \times \frac{1000L}{m^3}\right]}{sampled\ air\ volume} \quad (1)$$

Counts from settle plates were expressed as:

$$C_{settled} = \frac{cfu}{m^2 h} = \frac{\left[number\ of\ cfu \times \frac{60\ min}{exposure\ time}\right]}{\frac{(0.09\ m)^2 \times \pi}{4}} \quad (2)$$

The estimation of recontamination via the air was based on the methodology proposed by Whyte (1986), which assumed a relationship between the settling velocity, concentration of airborne microorganisms and exposed product area and time:

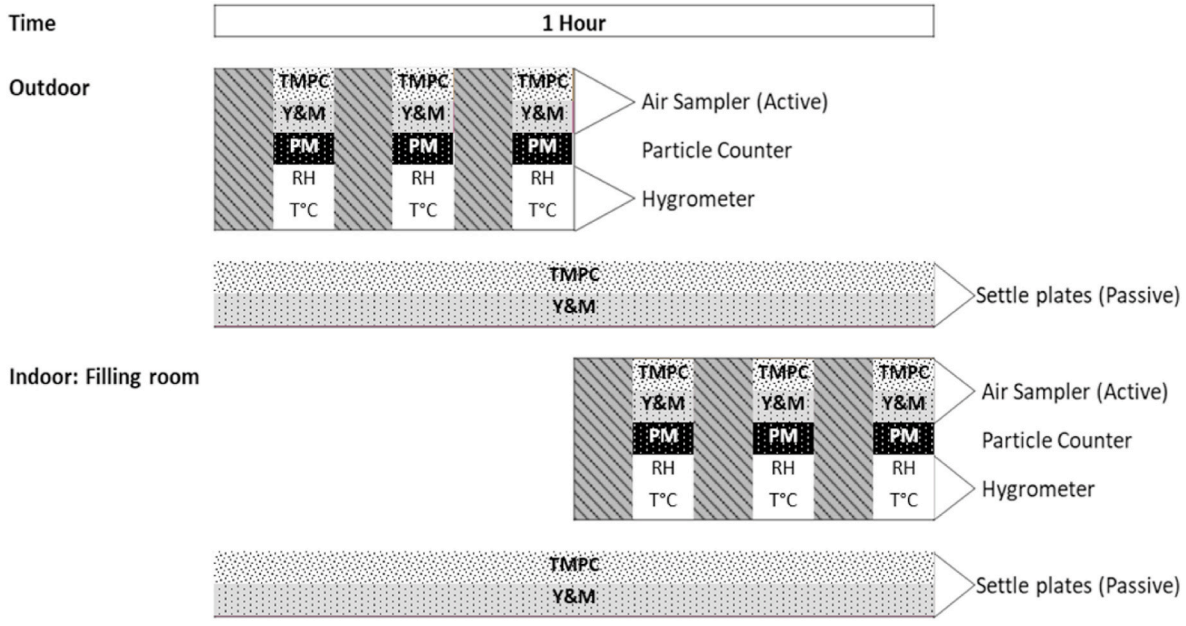

Fig. 1. Sampling plan over time for each filling room at selected companies, with a distinction between active and passive air sampling, particulate matter (PM) and environmental parameters (RH, T°C); with as microbial parameters: total mesophilic plate count (TMPC) and yeast and mould (Y&M) count.

$$L_c = v_s \times C_{air} \times A \times t \quad (3)$$

with L_c = contamination level (cfu); v_s = settling velocity (m/s); C_{air} = number of microorganisms in the air (cfu/m^3); A = exposed product area (m^2); t = exposure time (s).

Settling velocity v_s is estimated by dividing $C_{settled}$ (2) by C_{air} (1).

2.5. Statistical analysis

In the quantification of microorganisms, both settle plate and impaction techniques were utilized. Subsequently, for further assessment, only the impaction technique was employed and subsequently discussed.

Since the raw data was not normally distributed, a log transformation of TMPC, Y&M count and total particle count, was performed. Statistical analysis for this study was conducted using IBM SPSS Statistics (Version 28) for Windows.

The results of log cfu were compared using analysis of variance (ANOVA) with significance defined at the 95% level ($P < 0.05$). When assumptions of ANOVA (normality and homogeneity of variance) are violated, nonparametric Mann-Whitney tests (two independent groups), Kruskal-Wallis tests (> two independent groups) were applied to compare data sets. In case of normal distribution but unequal variances, Welch's ANOVA was used (> two independent groups). Post hoc Turkey's test (or Games-Howell test) were applied to further investigate the differences between groups.

Pearson's correlation coefficients were categorized as very weak when $0.000 \leq r \leq 0.200$; weak when $0.201 \leq r \leq 0.400$; moderate when $0.401 \leq r \leq 0.600$; strong when $0.601 \leq r \leq 0.800$; and very strong when $0.801 \leq r \leq 1.000$ (Christmann & Badgett, 2009).

3. Results

3.1. Airborne microorganisms in (semi) liquid food packaging areas

The results obtained by the impaction technique demonstrated a wide range of TMPC (< LOD 2–3.72 log cfu/m^3) as well as Y&M count (< LOD 2–3.50 log cfu/m^3) among the diverse companies (Tables 2 and 3). Welch's Analysis of Variance (ANOVA) revealed that the mean concentration of TMPC and Y&M count differed significantly among companies ($p < 0.05$). Post hoc Games-Howell revealed seven distinct groups with significant differences in their mean TMPC. This finding is noteworthy considering the lower standard deviation (SD = 0.66) for TMPC compared to Y&M count (SD = 0.74), which showed only five distinct groups with significant differences. This suggests that the TMPC exhibits more homogeneity in its data distribution despite having more distinct groups with significant mean differences. On the other hand, the Y&M count demonstrates greater variability among the distinct groups.

Application of the settle plate technique revealed a wide range of TMPC (< LOD 2–4.83 log cfu/m^{2}h) as well as Y&M count (< LOD 2–4.53 log cfu/m^{2}h) among the different companies (Tables 4 and 5). Welch's ANOVA underscored the presence of significant differences in the mean concentrations of TMPC and Y&M count among these companies ($p < 0.05$). Post hoc Games-Howell identified five distinct groups with

Table 2
Total mesophilic plate count (TMPC) of airborne microorganisms in the packaging areas of 14 (semi) liquid processing companies, determined by impaction technique.

