

Simple and affordable method for monitoring the microbiological quality of industrial air in developing countries

AHMAD KHALID NAYAB – LUBOMÍR VALÍK – ELENA PIECKOVÁ

Summary

Monitoring the microbiological quality of air in developing and least-developed countries, particularly in small and medium-sized food manufacturing enterprises, is a major challenge owing to economic and technical constraints. This study aimed to address the issue and observe the feasibility of using the gravimetric method as a less expensive and reliable alternative to the standard but the more expensive volumetric method. The gravimetric and volumetric methods were used to assess the microbiological quality of indoor air in the dairy industry. The gravimetric method was accomplished as per the index of microbiological air contamination (*IMA*) and the volumetric method using an aeroscope. Obtained results were compared using the statistical *t*-test and correlation test. Both methods produced quite similar results with a *p*-value > 0.210, which is much higher than the significance level of 0.05, and with Pearson's correlation coefficient $R > 0.996$, which shows a strong positive association between the two data sets. The obtained data suggest that the gravimetric method can give results similar to the volumetric method and it can be used to monitor the microbiological quality of industrial air as a good alternative. However, a comprehensive standardization of the gravimetric method is recommended prior to its application.

Keywords

gravimetric method; aeroscope; food industry; airborne; fungi; least-developed economies

Microbiological analysis of indoor and outdoor air revealed that filamentous fungi, moulds, are the dominant group of microorganisms there (77 %), while *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* are the dominant genera [1, 2]. Outside air is the primary source of indoor mycobiota [3]. Some other studies evaluated the microbiological quality of various medical [4] and educational [5] indoor atmospheres. *Penicillium* was the dominant fungal genus in the studied sites, followed by *Alternaria* and *Aspergillus*.

As people spend a large proportion of their time indoors [6], poor indoor air quality may result in ill health conditions [7]. When it comes to food spoilage and food-borne diseases, the food industry is a vital indoor space. In the food industry, air, personnel and surfaces are significant sources of microbial contamination [8], including recon-

tamination [9] and post-pasteurization contamination [10]. Airborne fungi and their mycotoxins have been studied extensively in a variety of food industries, including dairy [11], beverages [12], cereals [13], bakeries [14] and fruit industry [15]. Continuous objective monitoring of the environment [11], hygienic design of the food processing line [16], clean room clothing system [17] as well as adequate air treatment and ventilation [8] must be achieved to avoid airborne contamination in the food industry.

The application of air for drying [18], heating and cooling [19] as well as modified atmosphere packaging (MAP) raises concerns regarding microbiological safety of industrial air. When air is heated to a critical high temperature for heating purposes, it may no longer be suitable for vital microorganisms. However, in practice,

Ahmad Khalid Nayab, Lubomír Valík, Department of Nutrition and Food Quality Assessment, Institute of Food Sciences and Nutrition, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, 81237 Bratislava, Slovakia.

Elena Piecková, Department of Microbiology, Faculty of Medicine, Slovak Medical University in Bratislava, Limbová 12, 83303 Bratislava, Slovakia.

Correspondence author:

Lubomír Valík, e-mail: lubomir.valik@stuba.sk

Tab. 1. Comparison of the volumetric and gravimetric methods for monitoring the microbiological quality of industrial air [28–30].

Comparison	Methods		Superiority
	Volumetric	Gravimetric	
Acceptability by official guidelines	Yes	No	V
Sample collection speed	Rapid	Slow	V
Sensitivity to air movement	Low	High	V
Availability	Low	High	G
Taking many samples at the same time	No	Yes	G
Overall costs including maintenance	High	Low	G
Calibration needed frequently	Yes	No	G
Noise production during sampling	Yes	No	G
Device cleaning prior to sampling	Needed	Not needed	G
Extra training for an operator	Needed	Not needed	G

V – volumetric method, G – gravimetric method.

air usually does not reach such high temperatures to limit the survival of all microorganisms, including the thermotolerant ones. Consequently, it can endanger the safety of food items and subsequently consumers. The air is used in MAP in processing of fish [20], chicken [21] and other bird meat products [22], shrimp [23], cheese [24], fruits [25], bread [26] and pasta [27].

