



Incidence of spoilage fungi in the air of bakeries with different hygienic status



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ABSTRACT

This study aimed to determine the level of adequacy of bakeries to the requirements of good manufacturing practices (GMP) and the fungal contamination of the environmental air of these places, verifying the existence of a correlation between both. The checklist present in Ordinance No 78/2009 of good practices based on the current legislation was used to evaluate GMP adequacy and the contamination of environmental air was determined through the air sampling method. After the application of the checklist, it was observed that 75% ($n = 15$) of the bakeries evaluated were classified into group 2 (good) and 25% ($n = 5$) as group 3 (regular). On the other hand, only five (25%) and three (15%) bakeries presented values below of 300 CFU/m³ for the fungal contamination of air in food cooling and exposure areas, respectively. Fungi isolated from these areas were mainly species commonly reported as potential spoilers of bakery products. This study revealed that in spite of the evaluated bakeries presenting conditions considered “good” by the GMP, they presented, in most cases, high counts of fungi in the cooling and exposition areas for ready products. Only a weak correlation was found between the items of GMP and the load of airborne fungi present in the bakeries. More attention should be directed to hygienic aspects related to the microbiological quality of the air of bakeries, since these microorganisms could contaminate the surface of freshly baked products, compromising the final quality of these foods, as well as prompt its early deterioration.

1. Introduction

Bread has been a part of human nutrition for centuries, playing an important role in feeding people. The Brazilian consumption of bread is estimated at 32.6 kg per inhabitant per year (ABIP, 2017), a low value compared to the recommended amount of the World Health Organization (WHO), which is 60 kg, but represents a R\$ 90.3 billion in 2017 (ABIP, 2017).

Bakeries are a traditional form of retail of a wide diversity of manufactured products, which can deteriorate easily in a short period of time if the establishments do not follow sanitary adequate production procedures (Zimmermann et al., 2016). In Brazil, there are specific sanitary legislations such as the RDC No 216/2004 of the National Agency of Sanitary Surveillance (ANVISA), which aims to establish procedures of good practices for food services in order to guarantee hygienic-sanitary conditions of the prepared food (Brasil, 2004). Therefore, the adoption of good manufacturing practices (GMP) is of extreme importance for maintaining the quality and sanitary conditions of a food service, preventing the contamination of the products and also reducing

future losses due to a subsequent early deterioration (Nascimento and Barbosa, 2007).

In addition, fungal contamination of bakery products is extremely important, as it leads to economic losses in establishments due to the rejection of the deteriorated product by consumers (Legan, 1993). The physicochemical characteristics of bakery products, as well as the lack of hygienic care during their production and storage, are strongly related to the presence of these spoilage fungi in this class of foods (Pitt and Hocking, 2009; Smith et al., 2004).

Contamination of bread occurs after baking and airborne distribution of dust and mold spores is considered the main predisposing cause for bread spoilage. Researches have reported that air is a major source of food contamination as it is capable of carrying fungal spores among production areas. When they are deposited on the surface of the ready-to-eat bread, they can lead to its early deterioration (Garcia et al., 2018; Santos et al., 2016). Besides, a high number of fungal spores present in flour and grains used as raw materials, as well as poor hygiene and precarious processing conditions, can contribute to increasing the load of airborne fungi disperse in the environment and the contamination of

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the final product (Cook and Johnson, 2009).

Despite the relevance of the theme, there are no studies correlating the influence of good practices and the fungal contamination of air in environments that process bread. Based on the assumption that the adoption of quality programs and air contamination in food processing areas are important factors for maintaining the quality of ready-to-eat products, the objective of this study was to determine the level of good manufacturing practices adequacy and the presence of airborne fungi in bakeries, verifying the existence of a correlation between both.

2. Material and methods

2.1. Adequacy of good manufacturing practices in bakeries

A cross-sectional study consisted of a descriptive and observational field survey was carried out in bakeries ($n = 20$) from Santa Maria, RS, Brazil (Latitude: 29° 41' 03" S; Longitude: 53° 48' 25" W). Establishments were chosen by convenience. They were registered and supervised by the Municipal Sanitary Surveillance and have authorized the access to their facilities for research purposes.

The establishments were identified by letters (A to T), aiming to maintain secrecy regarding the name and avoid possible brand damages due to the nonconformities found. A checklist present in the Ordinance No. 78/2009 of the state of Rio Grande do Sul, Brazil, which approves the checklist of good practices and standards for training courses in good practices for food services (Rio Grande do Sul, 2009). This regulation is in accordance with RDC No 216/2004 of ANVISA (Brasil, 2004) was used in order to evaluate the GMP of the visited establishments.

The checklist shows 146 evaluation items, divided into the following blocks:

- Buildings, facilities, equipment, furniture, and utensils,
- Hygiene of facilities, equipment, furniture, and utensils,
- Integrated pest control,
- Water supply,
- Waste management,
- Food handlers (hygiene and competence in GMP),
- Raw material, ingredients, and packaging,
- Preparation of food,
- Storage and transportation of prepared food,
- Exposure to the consumption of prepared food,
- Documentation and registration,
- Responsibility.