Company	n	Impaction technique (log cfu/m^3)			
		Mean[1]	SD	Min.	Max.
1	24	1.36^{a}	0.46	0.30	2.27
2	24	2.29^{cd}	0.56	< LOD 1	2.91
3	12	3.42^{g}	0.16	3.13	3.72
4	24	1.99^{bc}	0.38	1.20	2.58
5	24	2.07^{bc}	0.39	1.45	2.71
6	12	2.30^{cd}	0.18	2.00	2.57
7	24	1.72^{ab}	0.41	< LOD 2	2.36
8	18	2.03^{bc}	0.33	1.48	2.72
9	18	2.83^{ef}	0.27	2.54	3.48
10	18	2.37^{cd}	0.14	2.04	2.60
11	24	2.72^{de}	0.21	2.43	3.18
12	12	3.21^{fg}	0.18	2.87	3.46
13	6	2.83^{ef}	0.09	2.72	2.95
14	24	1.47^{a}	0.42	< LOD 2	2.23
Total	264	2.20	0.66	< LOD 2	3.72

Values below the limit of detection (LOD) were considered as 1 log cfu/m^3 for 100L samples (LOD 1), and 0.3 log cfu/m^3 for 500L samples (LOD 2); Mean values followed by different letters are significantly ($p < 0.05$) different from each other.
SD: Standard deviation of the log transferred data.
[1] Each value is the mean of n samples.

Table 3
Yeast and mould (Y&M) count of airborne microorganisms in the packaging areas of 14 (semi) liquid processing companies, determined by impaction technique.

Company	n	Impaction technique (log cfu/m^3)			
		Mean[1]	SD	Min.	Max.
1	24	1.45^{a}	0.66	< LOD 2	2.12
2	24	2.55^{bcd}	0.62	1.00	3.03
3	12	2.56^{bcd}	0.64	1.30	3.50
4	24	2.13^{b}	0.60	< LOD 1	3.17
5	24	1.48^{a}	0.38	< LOD 1	2.27
6	12	2.40^{bc}	0.31	1.95	2.86
7	24	1.56^{a}	0.36	< LOD 2	2.09
8	18	2.19^{b}	0.33	1.60	2.70
9	18	3.06^{de}	0.28	2.61	3.48
10	18	2.55^{bcd}	0.22	2.11	2.79
11	24	2.85^{cde}	0.23	2.48	3.42
12	12	2.98^{de}	0.11	2.72	3.13
13	6	3.10^{e}	0.08	3.00	3.20
14	24	1.22^{a}	0.56	< LOD 2	1.90
Total	255	2.14	0.74	< LOD 2	3.50

Values below the limit of detection (LOD) were considered as 1 log cfu/m^3 for 100L samples (LOD 1), and 0.3 log cfu/m^3 for 500L samples (LOD 2); Mean values followed by different letters are significantly ($p < 0.05$) different from each other.
SD: Standard deviation of the log transferred data.
[1] Each value is the mean of n samples.

Table 4
Total mesophilic plate count (TMPC) in the packaging areas of 14 processing companies, determined by settle technique.

Company	n	Settle plate technique (log cfu/m^{2}h)			
		Mean[1]	SD	Min.	Max.
1	24	2.60^{a}	0.44	< LOD	3.54
2	24	3.13^{bcd}	0.41	< LOD	3.64
3	12	4.13^{e}	0.22	3.79	4.39
4	24	2.88^{abc}	0.42	< LOD	3.52
5	15	3.33^{cd}	0.59	2.23	4.09
6	12	3.19^{cd}	0.18	2.81	3.46
7	24	2.64^{ab}	0.45	< LOD	3.80
8	18	2.89^{abc}	0.38	< LOD	3.39
9	18	3.54^{d}	0.18	3.34	3.90
10	18	3.17^{cd}	0.2	2.80	3.52
11	24	3.58^{d}	0.55	2.50	4.80
12	12	4.16^{e}	0.47	3.28	4.83
13	6	3.57^{d}	0.21	3.29	3.83
14	24	2.54^{a}	0.36	< LOD	3.28
Total	264	3.13	0.62	< LOD	4.83

Values below the limit of detection (LOD) were considered as 2.2 log cfu/m^{2}h; Mean values followed by different letters are significantly ($p < 0.05$) different from each other; SD: Standard deviation of the log transferred data.
[1] Each value is the mean of n samples.

Table 5
Yeast and mould (Y&M) count in the air in the packaging areas of 14 (semi) liquid processing companies, determined by settle technique.

Company	n	Settle plate technique (log cfu/m^{2}h)			
		Mean[1]	SD	Min.	Max.
1	24	2.39^{a}	0.28	< LOD	3.04
2	24	3.17^{cdef}	0.62	< LOD	3.80
3	12	3.41^{defg}	0.62	2.28	4.14
4	24	3.04^{bcd}	0.39	< LOD	3.69
5	18	2.82^{abc}	0.39	< LOD	3.67
6	12	3.08^{bcde}	0.22	2.80	3.43
7	24	2.59^{ab}	0.32	< LOD	3.31
8	18	2.98^{bcd}	0.36	2.19	3.43
9	18	3.84^{g}	0.26	3.48	4.24
10	18	3.11^{cde}	0.43	< LOD	3.64
11	24	3.71^{g}	0.49	2.90	4.53
12	12	3.54^{efg}	0.21	3.28	3.84
13	6	3.65^{fg}	0.19	3.47	3.85
14	24	2.36^{a}	0.32	< LOD	3.34
Total	258	3.05	0.61	< LOD	4.53

Values below the limit of detection (LOD) were considered as 2.2 log cfu/m^{2}h; Mean values followed by different letters are significantly ($p < 0.05$) different from each other; SD: Standard deviation of the log transferred data.
[1] Each value is the mean of n samples.

significant variations in their mean TMPC values, compared to Y&M count, which showed seven distinct groups with significant differences. Strong correlation was found between TMPC and Y&M count, both for impaction and settle plate sampling techniques (with respective correlation coefficients of $r = 0.71$ and $r = 0.75$). Moreover, strong correlation was observed between these two sampling techniques, both for TMPC and Y&M count, with respective correlation coefficients of $r = 0.76$ for each)

Table 6 demonstrates a strong correlation between the active and passive air sampling methods and between TMPC and Y&M, for both active and passive sampling.

3.2. Estimated product contamination via the air for TMPC and Y&M in the (semi) liquid food packaging areas

The study reveals substantial variation in estimated airborne contamination of the products among the sampled companies (Table 7). For instance, company 8, categorized within risk class II-IV, exhibits an estimated −2.79 log cfu of contamination in terms of TMPC. This implies that one in 315 products is contaminated with a single cfu. In contrast, company 11, categorized in risk class III, poses a higher estimated contamination for TMPC, with 17 cfus settling on each product. The results of independent t-tests highlight a significant difference ($p < 0.05$) in settling velocities between Y&M count and TMPC. This disparity leads to lower airborne contamination levels for Y&M count, as they tend to remain suspended in the air for longer durations compared to TMPC. The highest contamination levels for both Y&M count and TMPC were devoted to companies 9, 11 and 13, primarily attributed to the prolonged filling time and a larger surface area exposed to the surrounding air.

