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Fungi in bottled water

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ABSTRACT

Bottled water is a product considered safe, although studies already proved fungi incidence even of potential pathogens. The Brazilian legislation does not set limits for this microorganism in water for human consumption, limiting only for bacteria. This study evaluated the fungi contamination in bottled water commercialized in the region of the city of Recife-PE, Brazil. The samples were collected between September and December of 2016. It was used 35 samples from seven companies, different lots, bottled in 500 mL polyethylene terephthalate (PET) bottles and coded from A to G and analyzed to fungi counting by mean of pour plate technique using acidified Potato Dextrose Agar (PDA) at pH 4.0 and incubated to 25°C ± 2°C for up to seven days, accordingly to the Standard Methods for the Examination of Water and Wastewater Methodology. pH data from the samples were obtained by information containing in the labels from the packages. It was observed the fungi presence in 20 samples (57.1%). From the positive samples, there were identified filamentous fungi in 14 (70%), only yeast in 1 (5%), and both in 5 (25%). Packages were considered contamination source. The predominant filamentous fungi in each sample were isolated, purified and submitted to morphological identification at the genus level. The results showed that *Aspergillus* spp., moreover, *Penicillium* spp., can be water contaminants. The samples pH, around 5.0, might have contributed for the found fungi maintenance. The filamentous fungi found may be pathogenic or toxigenic, which represents a risk to public health.

Keywords: Microorganisms, contamination, beverage.

Introduction

Natural mineral waters are obtained directly from natural sources or extracted from underground, characterized by the defined and constant minerals content trace elements few microorganisms and other constituents (Brasil, 2005a). Studies show a reduction of use of public supply waters for human consumption and the consequent increase of the bottled water consumption (Mendonça, Pitaluga & Figueiredo Neto, 2005; Brei, 2007).

The search for mineral water is due to the intimate relationship with the product quality and, consequently, to the food safety and wellbeing that it promotes. Besides, the shortage in some regions, as the northeast of Brazil and the consumer dissatisfaction with the water quality available by the public system supply increases this demand (MME, 2015). In the last years, the bottled mineral water consumption is showing rises even in

countries where the public supply is cheap and accessible practically to all, as the case in France (Brei, 2007; Nunes & Fuzihara, 2011).

In 2007, for the first time, the consumption of bottled water surpassed the soda, becoming the beverage with higher consumption in the world market, among non-alcoholic, reaching the 37.3% participation in the market compared to 36.8% of the soda. In this same year, there were consumed 206 billion liters of water, commercialized in bottles (ABINAM, 2009; SEBRAE, 2016). The global consumption of bottled water in 2013 was 266 billion liters, 7% more than in 2012. Brazil occupies the 5th place in the world ranking of bottled water consumption, ahead of countries such as Italy, Germany, and Spain. The country consumption in 2013 was of 18.2 billion liters, which represents a 4.1% growth compared to the year before, this shows that the consumption of this kind of product is rising, with high tendency,

accordingly to data from Mineral Summary of the National Department of Mineral Production of Brazil (DNPM, 2014; SEBRAE, 2016). Brazil owns little more than a thousand areas of mineral water mining, distributed by the five regions of the national territory, with the Northeast region being the second region with a higher concentration of these areas. Pernambuco-Brazil is registered as having 62 mining areas of mineral water until 2015, with the region of Recife with the most concessions concentration (MME, 2015).

Water can be contaminated by microbiologic agents, including bacteria, viruses, and parasites, even fungi (Hageskal, Lima & Skaar, 2009; Sessegolo et al., 2011). The hydric placement of etiologic agents of infectious character is responsible for the high incidence of diseases that affect the population, this being one of the most frequently referred problems of public health in developing countries. Each year 10 million people die in the world for drinking contaminated water (Fernandes, 2008; MS, 2014). Bottled mineral water should not offer any risk to the consumer. Its contamination can occur on the source, on the bottling or the recipient used in the package. The stages of the process used in the bottling should not alter its original composition and must obey the legislation over the Good Practices of Production (Brasil, 2005a; Yamaguchi et al., 2013).

In Brazil, the Resolution of the Collegiate Board, RDC 274/2005 of the National Agency of Sanitary Vigilance (ANVISA), determinates bottling procedures and physical-chemical parameters and the RDC 275/2005 establishes microbiological standards for bottled natural mineral water destined for human consumption (Brasil, 2005a; Brasil, 2005b). It is norm contemplates bacteria (Coliforms, *Escherichia coli*, *Enterococcus* spp., *Pseudomonas* spp. and *Clostridium* spp.).

The fungi research is not contemplated in the current Brazilian directives, despite widely having distributed in nature (Brasil, 2011; Oliveira et al., 2013). Studies have identified filamentous fungi and yeast in bottled water, among those, some are known as mycotoxin producers and opportunist pathogens of humans representing harm for the health mainly in immunocompromised individuals (Criado et al., 2005; Yamaguchi et al., 2007; Fernandes, 2008; Nunzio & Yamaguchi, 2010; Oliveira, 2010; Nunes & Fuzihara, 2011; Pontara et al., 2011).