The checklist was filled according to the observation made on the sectors of the establishments and verification of the items specified in the list, when necessary. The possible answers for the evaluation were: compliance (C), no compliance (NC) and not applicable (NA).

After calculating the adequacy index of the blocks and establishments, they were classified according to the accomplishment of items in: Group 1 - very good, with 91% to 100% attending; Group 2 - good, with 70% to 90%; Group 3 - regular, with 50% to 69%; Group 4 - bad, with 20% to 49%; and Group 5 - very poor, with 0% to 19% (Saccol et al., 2012).

2.2. Environmental fungal contamination in bakeries

Air sampling was carried out using an air sampler (Sampl'air™, Biomérieux®) in the cooling and exposure areas of the bakeries, where ready bread are most susceptible to environmental contamination. A total of 50 L of air was collected for the determination of filamentous fungi using DG18 (Dichloran Glycerol Agar 18%) culture medium. For the analysis, 90 mm diameter disposable Petri dishes containing about 27 mL of the culture medium (Apha, 1985) were used.

After collection, the plates were incubated under ideal conditions

for growth and enumeration of the fungi present in the air (25 °C for seven days). The analyses were performed in duplicate in each environment.

In order to classify the microbiological quality of the bakery's environmental air, the Fung Scale was followed. This scale was specifically developed for checking the air quality from environments where food is processed, where:

Values	Classification/recommendation
0 to 100 CFU/m ³	Clean air – no concern.
100 to 300 CFU/m ³	Acceptable air – intermediary level – slight concern.
> 300 CFU/m ³	Not acceptable air – serious concern - need for corrective actions.

Adapted by Al-Dagal and Fung (1993).

2.2.1. Enumeration of fungi

The colony count was performed visually, and the results obtained were corrected according to a table contained in the manual of the air sampler (Sampl'air™ Biomérieux). The total number of colonies obtained was equal to "n", with a corresponding "N" number in the table. Once the values were obtained, the final result was calculated using the formula:

$$\text{Contamination level (CFU/m}^3\text{)} = \frac{N}{V}$$

where:

N: number of microorganisms obtained by the conversion in the table;

V: volume, in m³, of air collected in the points.

In order to calculate the value of V, the sampling time (50 L = 30 s) and the air inlet velocity in the sampler (0.1 m³/min) were taken into account. In this case, V was equal to 0.05 m³/min.

2.2.2. Isolation and identification of fungi

About three fungal colonies from each fungus showing similar macroscopic characteristics (color and size of the colony, reverse, exudate production) were isolated for identification from each Petri dish. The fungal colonies were first isolated in Czapeck Agar Yeast Extract (CYA) medium and then identified according to the keys of each genus. *Penicillium* was identified according to Frisvad and Samson (2004) and Pitt (2000). *Aspergillus* spp., and other genera were identified according to Pitt and Hocking (2009) and *Aspergillus* spp. of the Circumdati Section according to Frisvad et al. (2004). The fungi were three points inoculated in different culture medium and had their diameters measured and microscopic characteristics observed after cultivation in different temperatures (5, 25 and 37 °C).

2.3. Correlation between environmental fungi and good manufacturing practices in bakeries

Statistical analyzes (correlation analyzes) were carried out with the software using the R environment, version 3.2.4 (R Development Core Team, 2016). Pearson's correlation coefficients (r) were calculated to identify possible associations between the incidence of fungi (CFU/m³) in the collection points and the adequacy percentage of GMP evaluated items in the checklist.

Interpretation of values was performed according to Pearson's coefficient (r) which are: very weak $0.000 \leq r \leq 0.200$; weak $0.201 \leq r \leq 0.400$; moderate $0.401 \leq r \leq 0.600$; strong $0.601 \leq r \leq 0.800$ and very strong $0.801 \leq r \leq 1.000$ (Christmann and Badgett, 2009).

Also, a heatmap was build clustering by Euclidean distance for the counts of the airborne fungi in the collection points areas of bakeries (Fig. 4) using XLSTAT-OMICS module (XLSTAT® evaluation 2018.6, www.xlstat.com). Bakeries with more similar counts are