Fig. 2 illustrates a matrix representing estimated airborne contamination with TMPC and Y&M. An acceptable threshold for airborne contamination depends on the extent to which a product is susceptible to spoilage. For instance, a company situated in risk class 1 will need to adhere to less stringent limits regarding the number of airborne microorganisms than a company in risk class 5. It is shown that company 12, which would have an equally high airborne contamination rate for TMPC per product as company 2, will not yield the same consequences on the quality (shelf life) of the final product.

Table 6
Correlation in settle plate technique (P) and impactor method (A), correlation in TMPC and Y&M count for indoor air samples.

	TMPC (P)	Y&M (A)
TMPC (A)	0.76*	0.71*
Y&M (P)	0.75*	0.76*

* Correlation is significant at the 0.05 level.

Table 7
Calculated contamination level in the product for 14 food processing companies based on the mean settling velocities of total mesophilic plate count (TMPC) and yeast & mould (Y&M) count in (semi) liquid food packaging areas, exposed surface and exposure time.

Company	n	v_s TMPC (cm/s)	v_s Y&M count (cm/s)	A (cm^2)	T (s)	L_c TMPC (log cfu)	L_c Y&M count (log cfu)
1	8	10.64 ± 15.51	4.01 ± 5.01	28 ± 0	10 ± 0	−2.47 ± 0.41	−2.70 ± 0.26
2	8	2.15 ± 0.10	1.37 ± 0.65	50 ± 0	10 ± 0	−1.70 ± 0.39	−1.64 ± 0.61
3	4	1.6 ± 1.00	5.90 ± 9.55	28 ± 0	30 ± 0	−0.49 ± 0.23	−1.13 ± 0.58
4	8	2.52 ± 1.90	3.63 ± 4.30	28 ± 0	10 ± 0	−2.20 ± 0.41	−2.04 ± 0.67
5	6	8.84 ± 7.66	6.11 ± 7.67	24 ± 0	15 ± 0	−1.65 ± 0.61	−2.13 ± 0.39
6	4	2.23 ± 0.72	1.38 ± 0.43	24 ± 0	15 ± 0	−1.79 ± 0.13	−1.90 ± 0.18
7	8	4.02 ± 5.06	3.61 ± 3.02	24 ± 0	10 ± 0	−2.50 ± 0.45	−2.53 ± 0.26
8	6	2.73 ± 2.03	2.28 ± 1.33	13 ± 6	6 ± 1	−2.79 ± 0.52	−2.68 ± 0.38
9	6	1.58 ± 0.78	1.70 ± 0.57	163 ± 165	66 ± 58	−0.62 ± 1.39	−0.32 ± 1.29
10	6	1.84 ± 0.72	1.14 ± 0.58	13 ± 0	10 ± 0	−2.27 ± 0.18	−2.34 ± 0.43
11	8	6.18 ± 12.52	4.13 ± 5.44	1128 ± 137	140 ± 21	1.23 ± 0.55	1.35 ± 0.54
12	4	3.50 ± 3.30	1.07 ± 0.31	18 ± 12	12 ± 3	−1.35 ± 0.38	−1.77 ± 0.24
13	2	1.60 ± 0.52	1.03 ± 0.36	314 ± 0	80 ± 0	0.42 ± 0.25	0.50 ± 0.25
14	8	3.63 ± 2.10	3.88 ± 4.29	24 ± 0	6 ± 0	−2.82 ± 0.34	−3.01 ± 0.33
Total	86	4.08 ± 6.81	3.18 ± 4.29	141 ± 323	28 ± 42	−1.63 ± 1.30	−1.72 ± 1.34

Settling velocity v_s estimated by dividing $C_{settled}$ (2) by C_{air} (1) of each sampling; Each value is the mean of n samples.

3.3. Outdoor versus indoor microbial air quality

In the active air sampling outdoor measurements, there was a relatively narrow range of TMPC (1.30–3.35 log cfu/m^3) and Y&M (1.78–3.72 log cfu/m^3) across different companies. Welch's ANOVA indicated a significant difference in the mean concentrations of outdoor TMPC and Y&M among the surveyed companies ($p < 0.05$). Further examination via the post hoc Games Howell test revealed that only the measurements at company 13 differed significantly from the other companies in terms of both TMPC and Y&M. It is worth noting that company 13 had a relatively small sample size of only six samples, which may account for the outlier counts. A weak positive relationship was found between indoor and outdoor air quality for TMPC ($r = 0.24$, $p < 0.05$) and Y&M ($r = 0.39$, $p < 0.05$).

The differences between the indoor and outdoor measurements were evaluated in relation to the seasons, namely winter and summer. During winter, the average count of mesophilic microorganisms in indoor environments was 2.05 (±0.75) log cfu/m^3 and 1.9 (±0.82) log cfu/m^3 for Y&M. In the summer, the average was 2.27 (±0.67) log cfu/m^3 for

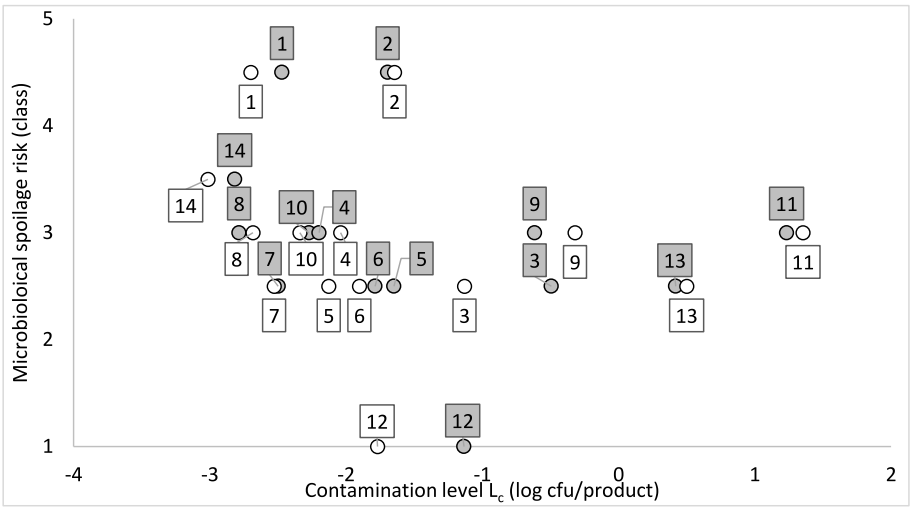

Fig. 2. Contamination with total mesophilic plate count (TMPC) ■ and yeast and mould (Y&M) count □ during filling of (semi) liquid food products in function of risk of microbiological food spoilage of the food product.