Economic constraints and the demands to keep the product cost low occasionally result in compromises in product quality, particularly in small and medium-sized food enterprises in the least-developed economies. Therefore, more affordable though reliable methods must be tested and suggested for application in various fields of industries. Active or passive methods of air sampling can be applied for microbiological air quality control. Active sampling methods are mostly based on impingement, centrifugation, filtration or impaction. The latter one is more usually applied as volumetric air sampling. On the other hand, the passive air sampling method is based on passive deposition of air particles on the surface of agar plates over time, without the application of forceful direction of the sampled air onto the agar plates. Tab. 1 represents a brief comparison of the volumetric and gravimetric methods based on the literature.

The most significant disadvantage in the case of the application of the gravimetric method is the random sedimentation velocities of particles of various masses, as well as the susceptibility of the particles to airflow and air dynamics. Relationship between particle size and sedimentation velocity has been studied. Eq. 1 fits the data present in the literature [28, 31], for the particle mass in the range of 0–100 μm with a high value of the determination coefficient ($R^2 = 0.999$), which

corresponds with the sedimentation velocity in the range of 0–0.25 $\text{m}\cdot\text{s}^{-1}$.

$$y = 0.0000215x^2 + 0.000348x \quad (1)$$

The objective of this research was to compare the microbiological quality monitoring of industrial air using two different methods (the gravimetric and the volumetric) and to assess the possibility of applying the gravimetric method for objective assessment as an alternative to the volumetric method in situations where aeroscopes are unavailable or unaffordable.

MATERIALS AND METHODS

Sampling

Two different methods of air sampling, active and passive, were applied to monitoring the industrial air quality in various sampling sites in a dairy company.

The aeroscope model A-AIR-010 HVP (Agea, Prague, Czech Republic) was employed for 9 min (the longest possible operation time at one run, with a flow rate of 1.11 $\text{l}\cdot\text{s}^{-1}$ and a total suction volume of 0.6 m^3 of air in a single run) in the volumetric method. In the gravimetric method, agar plates were exposed to air for a duration of a time interval of 5–30 min, according to the index of microbial air contamination (*IMA*) standard [28].

A total of 93 samples were taken in a four-months timeframe. Subsequently, Petri dishes containing nutrient agar (NA, Sigma Aldrich, St. Louis, Missouri, USA) were incubated for 48 h at 30 °C to observe total plate count (*TPC*) microbial numbers. Petri dishes containing malt extract agar (MEA, Sigma Aldrich) were incubated for 5 days at 25 °C to monitor fungal count.

The experiments were done according to all the requirements of good manufacturing practice, in all three sampling sites, namely cottage cheese production area, fermentation hall and yoghurt-filling area. Besides counting and recording the numbers of microbial colonies grown on each agar plate, fungal genera were identified according to their macroscopic and microscopic characteristics.

Gravimetric method

The correlation between logarithm of colony forming units per square meter of a Petri dish surface and logarithm of transformation of time (t , expressed in seconds) was evaluated. The fitness of this correlation was evaluated using Pearson's correlation coefficient R , value of which was > 0.967 for all sampling sites.

Volumetric method

The particular formula to calculate the number of the colonies per volume unit of air from the number of colonies growing on the surface of an agar plate, was given by the aeroscope producer as follows:

$$C = N \times \frac{15}{9} \quad (2)$$

where N is the total cultivable plate count (average number of duplicate plates exposed per sampling, expressed in colony forming units) and C is the number of microbial cells in the air (expressed in colony forming units per cubic metres of air).

Comparison of the results

Since the results of the gravimetric method were reported as logarithm of colony forming units per unit of agar plate surface divided by loga-

rithm of time (expressed in seconds) and the results of the volumetric method were presented as logarithm of colony forming units per unit of air volume, we were unable to directly compare these two data sets. Therefore, we computed the ratios between the outcomes of various sampling sites in both methods and compared them using the t -test and Pearson's correlation test.

RESULTS

Quantity of microorganisms

After the incubation period, the number of colonies that grew on the agar plate was recorded according to the time of their exposure to ambient air. Fig. 1 illustrates these findings, which show a linear correlation between the logarithm of time and the logarithm of the number of colony forming units per square meter of the plate surface with a correlation coefficient $R > 0.967$. The microbial counts increased with exposure time. However, the fact that the slopes within the independent experiments were similar to each other can confirm the real possibility of the gravimetric method to compare the findings earned exactly at the same time of exposure.

Data on numbers of colony forming units related to one cubic meter of indoor air sampled volumetrically are displayed in Fig. 2 in logarithm.