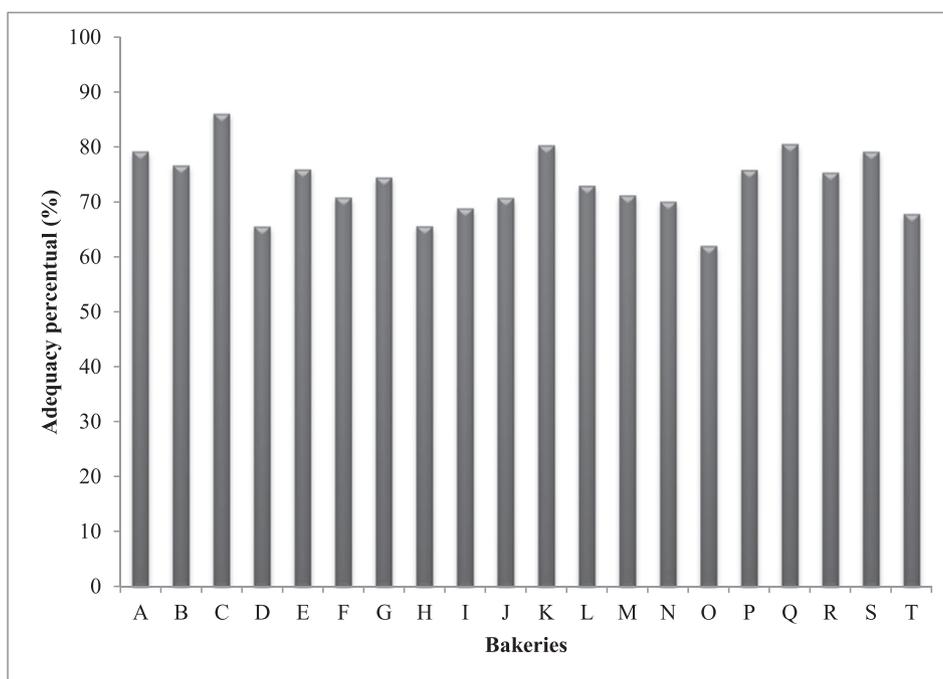


Fig. 1. Average of adequacy to good manufacturing practices requisites of bakeries from Santa Maria-RS, Brazil ($n = 20$).

mathematically clustered closer. The counts for airborne contamination were colored in shades of red (higher values) to yellow (lower values), as shown in the color key.

3. Results and discussion

3.1. Good manufacturing practices adequacy of bakeries

Fig. 1 shows the overall percentage of adequacy achieved by the evaluated bakeries with the application of the good manufacture practices checklist.

When considered by the average of adequacy, 75% of bakeries were classified in Group 2 - good, followed by Group 3 - regular (25%). Several studies have been evaluating good practices in bakeries, however, adequacy values reported are often considered low when compared to the results obtained in the present study. In studies conducted in Brazil, for example, Couss and Cezar (2009) obtained average percentages of compliance around 52.9%, while Guimarães and Figueiredo (2010) obtained a score of 44.8%. Also, Zimmermann et al. (2016), observed a general adequacy average of 42.8%.

In Fig. 2, we can observe the average of adequacy per item evaluated from the GMP by the checklist applied.

The water supply item showed the highest percentage of adequacy among the food services evaluated (100%), followed by integrated pest control (95%). According to Saccol et al. (2013), these items are, in general, the ones showing the highest adequacy with GMP in food services (Saccol et al., 2013). This is generally because the local water supply is carried out by the company responsible for the municipal distribution and also because the bakeries did not use an alternative water source (water tank). Integrated pest control is usually carried out by an outsourced company, which issues reports confirming pest control and prevention procedures.

Satisfactory results were obtained from buildings, installations, equipment, furniture and utensils (73.6%), sanitization of facilities, equipment, furniture and utensils (71.6%), food handlers (88.2%) and exposure of prepared food (78.9%). When evaluating possible causes of food contamination, these items are considered the most relevant, since they, alone or associated with other factors, can be a source of an

outbreak of food-borne diseases or for food alterations (Andrade and Salustiano, 2008; Doménech-Sánchez et al., 2011). Sanitization of facilities and equipment is crucial to obtain products of adequate microbiological quality; however, the microbiological quality of the air in such regulation. According to our perception, most of the people responsible for the sanitization of food-producing environments ignore the air as an important source of micro-organisms, especially fungi. Air contamination is still more relevant in bakeries, where, as previously mentioned, flour has a particular importance on dissemination of fungal spores; and fungi are the main spoilage agent of bakery products.

Application of sanitizer should also be addressed to the air. Still, the choice of sanitization agents effective against molds is crucial. The selection of sanitizer should considerate the antifungal efficacy of the compounds to species with potential to spoil bakery products. A recent study focused on the susceptibility of fungal species isolated from spoiled bakery products was carried out by Bernardi et al. (2019b). The authors found out variability in the sensitivity of different strains from the same species to common commercial sanitizers used in the food industries, highlighting the importance of check the sensitivity of spoilage fungi isolated from each industry, when the deterioration problem occurs. The most effective agents were peracetic acid and sodium hypochlorite, and only the highest recommended concentrations of those sanitizers were effective against all the tested species [*P. roqueforti*, *P. paneum*, *H. burtonii*, and *A. pseudoglaucus* (formerly *Eurotium repens*)].

In food industries, sanitization is the most practical and effective way to reduce the microbial contamination in the processing environment, including the air. Studies have been carried out to evaluate the efficacy of sanitizers in reducing the microbial load of ambient air in order to extend the shelf life of food produced. In the dairy industry, for example, a 15% reduction in fungal and yeast counts was observed using solutions of 1.000 mg/L chlorhexidine digluconate and 75 mg/L peracetic acid. Other results show that twice-weekly spraying of p-hydroxyphenylsilylamide to control fungal contamination showed reductions of 20–25% in the count of airborne fungal spores after a few weeks of treatment (Andrade and Salustiano, 2008).