TMPC and 2.30 (±0.76) log cfu/m^3 for Y&M. Outdoors, during winter, the averages were 2.29 (±0.29) log cfu/m^3 for TMPC, and 2.36 (±0.23) log cfu/m^3 for Y&M. In summer, the average TMPC was 2.75 (±0.30) log cfu/m^3, and 3.27 (±0.28) log cfu/m^3 for Y&M.

While both indoor and outdoor measurements display variations between winter and summer, the differences appear to be more

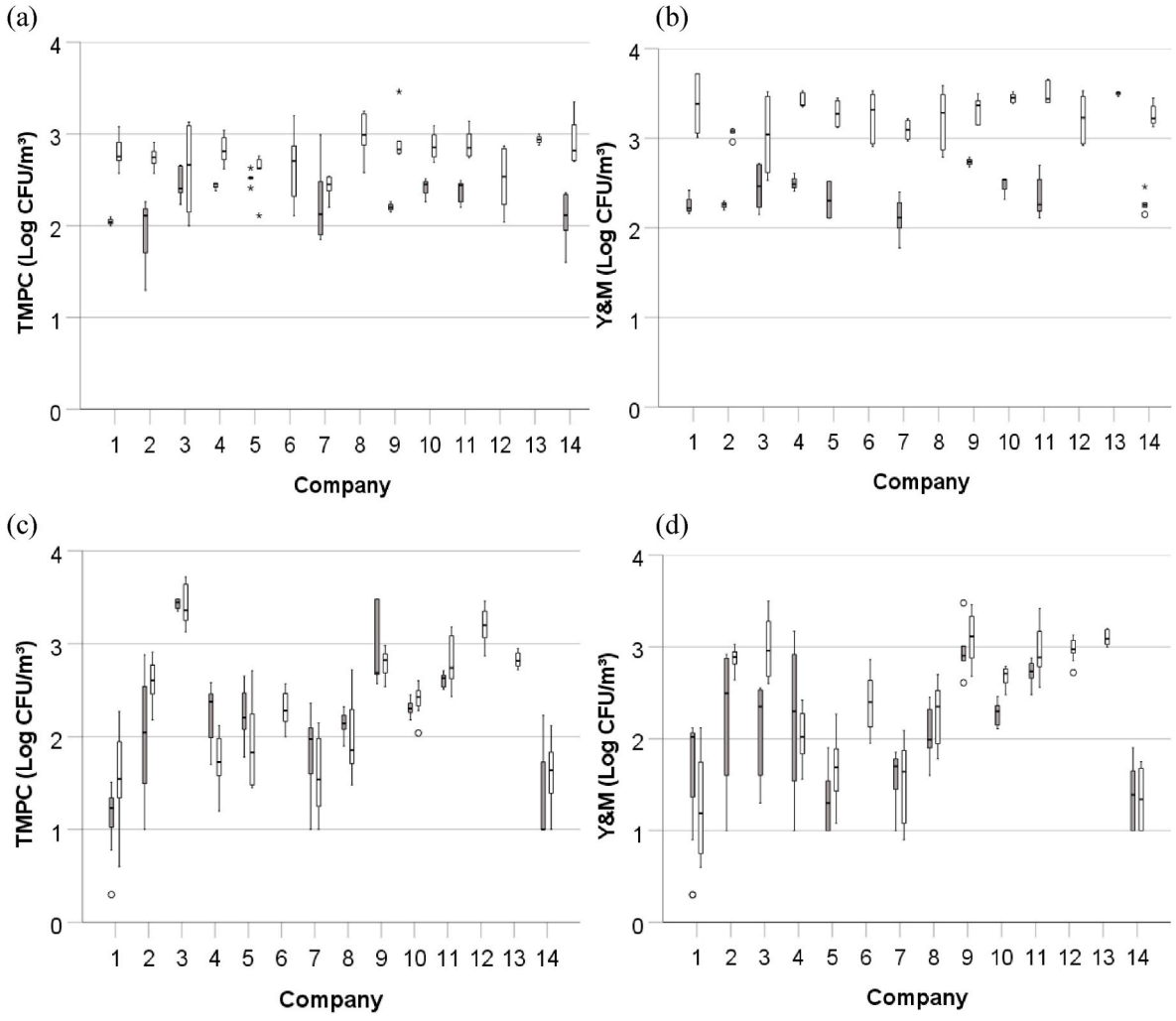

Fig. 3. Comparison of outdoor total mesophilic plate count (TMPC) (a), yeast and mould (Y&M) count (b) and indoor TMPC (c), Y&M (d) (log cfu/m^3), in winter ■ and summer.□

pronounced outdoors, particularly evident in the higher TMPC and Y&M counts during summer compared to winter (Fig. 3). However, the independent *t*-test results indicated no significant difference in TMPC or Y&M count between summer and winter for outdoor samples ($p = 0.985$ and $p = 0.244$, respectively). Indoor samples showed no significant difference in TMPC and Y&M between summer and winter as well ($p = 0.528$ and $p = 0.857$, respectively).

The outdoor microbial air quality exhibits significant variations ($p < 0.05$) across different locations (Fig. 4). However, the urban-rural categorisation of companies (urban, suburban, nonurban) does not show a significant impact on the indoor microbial air quality. This suggests that factors other than outdoor air quality play a crucial role in determining indoor microbial levels.

During this study, the temperature in the filling area ranged from 7.2 to 27.9 °C (Fig. 5a). The lowest temperatures were measured in the savory spreads (fish/meat) sector, where air conditioning units are used. The relative humidity (RH) ranged from 28.1 to 92.7 % (Fig. 5b). The impact of temperature on microbial counts was minimal at lower temperatures (<12.65 °C) and non-significant at higher temperatures within the filling area. Similarly, microbial counts were slightly lower both at low (<51.25%) and high (>72.76%) relative humidity levels. Furthermore, the influence of temperature and relative humidity on TMPC and Y&M count appeared to be uniform, with no significant differential effect between the two types of microbial counts.