As the results obtained by the tested methods were presented in different units, one per unit surface area of an agar plate (or the slope of the linear correlation between the logarithm of colony forming units per unit of agar plate surface area and the logarithm of time) and the other per unit volume of sampled air, therefore a direct compari-

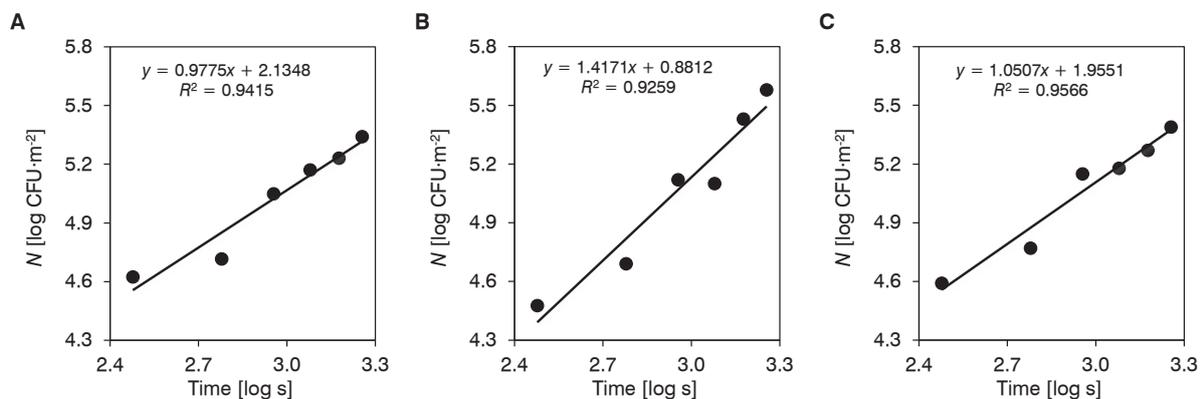


Fig. 1. Linear correlation of microbial counts per square meter of surface with time in samples from various food production sites.

A – cottage cheese production area, B – fermentation hall, C – yoghurt-filling area.
 N – total microbial counts.

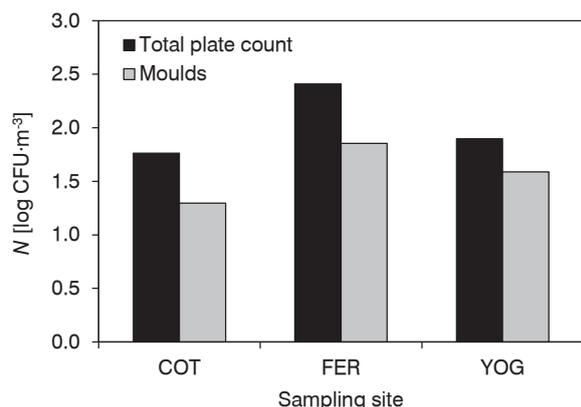


Fig. 2. Microbial counts per cubic meter of sampled air in various food production sites.

N – total microbial counts, COT – cottage cheese production area, FER – fermentation hall, YOG – yoghurt filling area.

Tab. 2. Data on microbiological contamination obtained by volumetric and gravimetric methods in various food production sites.

Sampling site	Gravimetric method	Volumetric method
	Linear correlation slope	Microbial counts [log CFU·m ⁻³]
COT	0.978	1.760
FER	1.417	2.410
YOG	1.051	1.900

COT – cottage cheese production area, FER – fermentation hall, YOG – yoghurt filling area.

Tab. 3. Proportional comparison of results obtained in various food production sites.

Comparison	Ratio		R	
	Gravimetric method	Volumetric method		
Cottage cheese production area				
A	COT/FER	0.690	0.730	0.996
B	COT/YOG	0.931	0.926	
A/B		0.741	0.788	
Fermentation hall				
C	FER/COT	1.449	1.369	0.996
D	FER/YOG	1.348	1.268	
C/D		1.075	1.080	
Yoghurt filling area				
E	YOG/COT	1.075	1.080	0.999
F	YOG/FER	0.742	0.788	
E/F		1.449	1.371	

COT – cottage cheese production area, FER – fermentation hall, YOG – yoghurt filling area.