Moreover, recent studies have demonstrated the in vitro fungicidal efficacy of sodium hypochlorite and peracetic acid solutions for

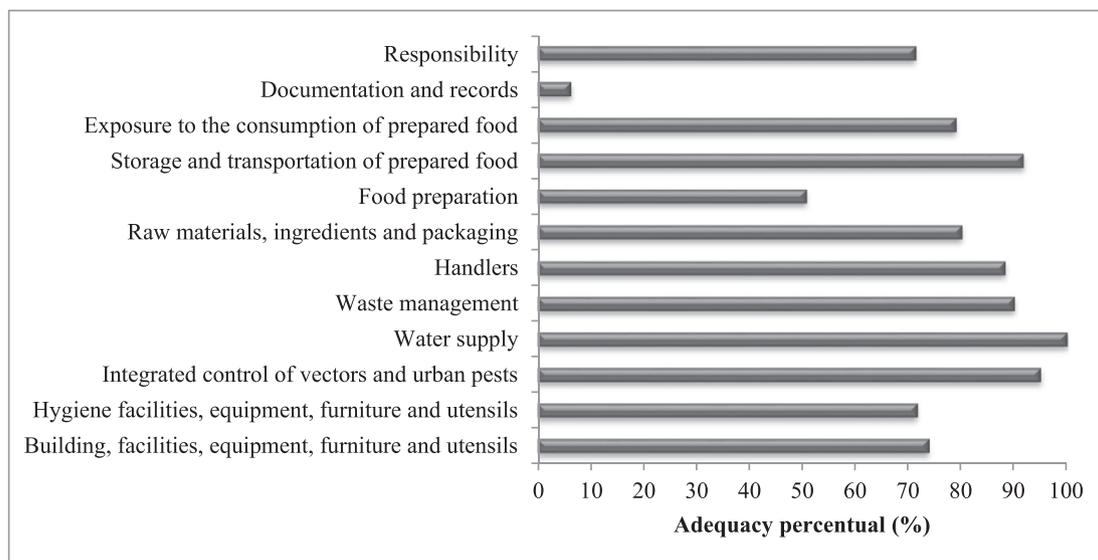


Fig. 2. Average of adequacy, per item of good manufacturing practices requisites, of bakeries from Santa Maria-RS, Brazil ($n = 20$).

inactivation of deteriorating fungi of importance in food industries. Among the tested species were *Aspergillus brasiliensis*, *Penicillium roqueforti*, *Penicillium commune*, *Penicillium polonicum*, *Cladosporium cladosporioides* and *Candida albicans* (Bernardi et al., 2018). On the other hand, a smoke generation agent, based on orthophenylphenol presented only limited action on reducing fungal species commonly associated with food spoilage (Bernardi et al., 2019a). The use of an efficient air cleaning program in these food processing environments is described as the main methodology for microbial control, whether they are deteriorating and/or pathogenic, however, would be important to evaluate the antifungal activity from the sanitizer prior their application in the facility (Bernardi et al., 2018).

Also, if surfaces used for food preparation remain moist and with food residues, it can allow the adhesion of micro-organisms. Microbial adhesion is often a survival strategy, which can generate extracellular matrix and subsequent formation of microbial biofilms, making difficult micro-organism's elimination and perpetuating their occurrence in the facilities environment (Andrade and Salustiano, 2008; Doménech-Sánchez et al., 2011; Kusumaningrum et al., 2003).

In addition, the importance of the food handler, which is any person of the food service that comes in direct or indirect contact with the food, as responsible for the contamination and vector of food-borne pathogens, has also been reported (Almeida et al., 1995; Brasil, 2004; Coelho et al., 2010; Tomich et al., 2005). Oliveira et al. (2003) reported that most microbial contamination of food originates in the negligence of food handlers, in inadequate sanitary conditions of the place of production, out-of-specification buildings, lack of hygiene in equipment and utensils, as well as in the poor quality of the raw material used. Andrade and Salustiano (2008) also highlighted that food handlers are responsible for up to 26% of foodborne outbreaks. According to the authors, the use of inadequate handling and hygiene methods, unhygienic attitudes in food preparation, and the use of poorly sanitized equipment and utensils have also been responsible for outbreaks of food-borne diseases. The competency and the training of these professionals are considered in the legislation used to evaluate the GMP (Rio Grande do Sul, 2009).

The items documentation, food registration, and preparation were the ones presenting the lowest adequacy scores, with 6.30% and 50.7%, respectively. Despite it is mandatory and considered fundamental by the sanitary laws in Brazil, the item documentation and registration has stood out as the most problematic in food services in general. The main irregularity is related to the absence of a manual of good practices at these food processing facilities, a document that describes the main

measures that should be taken to obtain safe foods (Brasil, 2004). The lack of the industry-specific good practices manual may be related to the low commitment of both food service providers and food handlers to meeting routine quality requirements, which often requires investment and the hiring of a qualified professional.

These professionals should be vigilant and follow the recommendations prescribed in the manual of good practices and standard operating procedures, for example (Brasil, 2004; Garcia and Centenaro, 2016; Rio Grande do Sul, 2009; Schimanowski and Blümke, 2011; Zimmermann et al., 2016).