3.4. Number of total (non) viable particles

The concentrations of the six size fractions of particles (<0.3 μm. 0.3–0.5 μm. 0.5–1 μm. 1–2.5 μm. 2.5–5 μm. 5–10 μm. and >10 μm) displayed statistically significant correlations with each other, as evidenced by Pearson correlation coefficients (r) ranging from 0.89 to 0.99. However, no significant correlations were found between the concentrations of TMPC & Y&M with any of the PM fraction concentrations, where the correlation coefficients (r) ranged from 0.01 to 0.16. Most companies showed similar levels in the total particle count within the 2.5–5 μm particle range (Fig. 6). However, company 6 challenged the conventional association between high particle counts and elevated TMPC levels, indicating an inverse relationship. Conversely, company 3 demonstrated that low or moderate particle counts did not consistently correspond to equally low or moderate TMPC levels.

The lack of significant correlations between TMPC and PM fraction concentrations prompts a closer examination of the impact of air filter classes in air handling units on microbial levels, as illustrated in Fig. 7. It shows a decline in mean TMPC as the final filter class of air handling units increases, implying the efficacy of higher-class filters in reducing airborne microorganisms. However, this trend does not consistently apply to particles within the 1 to 2.5 or 2.5–5 μm range, indicating that the final filter class may not exert the same impact on TMPC in the same manner as it does on total particle counts. An exception is noted for companies equipped with HEPA 13 filters, though, it is worth noting that this result is based on the sampling of only one company, which may not be representative of all cases.

Moreover, companies equipped with higher final filter classes in their air handling units exhibit more pronounced variations in TMPC compared to those without air handling units. This finding suggests that the presence of an air handling unit may contribute to increased variability in the TMPC within a company. Conversely, the absence of such equipment in the mentioned companies appears to result in a more stable, consistent, yet elevated microbial environment. Similar results are observed for Y&M count (data not shown). Additionally, it is worth noting that despite the 24/7 operation of AHUs and regular maintenance conducted 1–2 times a year at these companies, the higher variability observed cannot solely be attributed to filter contamination.

4. Discussion

The quantification of airborne microorganisms in the high care zone of (semi) liquid food producers, where the transfer of (heat-treated) food products into various packaging materials takes place, has been a relatively unexplored area in previous scientific works (Brandl et al., 2014; Theisinger et al., 2021). This study provides a contribution in conducting a comprehensive sampling campaign that quantifies airborne microorganisms using both the settle plate method and impaction technique. While active air sampling might entail higher costs compared to passive sampling, the strong correlation implies that the two methods can provide consistent and comparable results. The correlation between the two sampling techniques in this study is stronger than the correlation ($r = 0.63$) reported by Asefa et al. (2009), although it is slightly weaker than the very strong correlation ($r = 0.83$) reported by Verhoeff et al. (1990). The choice between active and passive sampling should be made based on specific research objectives and budget constraints.

The variability in airborne TMPC and Y&M observed among different companies in the packaging areas of (semi) liquid food producers aligns with prior research highlighting the diverse nature of airborne microorganisms in food processing facilities (Holah et al., 1995; Zorman & Jeršek, 2008). It is crucial to contextualize the contribution of airborne microorganisms to the overall concentration of microorganisms in these products. Therefore, the estimation of food contamination via the air was conducted by applying existing knowledge, assuming that the main driving force determining deposition of microorganisms is gravitation, and as such is depending on the settling velocity of the particles (Whyte, 1986). Other studies have shown that as bacterial contamination in the air increases linearly, the concentration in the product increases quadratically (Radmore et al., 1988). No matter

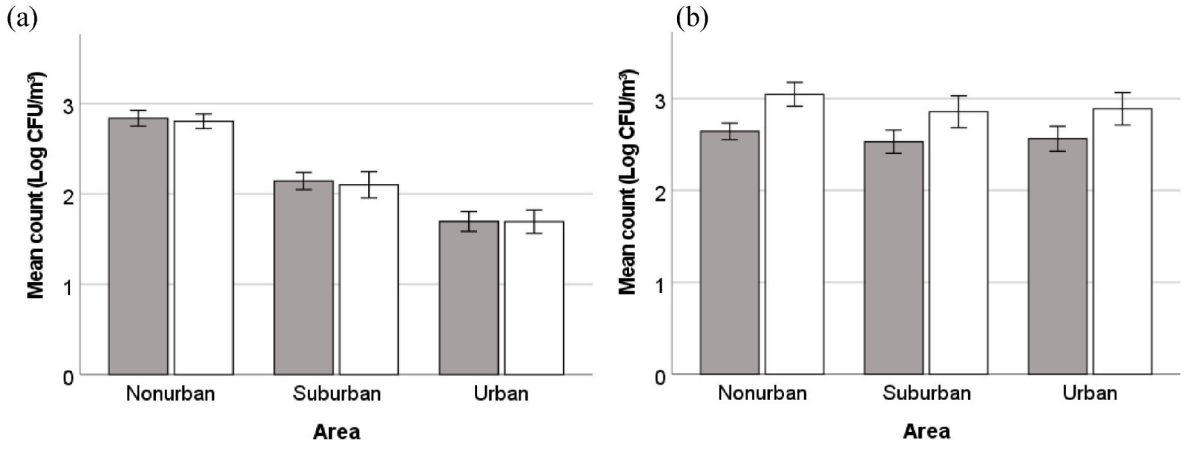

Fig. 4. Influence of location on the airborne concentration of TMPC ■ and Y&M □, outdoor (a) and indoor, around the filling area (b).