R – Pearson's correlation coefficient (calculated using the correlation test).

son of the methods was not possible. So, first we compared ratios between data from all sampling sites within each data set and subsequently, we compared the calculated ratios, which were both similarly based on the ratios of the same sampling sites.

After computing the ratios between the results of various sampling sites obtained by both methods and comparing them using the t -test and correlation test using Microsoft Excel program (Microsoft, Redmond, Washington, USA), we got Pearson's correlation coefficient $R > 0.996$ for all sampling sites. This showed a strong positive association between the two data sets and a p -value of > 0.210 for all observed sampling areas, which was much higher than the significance threshold level of 5%. The total plate microbial counts obtained by the volumetric method expressed in colony forming units per cubic meter of air and the slopes of the linear correlations obtained by the gravimetric method are given in the Tab. 2. The proportional comparison of results obtained by both methods is detailed in Tab. 3.

Fungal genera

Alternaria was phenotypically identified as the predominant fungal genus present in the air of the cottage cheese production area, whereas the *Penicillium* genus outnumbered other airborne fungal genera in the fermentation hall and the yoghurt-filling sampling sites (Fig. 3). Furthermore, *Scopulariopsis* and *Aspergillus* were found in the air of the cottage cheese production area, the latter also being found at the yoghurt-filling sampling site. The highest number of colonies of the *Cladosporium* genus was recorded in the fermentation hall, whereas it was absent in the cottage cheese production area. In addition to these, air samples obtained from the yoghurt-filling sampling site also indicated the presence of a few other fungal genera such as *Mucor* and *Rhizopus*, which were absent in samples taken from other sampling sites.

DISCUSSION

The study findings showed a linear correlation between the number of microorganisms sedimented on the surface of the agar plates per the logarithm of time of exposure (expressed in seconds). However, the slope of this linear correlation varied with the microbial density in the air at the sampling site. The higher the number of microorganisms in the air of the sampling site, the greater the value of the slope of this linear correlation. The findings of this investigation demon-

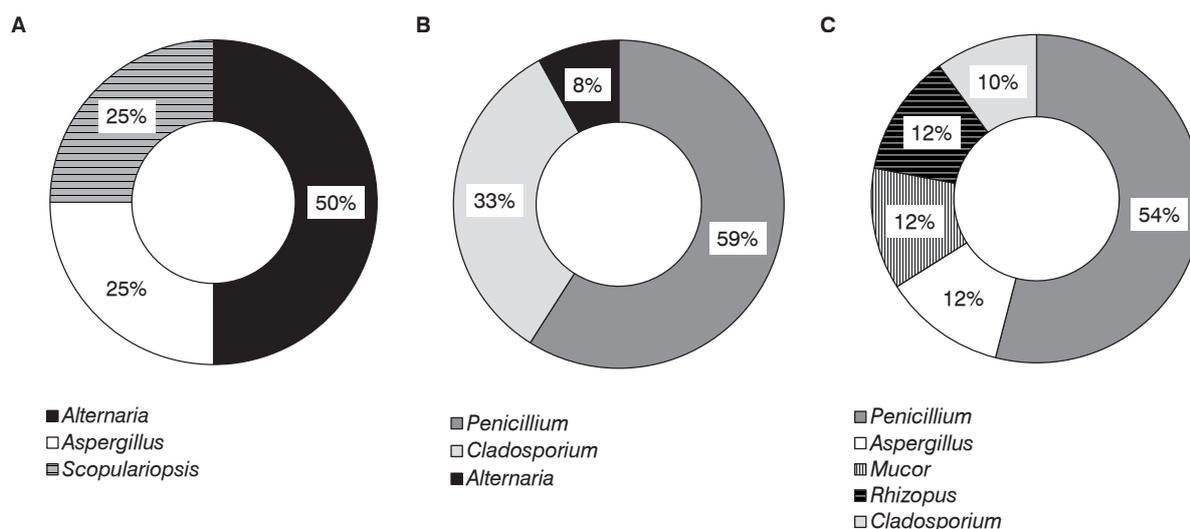


Fig. 3. Proportional presentation of genera of filamentous fungi in sampled air in various food production sites.