3.2. Airborne fungi in bakeries

Table 1 shows the mean air contamination counts in bakery environments. When compared to the scale proposed by Al-Dagal and Fung (1993), fifteen (75%) bakeries presented counts > 300 CFU/m³ in the cooling area and seventeen (85%) in the food exposure areas. Even fungal counts in the air were in the level considered acceptable (< 300 CFU/m³) in six bakeries (30%), it was possible to recover a wide variety of fungal species (Table 1). When compared to the current Brazilian legislation on the microbiological quality of air in artificially acclimatized environments for public and collective use, twelve (60%) of bakeries presented values above 750 CFU/m³, which is the maximum recommended by this legislation (Brasil, 2000). Currently, there's no regulation available specifying limits for micro-organisms (both airborne fungi and bacteria), neither correlating them to the air quality in food industries or food processing environments in Brazil.

It is important to emphasize that during the air sampling in the areas, there was human activity in these places. The movement of air caused by the movement of people may influence the high fungal counts found in some sampled sites (Fig. 3). It should be noted that in two bakeries, the fungal propagules present in the air represented counts higher than 24,000 CFU/m³ in the cooling area (above the detection capacity by the air sampler used).

Knight and Menlove (1961) postulated that the level of fungal spores in the air commonly associated with the bakery industries can range from 7000 to 8000 m² for an hour. In an investigation carried out in bakeries in England, the counts ranged from 85 to 2850 CFU/m³, while in Canadian bakeries it varied from 50 to 2000 and 60 to 210 in preparation and packaging areas, respectively (Ooraikul et al., 1987). Evaluations conducted in France detected counts varying from 38 to 5170 CFU/m³ (Simeray et al., 1995). In Brazil, Freire (2011) highlighted that the air counts in Brazilian bakeries ranging from 65 to

Table 1
Fungal genus and species present in the environment air of bakeries from Santa Maria-RS, Brazil (n = 20).

Bakeries (n = 20)		
Collection point	Cooling area	Exhibition area
Range (CFU/m ³)	2.4 × 10 ² to > 10 ⁴	1.32 × 10 ² to 4.16 × 10 ³
Average (CFU/m ³)	1.40 × 10 ³	1.47 × 10 ³
Fungi	FO (%)	FO (%)
<i>Alternaria</i> sp.	10	10
<i>Aspergillus</i> sp.	70	85
<i>Aspergillus candidus</i>	ND	15
<i>Aspergillus chevalieri</i>	10	10
<i>Aspergillus montevidensis</i>	ND	5
<i>Aspergillus niger</i> complex	15	15
<i>Aspergillus flavus</i>	20	ND
<i>Aspergillus ochraceus</i>	ND	15
<i>Aspergillus penicillioides</i>	5	5
<i>Aspergillus pseudoglaucus</i>	ND	5
<i>Aspergillus ruber</i>	5	5
<i>Aspergillus sydowii</i>	ND	5
<i>Aspergillus tamarii</i>	15	ND
<i>Aspergillus terreus</i>	ND	ND
<i>Aspergillus versicolor</i>	20	15
<i>Aspergillus wentii</i>	ND	5
<i>Aspergillus</i> sp. (<i>Neosartorya</i>)	5	ND
<i>Cladosporium</i> sp.	85	90
<i>Fusarium</i> sp.	10	5
<i>Mycelia sterilia</i>	10	10
<i>Penicillium</i> sp.	65	70
<i>Penicillium brevicompactum</i>	ND	10
<i>Penicillium citrinum</i>	10	5
<i>Penicillium claviforme</i>	ND	5
<i>Penicillium commune</i>	ND	5
<i>Penicillium corylophilum</i>	5	ND
<i>Penicillium crustosum</i>	5	10
<i>Penicillium digitatum</i>	5	5
<i>Penicillium expansum</i>	ND	5
<i>Penicillium italicum</i>	ND	5
<i>Penicillium lividum</i>	5	ND
<i>Penicillium olsoni</i>	15	15
<i>Penicillium oxalicum</i>	5	ND
<i>Penicillium paxilli</i>	15	15
<i>Penicillium sclerotiorum</i>	ND	5
<i>Penicillium simplicissimum</i>	ND	5
<i>Penicillium spinulosum</i>	5	ND
<i>Rhizopus</i> sp.	5	10
<i>Syncephalastrum racemosum</i>	ND	ND
<i>Talaromyces islandicum</i>	5	5
<i>Talaromyces variable</i>	ND	5
<i>Trichoderma</i> sp.	5	ND
<i>Wallemia sebi</i>	10	ND
Yeasts	20	15

*FO: frequency of occurrence: number of samples that the microorganism was present / total number of air samples × 100.

*ND: not detected.

2290 CFU/m³. The minimum values found in our work were about 10 times higher than those reported by other authors, as well as the maximum detected in the cooling area. This factor can be related to the high values of relative humidity (RH) registered in the city of Santa Maria, RS. Sartori (2003) highlight that the mean levels of RH in the state of Rio Grande do Sul ranges from 70% to 85% (mean values of 68–85% in the summer and 76–90% on winter). Flours, as well as other raw materials, are hygroscopic. In high RH environments, they absorb water vapor from the atmosphere, elevating their water activity, and consequently, influencing the multiplication of fungi (Pitt and Hocking, 2009). As higher is the water activity of these substrates, higher can be the fungal multiplication on it, elevating the load of spores released in the environment. Water activity is numerically equal to equilibrium relative humidity expressed as a decimal.