Fungi can develop in PET (polyethylene terephthalate) packages, where, conventionally, the water is bottled to commercialization in bottles of until two liters. Components of these packages can serve of nutrients for the development of these

microorganisms (Criado et al., 2005). When present in the potable water used for use and food preparation, the fungi can cause color and odor alterations and even produce mycotoxin when in contact with the nutritive environment. Fungi have metabolic strategies that allow it to survive in oligotrophic environments such as in the clean water (Gonçalves et al., 2006; Oliveira, 2010). Water contaminated by fungi, when ingested by healthy individuals, the risk of diseases can be limited, but for immunocompromised ones, such as kids, elderlies, transplanted or immunologic disease carrier, represents significant danger, by its susceptibility to infections (Nunzio & Yamaguchi, 2010; Pontara et al., 2011). Spores can be found in water; these can be used to evaluate the capacity of species that can cause respiratory infections. Spores size greater than 5 μm is considered pathogenic to humans and animals, due to its capacity of causing lung diseases for affecting the alveolus (Oliveira et al., 2013). There was intended for this study to evaluate the fungi contamination in bottled water commercialized in the region of the City of Recife-PE, Brazil.

Material and Methods

Sampling

There were collected in a period between September and December of 2016, 35 samples of non-carbonated water, in 500 mL bottles of polyethylene terephthalate-PET, of seven different bottling companies, commercialized in the region of the City of Recife-PE, Brazil. Five samples of each brand, from different lots, identified as A, B, C, D, E, F, and G, were obtained and transferred in the same day for the Laboratory of Food Processing and Analysis of the Universidade Federal Rural de Pernambuco-UFRPE.

Experiment preparation

The packages were washed with tap water, and liquid detergent and the top part sanitized with alcohol at 70%. Each bottle was homogenized 25 times with the purpose to remove possible contamination stuck in the internal walls of the packages.

Fungi analysis

In the fungi counting there was used the methodology 9610 of the Standard Methods for the Examination of Water and Wastewater (Apha, 2012), a pour-plate method with Potato Dextrose Agar, acidified to pH 4.0 with tartaric acid to 10%. The final pH of the environment was verified using indicator tapes of pH MColorpHast™. The inoculated plates were incubated to 25°C \pm 2°C for up to seven days in incubator BOD SPLabor. The

developed colonies were counted in separate, considering the cottony aspect, like molds and smooth, rusty, shiny or pale, like yeast. The confirmation of yeast colonies there was obtained due to coloration and microscopic analysis. The results were expressed in Colonies Forming Units for 5 mL of water (CFU.5 mL⁻¹). The laboratory control was assured by inoculated plate without a sample, maintained at the same used conditions in the experiment. The pH data of the samples were obtained from information contained on the labels of the packages.

Main fungi identification

The plates that showed colonies of the filamentous fungus were reserved. The predominant filamentous fungi in each sample were isolated removing a block containing mycelium and reproductive structures along with some culture medium (Potato Dextrose Agar-PDA), being deposited in the center of another plate containing the same culture medium. The plates were incubated in the same conditions used on the counting (25°C ± 2°C per three to seven

days). The isolated and purified colonies were subjected to morphological identification. Therefore, the isolated filamentous fungi were cultivated on malt agar extract (MAE) for further identification at the genus level.

The identification of the filamentous fungi was based on macromorphology analysis, by observation of characteristics of the colonies as edge, zonation, texture, color, diameter and pigment production; and micromorphology by evaluation in microculture of the septation of the hyphae, presence or absence of the conidiophore, its organization and type of conidium according to the established literature using identification keys (Samson et al., 2007; Rossman & Seifert, 2011; Samson et al., 2011).

Results

From the 35 analyzed samples, there was observed the fungi presence in 20 (57.1%), which 14 (70%) being only with filamentous fungi, 1 (5%) with only yeasts and 5 (25%) with both kinds of fungi (molds and yeasts) as observed in Table 1.

Table 1. Fungi in bottled water. Expressed results as <1=development absence, considering the method limit; CFU= Colony Forming Units; X= Arithmetic average; SD= Standard deviation.

Samples	Filamentous fungi CFU.5 mL ⁻¹	Yeast CFU.5 mL ⁻¹	pH	Prevailing fungi
A1	<1	<1		
A2	<1	<1		
A3	1	<1	5.21	<i>Aspergillus</i> sp.
A4	1	<1		
A5	5	<1		
X±SD	1.4 ± 1.44	<1 (0)		
B1	20	<1		
B2	<1	<1		
B3	<1	<1	5.67	<i>Aspergillus</i> sp.
B4	<1	<1		
B5	<1	<1		
X±SD	4 ± 6.4	<1 (0)		
C1	3	2		
C2	<1	<1		
C3	1	<1	5.22	<i>Aspergillus</i> sp.
C4	<1	1		
C5	1	<1		
X±SD	1 ± 0.8	0.6 ± 0.72		
D1	<1	<1		
D2	<1	<1		
D3	<1	<1	5.30	-
D4	<1	<1		
D5	<1	<1		
X±SD	<1 (0)	<1 (0)		
E1	48	<1		
E2	28	306	5.34	<i>Penicillium</i> sp.
E3	3	11		
E4	2	<1		