A – cottage cheese production area, B – fermentation hall, C – yoghurt-filling area.

strated a significant correlation between exposure time and the number of microorganisms growing on the surface of the plate, with a Pearson's correlation coefficient $R > 0.966$ for all sampling sites. The data from the fermentation hall showed the highest value of this slope (1.417). Data from the other two sampling sites had slightly lower values, 1.051 and 0.978, for the yoghurt-filling sampling site and the cottage cheese production area, respectively. The higher value of this slope indicated the presence of more microorganisms in the air tested compared to other sampling sites. A similar trend was also seen in the case of results obtained by the volumetric method. The logarithm of the number of colony forming units per cubic meter of air was the highest in the fermentation hall (2.410), while it was slightly lower in yoghurt-filling and cottage cheese production areas (1.900 and 1.760, respectively). Both of these results indicated that the fermentation hall was the most polluted and the cottage cheese production area was the least polluted sampling site among the experimental sites.

Correlation coefficient between the two tested data sets, one related to gravimetric method and the other to the volumetric method, at all sampling sites was in the range of 0.996 to 0.999, which is very near to 1.0. This showed a very high correlation of the data obtained by gravimetric method to the data obtained by the standard volumetric method (Tab. 3). Besides, the calculated p -values were higher than the 5% significance level (> 0.05) and in the range of 0.210–0.828, which means that the two data sets were significantly correlated to

each other. This again implied that the results of the gravimetric method were comparable to the standard volumetric method.

The results of our research are in correlation with the results of similar research conducted previously. PUCHIANU et al. [32] studied active and passive monitoring of the microflora in a milk processing unit. The authors concluded that the sedimentation method allowed an approximate quantitative determination, while the other tested method allowed accurate quantitative determination. Another similar research of VERONESI et al. [33] was performed in some dental clinics to compare active and passive methods. The authors found that both methods were capable of evaluating the microbiological quality of the sampled air and highlight critical situations.

Macroscopic and microscopic observations of the colonies recovered from the indoor air yielded the following genera of filamentous fungi: *Alternaria*, *Aspergillus*, *Cladosporium*, *Penicillium*, *Scopulariopsis*, *Rhizopus* and *Mucor*. *Alternaria* is a widespread fungal genus that damages agricultural goods at both pre- and post-harvest levels [34]. *Aspergillus* is an essential airborne fungus that affects human life in various ways, including agriculture, medicine and biotechnologies [35]. *Cladosporium* is a common melanized micromycete that can survive under refrigeration storage conditions and cause black spots on foods including nuts, cereal grains fruits, and chilled meat [36]. *Penicillium* is a particularly influential airborne fungal genus due to its widespread distribution and the role of its numerous species

also in food deterioration and the production of mycotoxins [37]. *Scopulariopsis* is a saprobic fungus of keratinophilic character, which is prevalent in the environment and is only rarely pathogenic [38]. *Rhizopus* is a genus of Zygomycota, growing on dead and decaying plant material [39]. *Mucor* species can grow over an extensive range of temperatures and utilize various carbon and nitrogen sources, making this genus common in various environments, including foods [40].

In the dairy plant, the numbers of colony forming units of microorganisms did not exceed 10^3 CFU·m⁻³ in any of the sampling sites [41, 42]. However, due to their potential pathogenicity, in particular to immunocompromised patients, and their capacity to spoil food, monitoring of air and surfaces, including the packaging materials, consistent monitoring in the food industry is required.

CONCLUSIONS

The involvement of air in spreading microbial and non-microbial substances causing diseases and/or food spoilage is evident and has long been studied. Relevant and reliable microbiological monitoring of air quality is required, particularly in the food industry, where it can influence human health not only directly through breath (especially in personnel), but also through the contamination of food materials (foodborne infections and intoxications). In terms of food safety, consistent control of the microbiological quality of industrial air in developing and least-developed nations has always been a concern. Aeroscopes required to evaluate the microbiological quality of air are expensive and do not provide multisampling, thus, they are not widely used by small and medium-sized businesses in lower-income countries for economical and technical reasons. However, more economically feasible, still technically reliable methods are required for this purpose. The findings of this study suggest that the gravimetric method provides results that are quite similar to the volumetric method, with a Pearson's correlation coefficient $R > 0.996$ and a p -value > 0.210 while comparing using the t -test and the correlation test. According to our results, the gravimetric method can be applied as an alternative to the precise volumetric method, as it provides reliable information on microbiological air quality when monitored regularly, e. g. daily. However, more studies in different experimental settings, and thus standardization of this method, as well as further development of the *IMA* standard, are required before the gravimetric method is universally applied.

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