The culture media can also influence the recovery of fungi. The DG18 medium was chosen because the bakery products have intermediate water activity and our objective was to isolate fungi commonly responsible for the deterioration of these products. This medium was first designed for enumeration of a range of nonfastidious xerophilic fungi, however, now DG18 is considered as a general purpose medium since has been shown that it supports the growth of the common *Aspergillus*, *Penicillium* and *Fusarium* species, as well as most yeast and many others common food and airborne fungi (Pitt and Hocking, 2009; Samson et al., 2002).

Considering the results mentioned above, the fungal counts recommended in the scale proposed by Al-Dagal and Fung (1993) are quite low and cannot reflect the reality of bakeries. Although they used the same methodology as in our study (air sampling), that scale was constructed based on the count of airborne fungi found in a meat complex (meat coolers, meat fabrication, and slaughtering room). In bakeries and bread industries, particles of flour coming from cereal grains contaminated with fungal spores are commonly dispersed in the air during the production steps. So, the quality of raw material can strongly influence the microbial load in the air (Legan, 1993; Santos et al., 2016).

According to our experience and data available in the scientific literature, a new scale was proposed for the evaluation of the air quality in bakeries (Table 2). We believe it also could guide factories of matured food products, such as cheese and fermented meat/sausages, which usually show fungi in their surface and consequently influence the environmental fungal counts when spores are released in the air.

By our proposed scale, only nine bakeries (45%) would be classified with a high degree of likelihood for early spoilage occurrence regarding the mycological quality of the air. Otherwise, the others would be classified with a moderate and low risk for the occurrence of early deterioration in the products (Fig. 3).

From Fig. 4, we can verify that the cluster analysis carried out a grouping between the bakeries that showed higher airborne fungi contamination, in the two studied areas (cooling and exposure). The bakeries were the ones with the highest scores was the M, S, and Q (Group 2, cooling area) and the A, B, C, K, S (Group 2, exhibition area). While the bakeries E, F, G, H, N, P, R presented the lowest values of the load for fungal contamination in the cooling and exhibition areas.

In relation to the identification of fungal contamination of the air, 11 genera and 33 fungal species were recovered in the areas of cooling and product exposure. The genus *Cladosporium* was the most frequent, both in the cooling (85%) and exposure (90%) areas. Species of the genus *Aspergillus* and *Penicillium* also had a high frequency of occurrence (Table 1).

Species known as spoilers of bakery products (*Aspergillus montevidensis*, *Aspergillus chevalieri*, *Penicillium brevicompactum*, *Penicillium commune*, *Penicillium crustosum*, *Rhizopus* sp. and *Wallemia sebi*) and some potentially toxigenic (such as *Aspergillus flavus* and *Aspergillus ochraceus*) (Pitt and Hocking, 2009) were isolated.

Our interest in identifying the *Aspergillus* and *Penicillium* species is because these genera are directly related to the contamination and deterioration of raw materials and bread, respectively. *Aspergillus* spp. (e.g. *Aspergillus candidus*, *Aspergillus flavus*) have been isolated from the main raw material used in the bakery industry, such as rye, wheat flour, linseed and oat flakes (Garcia et al., 2018; Santos et al., 2016). On the other hand, *Penicillium* spp., like *Penicillium citrinum*, *Penicillium paneum*, and *Penicillium roqueforti*, are the major species related to the occurrence of moldy bread (Garcia et al., 2018; Pitt and Hocking, 2009; Santos et al., 2016).

Sveum et al. (1992) report that air quality in food processing facilities may not directly affect microbiological safety or maintenance of quality in the case of stable foods. However, more perishable foods are particularly susceptible to contamination by micro-organisms transported by the air. This can be directly applied to bakery processing environments. In bakeries, the greatest concern is due to fungal spores