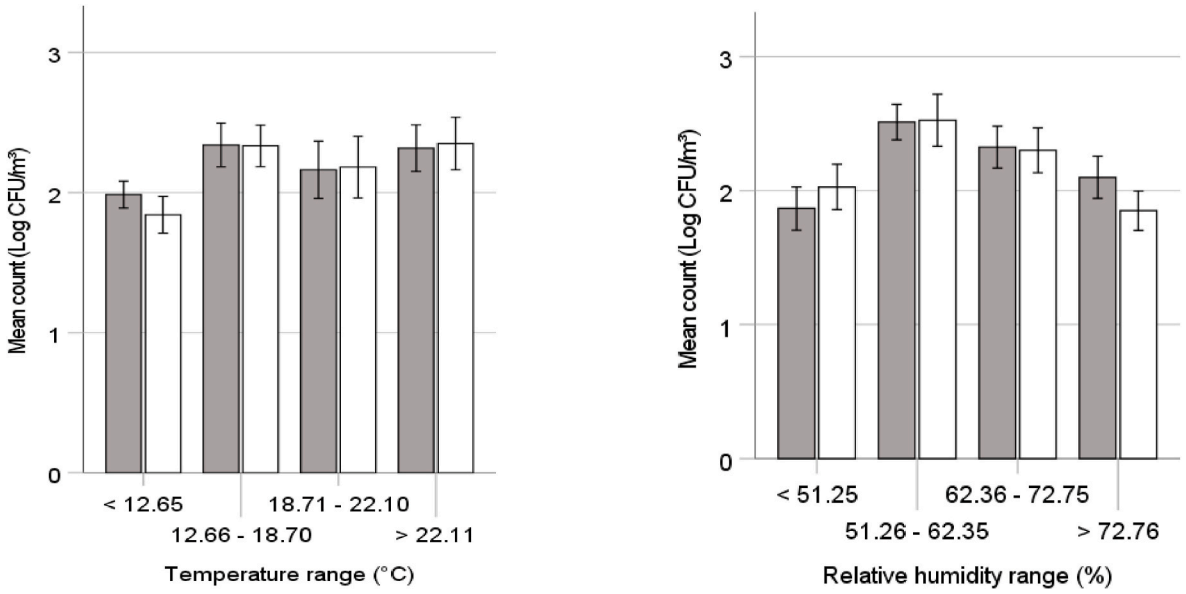

Fig. 5. Influence of temperature (a) and relative humidity (b) at the filling area on total mesophilic plate count (TMPC) ■ and yeast and mould (Y&M) count. □

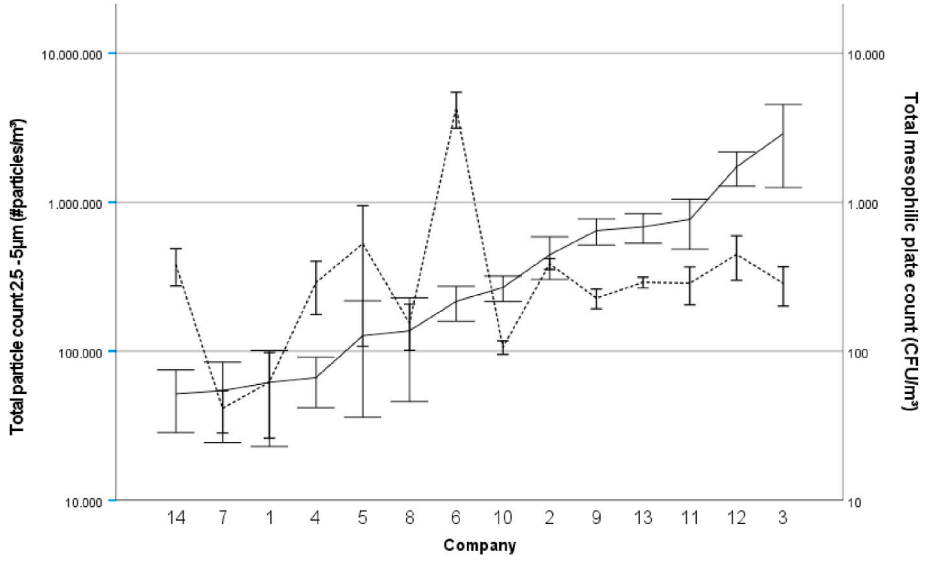

Fig. 6. Total particle count 2.5–5 µm (dotted line) across companies sorted by increasing total mesophilic plate count (TMPC) (full line).

which model is used, a microbial environmental monitoring should be conducted, i.e. the contribution of airborne contamination should be evaluated in comparison with the microbial concentration in the packaged product and other potential sources of recontamination, such as contact surfaces and personnel.

In the context of controlling and preventing airborne microorganism concentrations, investigations were conducted to assess the influence of outdoor air on indoor air quality. The findings indicate that outdoor air has a limited impact on indoor air quality, and seasonal variations may not be a major driver of microbial variability in food facility packaging areas. These results contradict other studies, which demonstrated a seasonal effect (Anaya, Gámez-Espinosa, Falco, Benítez, & Carballo, 2019; Tsai & Liu, 2009); however, these conclusions were based on factories in Cuba and Taiwan, which experience different seasonal patterns than companies in Flanders, Belgium.

In contrast to earlier work by Griffiths and DeCosemo (1994), our findings suggest that controlled environmental parameters, such as temperature and relative humidity, may not be the primary driving forces in preventing airborne microorganisms. In a related context, Salustiano et al. (2003) conducted research within a dairy processing facility and observed that fluctuations in temperature had no significant impact on airborne microbial counts. However, they noted a positive correlation between increasing relative humidity and higher concentrations of airborne microorganisms. Other investigations have proposed that spores exhibit prolonged survival at higher relative humidity ranges (70–80%) compared to lower levels (50–60%). This phenomenon is possibly attributed to the tendency of elevated relative humidity to stimulate fungal growth and spore production, potentially contributing to an increased presence of airborne spores. It is important to note that interpreting these findings can be challenging, as different bacterial strains within the same structural classification may exhibit divergent responses to variations in temperature and relative humidity. Additionally, variations in collection and cultivation methods can introduce significant variability into the outcomes (Cox & Wathes, 1995). Furthermore, it is worth acknowledging that much of the existing literature draws from atmospheric environmental studies, which may

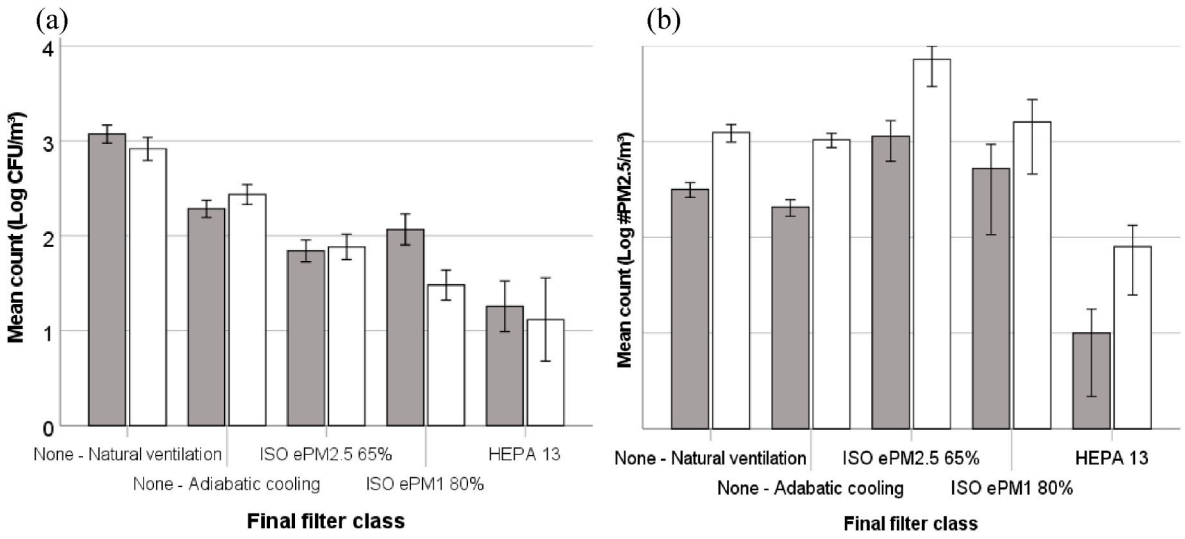

Fig. 7. Influence of final filter class (ISO 16890) on (a) TMPC ■ and Y&M □ and (b) particle size range 1–2.5 μm □ and 2.5–5 μm ■, around the filling area.

not be directly reflective of the conditions encountered within enclosed enviroments such as the food industry.