E5	4	42		
X±SD	17 ± 16.8	71.8 ± 93.8		
F1	2	<1		
F2	158	<1		
F3	<1	<1	5.28	<i>Aspergillus</i> sp.
F4	<1	<1		
F5	<1	<1		
X±SD	32 ± 50.4	<1 (0)		
G1	4	<1		
G2	49	<1		
G3	4	<1	5.46	<i>Aspergillus</i> sp.
G4	4	<1		
G5	8	2		
X±SD	13.8 ± 14.08	0.4 ± 0.64		

Discussion

In the fungi research in the water, there was observed the presence of 333 samples, which 218 of them, were confirmed as filamentous fungi (Oliveira, 2010). Other authors also identified fungi in water samples and among them, pathogenic species and toxin producers, suggesting harm for the health, mainly to children, elderly and patients with impaired immunologic system (Yamaguchi et al., 2007; Nunzio & Yamaguchi, 2010; Oliveira, 2010; Nunes & Fuzihara, 2011; Pontara et al., 2011). The presence of fungi in water was also referred by another author, who suggested being, the microorganisms, the responsible for sensorial alterations in the water, such as color and odor (Gonçalves et al., 2006).

The results obtained in this research shows considerable variation in the counting which suggests that the package might affect the fungi contamination in the water. It is because, in the same brand, the results were discrepant, as observed in the E, F and G samples. Nunzio & Yamaguchi (2010) also observed variations in the levels of fungi contamination in the water.

On the other hand, the contamination by yeast was observed in well lower levels and incidences when compared to molds. Also, for this group of microorganism was not observed constancy which reinforces the suspicion that the package might be responsible for this kind of contamination. Pontara et al. (2011) also verified lower yeast incidence when in 15 samples there were observed 32 colonies related to fungi, being 22 molds and only 10 yeasts.

The pH of the samples, closer to 5.0 can favor fungi maintenance, even considering that the water is not a culture medium for multiplication of this kind of microorganism. In this understanding, the water might be considered a carrier for the transmission of fungi, when used as prime matter in the food and beverage formulation. It was also admitted by another researcher when observed, in

sanitation water samples, pH varying between 3.2 to 5.9 and considering that when the water has a favorable pH for the fungi development, there is a high risk of food contamination and harm for the consumer health (Oliveira, 2010).

Aiming to verify the fungi origin of sample E, 41 empty packages were analyzed. Thus, 8 showed positive fungi results. In parallel, there were analyzed 15 samples of the water from the source used in the bottling, which showed the absence of fungi. It proved the possible influence of the package in the water contamination.

Considering the isolated filamentous fungi in this research, the main ones in each company were identified (Table 1). The same genera identified in this work has been reported by Cabral & Pinto (2002) when analyzed samples of bottled mineral water such as *Penicillium* spp. among others as *Cladosporium cladosporioides* and *Alternaria alternate*. It is important to highlight that the fungal genera found in this work have several toxigenic species (Pitt, 2000; Ameen et al., 2017).

The bottled water can be a path for fungi dissemination even though is not considered a quality indicator for potable water by the Brazilian legislation, differently from the importance given to bacteria and viruses even in case of outbreaks. Besides that, the majority of researches about the microbiological contamination of the water does not emphasize the fungi presence (Carvalho et al., 2015; Lima et al., 2015; Paula & Novais, 2015; Vasconcelos, Melo & Fontenelle, 2015; Oliveira et al., 2016; Leite, 2017). In Brazil, in 2017, almost 100 deaths were linked to diarrhea with 149.640 notified cases involving 47 outbreaks by ingestion of water and food contaminated. In these cases, also was not mentioned the possibility of fungi also contributing to the referred outbreaks (Leite, 2017).

Due to the results of this research corroborate others authors that observed fungi contamination in bottled water (Criado et al., 2005;

Nunzio & Yamaguchi, 2010; Nunes & Fuzihara, 2011; Pontara et al., 2011; Yamaguchi et al., 2013), might infer that, differently from what expects and believes, the bottled water is not entirely safe over the microbiological aspects, even considered by consumers as “refined”, “smooth” and “fancy” (Arrais, 2011; Castro, Coswosk & Fraga, 2014). Even considering that the underground water is protected by nature, when bottled not always keep the safety characteristics probably by capitation, stocking, bottling and packing failure.

Conclusion

The current study had observed the occurrence of filamentous fungi and yeast in bottled mineral water in PET bottles that are commercialized in the region of the city of Recife-PE, Brazil with a predominance of filamentous fungi. It was observed the presence of the fungal genera *Aspergillus* spp. and *Penicillium* spp. Such contamination can be attributed to the packages used as well as to the water pH analyzed that it is located in the favorable zone for the fungi maintenance.

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