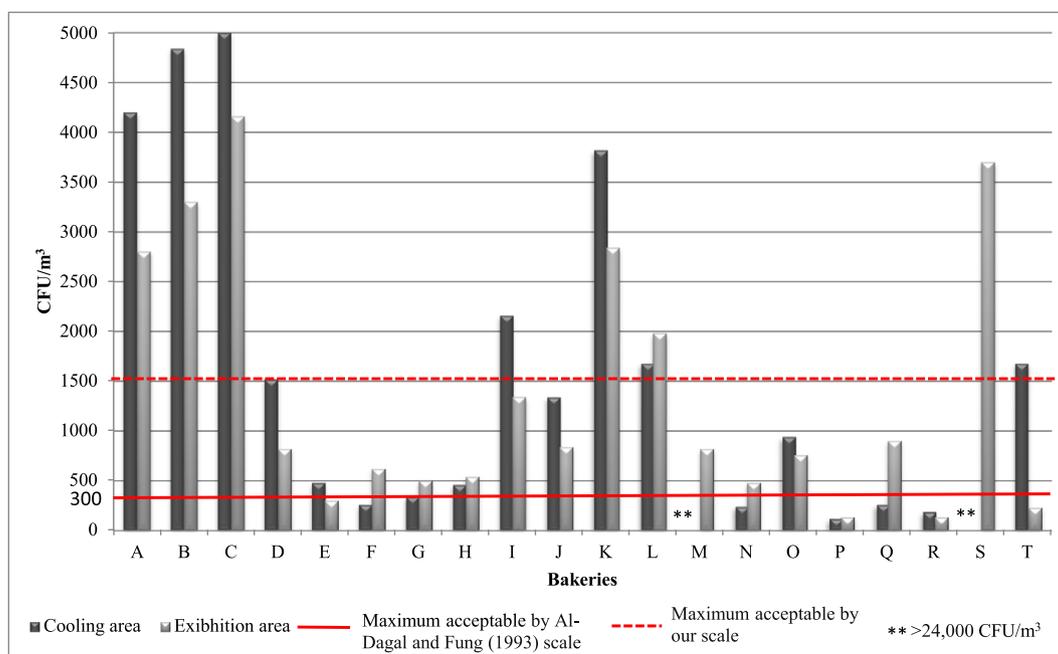


Fig. 3. Level of airborne fungi contamination in the cooling and exposure areas of bakeries from Santa Maria-RS, Brazil (n = 20).

contaminating the air in the processing environment, since they are potential deterioration agents and can reduce the shelf life of bread and other bakery products (Legan, 1993).

Yeasts were also present in the analyzed areas (Table 1). These microorganisms, like molds, do not survive to bake temperatures but can contaminate the bread if they are present in the environment where this product is sliced or cooled. Contamination will occur mainly after physical contact with dirty surfaces and equipment, or with other already contaminated bakery products (Cauvain and Young, 2007).

3.3. Correlation between environmental fungi and good manufacturing practices

Results of correlation analyses are showed in Table 3. It was possible to observe negative results, e.g., increase of adequacy to the GMP and decrease of air contamination of bakeries only in the blocks “Building, facilities, equipment, furniture, and utensils”; “Food handlers”; and “Food preparation” versus “Fungal contamination in cooling areas” and in “Hygiene of facilities, equipment, furniture and utensils”; and “Raw materials, ingredients and packaging” versus “Fungal contamination in the exposure areas”. Even then, the results were classified as “very weak” or “weak” from the correlation scale adopted.

The observed correlation results between the evaluated parameters, in general, contrast with the predicted. Generally, lower the level of adequacy, higher should be the air contamination, since it is believed that poor environmental hygiene in producing areas will contribute for dispersion of micro-organisms in the air. On the opposite, good hygiene would prevent the source of contaminants or environmental conditions to act as a co-adjutant in the process of contamination and deterioration of food (Southier and Novello, 2008). In addition, disrespect for

hygienic-sanitary standards can lead to the contamination of any food, since microorganisms may have their proliferation, growth and development favored due to the deficiency of several criteria (Benevides and Lovatti, 2004; Fernandez et al., 2003).

However, the factors involved in the fungal contamination of air in food processing environments go beyond the requirements of good manufacturing practices addressed by the checklist. The ventilation system, for example, when present in the processing facilities, can contribute to airborne micro-organisms dispersion. In order to obtain an adequate design or a proper maintenance of this system, one must know the movement of air through the factory, as well as the diffusion of the particles through the air. An efficient ventilation system could help to control the airborne microorganisms, contributing to improve the microbiological quality of the air, as well the ambient temperature and the relative humidity of the environment (Andrade and Salustiano, 2008).

On the other hand, mycological quality of raw material, especially flours, could have a high influence on a load of fungal spores disperse in the processing air, but it is not evaluated on the good manufacturer checklist. The literature report that flour contains a substantial number of fungal spores (Pitt and Hocking, 2009; Poisson, 1975; Rogers and Heseltine, 1978) and even than these spores are reduced during the cooking of the products (Garcia et al., 2019; Knight and Menlove, 1961), the presence of flour particles with fungal spores can contaminate the surface of ready products and contribute to the spoilage of bread. Therefore, for bread to suffer fungal deterioration, it should be contaminated after cooking, during the cooling, exposure, slicing or packaging processes (Burfoot et al., 2000; Den Aantrekker et al., 2003; Legan, 1993; Viljoen and Von Holy, 1997), being the mycological quality of air in the cooling environment crucial for definition of breads

Table 2
Scale for evaluation of mycological air quality in bakeries.

Fungal counts (CFU/m ³)	Classification	Likelihood of early spoilage occurrence
0 to 100	Clean air.	Remote.
101 to 500	Low contaminated, acceptable air.	Low, minor concern.
501 to 1500	Moderately contaminated, acceptable air.	Moderate, major concern.
> 1500	Highly contaminated, unacceptable air.	High, critical concern.