This study highlights the intricate relationship between total particles, airborne microorganisms, and the filter class of air handling units in the context of preventing airborne microorganisms in the food industry. While there is a well-recognized correlation between total particles and airborne microorganisms in atmospheric studies, this link may not be as straightforward within the unique environments of food processing facilities (Degobbi et al., 2011; Parat et al., 1999). Research on this subject is scarce in the context of the food industry, especially within the high care zones of food processors. Existing literature has identified a connection between total particles and airborne microorganisms in a dairy processing plant (Brandl et al., 2014). However, it is crucial to emphasize that these findings were derived from a single company. Our study, in contrast, encompasses evaluations from 14 companies and disproves the association between particulate matter and airborne microorganisms. The absence of a significant correlation between particulate matter and airborne microorganisms in food packaging areas may be attributed to the unique environmental conditions and activities inherent to these facilities. Due to the distinct sources and dynamics of particulate matter and microorganisms, their dispersion patterns may not directly correlate. The lack of a direct correlation observed in this study suggests that other site-specific factors, such as personnel movements, equipment design, and cleaning protocols, may exert a stronger influence on the microbial quality of air in food packaging environments.

Furthermore, it was observed that the final filter class of air handling units in the filling area is a significant determinant of microbiological air quality. This aligns with the study of González et al. (2016), where the performance of air handling unit filters for PM10 particles and microbial aerosols was investigated experimentally. In that study, the F7/F9 configuration was slightly more efficient for particles and culturable airborne microorganisms than the G4/F7 configuration. However, this was not confirmed in a real factory with full-scale HVAC-systems for TMPC, where the G4/F7 configuration showed lower counts than the F7/F9 configuration.

These findings underscore the pivotal role of air handling filters in regulating airborne microorganisms and suggest that specific guidelines targeting filter standards could be instrumental in maintaining suitable microbiological air quality for high care product packaging. Still, caution is warranted as filter efficiencies are presently determined using mineral aerosols. Given that particles may not necessarily correlate with airborne microorganisms in actual factory settings, there is a question concerning the adequacy of these guidelines in establishing air quality standards for the food industry.

5. Conclusion

In conclusion, this study provides a contribution in the quantification of airborne microorganisms within the high care zones of (semi) liquid food production facilities, particularly during the process of transferring pretreated food products into various packaging materials. The mean concentrations of airborne TMPC and Y&M exhibit considerable disparities among different companies, emphasizing the significance of intrinsic company-specific factors in contributing to airborne microbial contamination. Contrary to some prior research, this study suggests that environmental factors such as temperature and relative humidity may not be the primary drivers of airborne microorganism abundance in food packaging areas. Instead, the filter class of air handling units emerges as a critical factor in regulating microbiological air quality. However, it's important to acknowledge that in real factory conditions, the relationship between particulate matter and microbiological air quality may not hold. These insights emphasize the need for a nuanced understanding of airborne contamination, suggesting that relying solely on total particle counts, environmental factors or outdoor air as an indicator of microbial contamination can be misleading. These findings highlight the complex and context-specific nature of airborne microorganism dynamics in the high care zones of the food industry, emphasizing the importance of tailored preventive measures and further research in this field.

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CRediT authorship contribution statement

Pieter-Jan Loveniers: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Frank Devlieghere:** Supervision, Writing – review & editing. **Imca Sampers:** Conceptualization, Funding

acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Aichinger, K., Burfoot, D., Fahldieck, A., Freund, M., Germain, J. P., Hamer, A., Klemetti, I., Lacroix, N., Nakatani, S., Sasia, G., Sun, X., Van Beek, P., & Wray, S. (2006). Guidelines on air handling in the food industry. *Trends in Food Science & Technology, 17*, 331–336. https://doi.org/10.1016/j.tifs.2005.12.008

Anaya, M., Gámez-Espinosa, E., Falco, A. S., Benítez, E., & Carballo, G. (2019). Characterization of indoor air mycobiota of two locals in a food industry. *Cuba Air Quality*. *Atmosphere & Health, 12*, 797–805. https://doi.org/10.1007/s11869-019-00707-7

Asefa, D. T., Langsrud, S., Gjerde, R. O., Kure, C. F., Sidhu, M. S., Nesbakken, T., & Skaar, I. (2009). The performance of SAS-super-180 air sampler and settle plates for assessing viable fungal particles in the air of dry-cured meat production facility. *Food Control, 20*(11), 997–1001.

Botondi, R. (2019). Hygiene behavior assessment of a hazelnut processing plant. *British Food Journal, 121*, 400–410. https://doi.org/10.1108/bfj-03-2018-0175

Brandl, H., Fricker-Feer, C., Ziegler, D., Mandal, J., Stephan, R., & Lehner, A. (2014). Distribution and identification of culturable airborne microorganisms in a Swiss milk processing facility. *Journal of Dairy Science, 97*(1), 240–246.

Brown, K. L., & Wray, S. (2014). Control of airborne contamination in food processing. In *Hygiene in food processing: Principles and practice* (2th ed., pp. 174–202). Sawston: Woodhead Publishing Limited.

Christmann, E. P., & Badgett, J. L. (2009). Chapter 5: Correlation. In *Interpreting assessment data: Statistical techniques you can use* (pp. 97–114). Arlington: NSTA Press.

Cox, C., & Wathes, C. (1995). Chapter 9: Inertial samplers: Biological perspectives. In *Bioaerosols handbook* (pp. 247–268). Boca Raton: CRC Lewis Publishers.

Degobbi, C., Saldiva, P. H. N., & Rogers, C. (2011). Endotoxin as modifier of particulate matter toxicity: A review of the literature. *Aerobiologia, 27*(2), 97–105. https://doi.org/10.1007/s10453-010-9179-6

den Aantrekker, E. D., Beumer, R. R., van Gerwen, S. J. C., Zwietering, M. H., van Schothorst, M., & Boom, R. M. (2003). Estimating the probability of recontamination via the air using Monte Carlo simulations. *International Journal of Food Microbiology, 87*(1–2), 1–15. https://doi.org/10.1016/S0168-1605(03)00041-2.