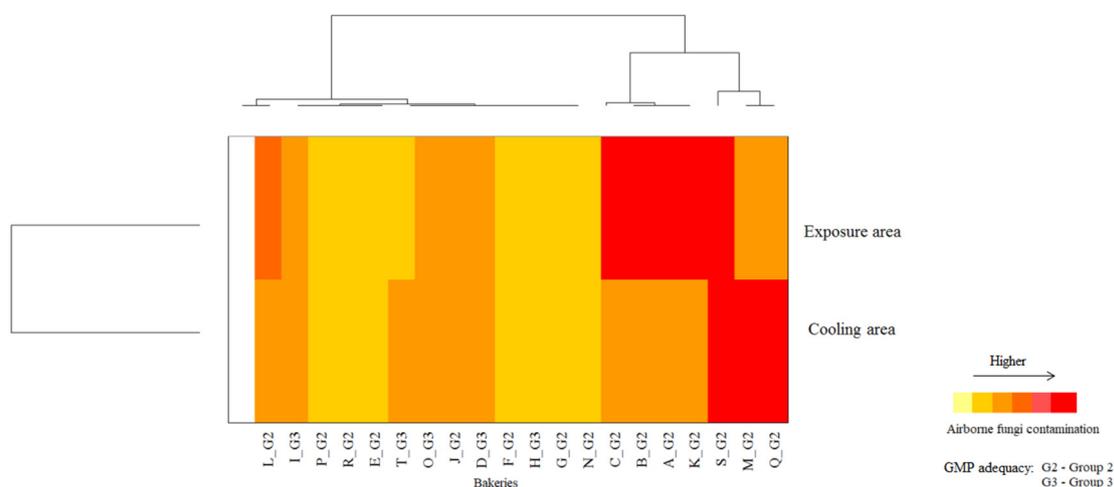


Fig. 4. A cluster of airborne fungi contamination in the cooling and exposure areas of bakeries from Santa Maria – RS, Brazil (n = 20).

Table 3

Correlation coefficients (r) of some evaluated parameters of good manufacturing practices versus the counts of airborne fungi contamination in bakeries.

Adequacy parameters	Fungal contamination	
	Cooling area	Exposure area
Building, facilities, equipment, furniture, and utensils	-0.041	0.072
Hygiene of facilities, equipment, furniture, and utensils	0.168	-0.112
Waste management	0.147	0.186
Food handlers	-0.383	0.192
Raw materials, ingredients, and packaging	0.030	-0.245
Food preparation	-0.042	0.210
Exposure to the consumption of prepared food	0.013	0.293

shelf life.

In our opinion, the microbiological quality of the air from food facilities should be specified in a regulation, as well it is for water and raw material quality. It should be emphasized that good manufacturing practices were mainly designed to avoid the occurrence of foodborne diseases in food services (Brasil, 2004). It means, bacteria are the main target micro-organism of this kind of regulation; and they don't have the same facility of dispersion through the air as fungi. Also, besides the hygiene of equipment, furniture, and utensils, the food handler is reported as one of the main sources of food contamination by pathogenic bacteria (Campos et al., 2009; Rebouças et al., 2017). Bacteria, such as *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Bacillus cereus*, Coliforms, and others, are the main microorganisms responsible for the occurrence of these diseases. Recent data show that, despite a small decrease in reported cases, *Clostridium perfringens* remain the major responsibility for the occurrence of these diseases in Brazil. In addition, residences are the places where the cases mostly occur, but restaurants and bakeries remain the second in the Brazilian ranking of places of origin of food-borne disease outbreaks (Brasil, 2017).

Therefore, even the GMP status of bakeries was not well correlated with the fungal contamination of the air, it is believed that the adoption of good manufacturing practices in bakeries could allow the reduction of food-borne diseases occurrence and minimize the early deterioration of bread. Care should be taken, especially with air quality in cooling and exposure environments, through the use of sanitizing agents that may significantly reduce air contamination and consequently provide a longer shelf life of bakery products in these food services.

In our opinion, also a code of practices of hygienic production of bakery products should be elaborated, highlighting some key controls

measures at each stage. This document could encompass both building design [e.g. recommending segregation of the area of raw material, ingredients, mixing and bread making (dirty area) from the post-baking cooling area (clean area)]; sanitization procedures (e.g. the choice of effective antifungal sanitizers, cleaning of environmental at the end of the production day and sanitizing both facilities and equipment previously of personal entrance in the next turn, when most spores are deposited in the ground and equipment surfaces); as well hygienic practices (e.g. avoid air circulation between dirty and clean areas, avoid sweep the floor, which promotes the air circulation in the presence of not packed fresh baked products).

4. Conclusion

Although most of the bakeries (75%) were classified as “good”, according to the results obtained from the checklist of good manufacturing practices applied, the fungal contamination in the air in these facilities was generally high, allowing for recovering a wide variety of fungi with the potential to spoil bakery products. The Pearson correlation showed a “weak” correlation between the attendance of good manufacturing practices and the fungal contamination of the air in the areas of cooling and exposure in the bakeries. Therefore, even attending the good manufacturing practices requirement, the adoption of an air cleaning program, with the use of an effective antifungal sanitizer in these food processing environments, is described as the main method for controlling these food spoilage agents.

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