European Committee for Standardization. (2020). *Cleanrooms and associated controlled environments—Biocontamination control (EN 17141)*. Retrieved from https://app.nbn.be/data/r/platform/frontend/detail?p40_id=214516&p40_language_code=en&p40_detail_id=92296&session=1321632520746.

González, L. F., Joubert, A., Andrès, Y., Liard, M., Renner, C., & Le Coq, L. (2016). Filtration performances of HVAC filters for PM10 and microbial aerosols—influence of management in a lab-scale air handling unit. *Aerosol Science and Technology, 50*, 555–567.

Griffiths, W. D., & DeCosemo, G. A. L. (1994). The assessment of bioaerosols: A critical review. *Journal of Aerosol Science, 25*, 1425–1458.

Holah, J. T., Hall, K. E., Holder, J., Rogers, S. J., Taylor, J., & Brown, K. L. (1995). *Airborne microorganism levels in food processing environments (R&D Report No. 12)*. Chipping Campden, UK: Campden & Chorleywood Food.

International Organization for Standardization. (2016). *Air filters for general ventilation—Part 1: Technical specifications, requirements and classification system based upon particulate matter efficiency (ePM) (EN ISO 16890-1)*. Retrieved from https://www.iso.org/standard/57864.html.

Kang, Y.-J., & Frank, J. F. (1989). Biological aerosols: A review of airborne contamination and its measurement in dairy processing plants. *Journal of Food Protection, 52*, 512–524. https://doi.org/10.4315/0362-028x-52.7.512

Masotti, F., Cattaneo, S., Stuknytė, M., & De Noni, I. (2019). Airborne contamination in the food industry: An update on monitoring and disinfection techniques of air. *Trends in Food Science and Technology, 90*, 147–156. https://doi.org/10.1016/j.tifs.2019.06.006

Miaskiewicz-Peska, E., & Lebkowska, M. (2012). Comparison of aerosol and bioaerosol collection on air filters. *Aerobiologia, 28*, 185–193.

Parat, S., Perdrix, A., Mann, S., & Baconnier, P. (1999). Contribution of particle counting in assessment of exposure to airborne microorganisms. *Atmospheric Environment, 33*(6), 951–959.

Parussolo, G., Bernardi, A. O., Garcia, M. V., Stefanello, A., Silva, T., dos, S., & Copetti, M. V. (2019). Fungi in air. raw materials and surface of dry fermented sausage produced in Brazil. *LWT - Food Science and Technology, 108*, 190–198. https://doi.org/10.1016/J.LWT.2019.03.073

Pasquarella, C., Pitzurra, O., & Savino, A. (2000). The index of microbial air contamination. *Journal of Hospital Infection, 46*, 241–256. https://doi.org/10.1053/jhin.2000.0820.

Radmore, K., Holzapfel, W. H., & Lück, H. (1988). Proposed guidelines for maximum acceptable airborne microorganism levels in dairy processing and packaging plants. *International Journal of Food Microbiology, 6*(1), 91–95.

Ren, T. J., & Frank, J. F. (1992). Measurement of airborne contamination in two commercial ice cream plants. *Journal of Food Protection, 55*(1), 43–47. https://doi.org/10.4315/0362-028X-55.1.43

Salustiano, V. C., Andrade, N. J., Cardoso Brandão, S. C., Cordeiro Azeredo, R. M., & Kitakawa Lima, S. A. (2003). Microbiological air quality of processing areas in a dairy plant as evaluated by the sedimentation technique and a one-stage air sampler. *Brazilian Journal of Microbiology, 34*(3), 255–259. https://doi.org/10.1590/S1517-83822003000300015

Theisinger, S. M., de Smidt, O., & Lues, J. F. (2021). Categorisation of culturable bioaerosols in a fruit juice manufacturing facility. *PLoS One, 16*, 4. https://doi.org/10.1371/journal.pone.0242969

Tsai, M. Y., & Liu, H. M. (2009). Exposure to culturable airborne bioaerosols during noodle manufacturing in central Taiwan. *Science of The Total Environment, 407*, 1536–1546.

Valle Garcia, M., Sonnenstrahl Bregão, A., Parussolo, G., Olivier Bernardi, A., Stefanello, A., & Venturini Copetti, M. (2019). Incidence of spoilage fungi in the air of bakeries with different hygienic status. *International Journal of Food Microbiology, 290*, 254–261. https://doi.org/10.1016/j.ijfoodmicro.2018.10.022

VDMA. (2007). *Hygienic filling machines for liquid and viscous foods – classification and typical fields of application, Document No. 2/2000. English revised edition*. Retrieved from: https://www.vdma.org/documents/34570/15113577/FS_2_2000_revision+2016_English.pdf/5f43d0be-ceb3-7874-b03e-40aa039c82e5?t=1618997470059.

Verhoeff, A. P., Van Wijnen, J. H., Boleij, J. S. M., Brunekreef, B., Van Reenen-Hoekstra, E. S., & Samson, R. A. (1990). Enumeration and identification of airborne viable mould propagules in houses: A field comparison of selected techniques. *Allergy, 45*(4), 275–284.

Whyte, W. (1986). Sterility assurance and models for assessing airborne bacterial contamination. *PDA Journal of Pharmaceutical Science & Technology, 40*(5), 188–197.

Whyte, W., Green, G., & Whyte, W. M. (2012). Removal of microbe-carrying particles by high efficiency air filters in cleanrooms. *International Journal of Ventilation, 10*(4), 339–351.

Zand, E., Brockmann, G., Schottroff, F., Zunabovic-Pichler, M., Hartmann, A., Kriegel, M., & Jaeger, H. (2022). Identification of microbial airborne contamination routes in a food production environment and development of a tailored protection concept using computational fluid dynamics (CFD) simulation. *Journal of Food Engineering, 334*. https://doi.org/10.1016/j.jfoodeng.2022.111157

Zhivov, A., Skistad, H., Mundt, E., Posokhin, V., Ratcliff, M., Shilkrot, E., … Zhao, F. (2020). In H. D. Goodfellow, & R. Kosonen (Eds.), *Chapter 7—principles of air and contaminant movement inside and around buildings. Industrial Ventilation Design Guidebook* (2nd ed., pp. 245–370).

Zorman, T., & Jeršek, B. (2008). Assessment of bioaerosol concentrations in different indoor environments. *Indoor and Built Environment, 17*(2), 155–163. https://doi.org/10.1177/1420326X08089251