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Research Paper

Yeasts and hygienic-sanitary microbial indicators in water buffalo mozzarella produced and commercialized in Minas Gerais, Brazil

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Abstract

The aim of this work was to study the yeast populations and the main hygienic-sanitary microbial indicators in water buffalo mozzarella produced and commercialized in Minas Gerais, Brazil. Forty-two water buffalo mozzarella samples were purchased from retail outlets in Belo Horizonte. In addition, five samples of consecutive starter cultures, curd before acidification, acidified curd and mozzarella were collected at an industry in the city of Oliveira. Only three of the five water samples analyzed were suitable for consumption according to Brazilian sanitary standards. Four milk samples were highly contaminated with fecal coliforms, and did not meet the minimal hygienic-sanitary standards according to Brazilian regulations. Only one sample of buffalo muzzarela purchased from retail outlets exceeded the limit for coagulase-positive *Staphylococcus*. Eleven samples showed counts of thermotolerant coliforms higher than 5 x 10^3 CFU.g^{-1}, but still lower than the maximum permitted by the Brazilian laws. *Salmonella* spp. and *Listeria monocytogenes* were not isolated. *Debaryomyces hansenii, Candida lusitaniae* and *C. parapsilosis* were the prevalent yeast species isolated from cheese. Among samples from the production stages, the acidified curd presented the highest numbers of yeasts, with *C. catenulata* being the most frequent species isolated. Some opportunistic yeast species such as *C. guilliermondii, C. tropicalis, C. parapsilosis, C. lusitaniae, C. catenulata, C. rugosa* and *C. krusei* occurred in the mozzarella cheese samples analyzed. The mozzarella cheese presented a low microbial load as compared to other cheese already studied, and the yeast biota included species typical of cheese and also opportunistic pathogens.

Key words: water buffalo mozzarella; yeast diversity; opportunistic yeast; hygienic-sanitary microbial indicators.

Introduction

Water buffalo mozzarella is a fine cheese of Southern Italian origin with a good acceptance in Brazil. The cheese is made from pasteurized water buffalo milk by adding a commercial starter culture and rennet. After the curd undergoes a ripening phase for 4.0 to 18 h at a temperature of 35 to 37 °C, the optimal pH (4.9 to 5.1) is reached and the drained curd is stretched in hot water (90 to 95 °C) (Pandya *et al.*, 2008). The mass is then salted and molded, and the cheese is ready to eat without going through a ripening process. Romano *et al.* (2001) described the final product as an unripened soft cheese of milk-white color, which releases whey under pressure and has a fresh and slightly acidic taste and a pleasant aroma.

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Yeast frequently form part of the cheese microbiota due to their ability to grow under unfavorable conditions and to metabolize milk constituents. These microorganisms play a significant role in the spoilage of dairy products as well as in the ripening of some cheese varieties (Irlinger and Mounier, 2009). Among positive contributions of some yeast species to cheese production, their ability to inhibit undesirable microorganisms and to contribute to the development of flavor and texture of cheese are well established; and these characteristics are directly attributed to their ability to ferment lactose, produce aromatic compounds and to their proteolytic and lipolytic activities (Westall and Filtenborg, 1998). However, spoilage of dairy products due to excessive growth of yeasts has been reported and they can cause fruity flavor, gas production, discoloration, slime formation and changes in texture (Westall and Filtenborg, 1998; Jacques and Casaregola, 2008). *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Yarrowia lipolytica* are the most frequent yeast species associated with cheese and dairy products (Romano *et al.*, 2001; Borelli *et al.*, 2006a; Álvarez-Martin *et al.*, 2007). *Saccharomyces cerevisiae, K. lactis, K. marxianus*, and *Candida lusitaniae* have previously been isolated from water buffalo mozzarella (Minervini *et al.*, 2001; Romano *et al.*, 2001; Tornadijo *et al.*, 2001; Aponte *et al.*, 2010). Several studies performed buffalo mozzarella found that yeasts represent a significant part of the natural microbiota, with total counts ranging from 10^4 to 10^6 cfu/g (Aponte *et al.*, 2010).

The presence of hygienic-sanitary microbial indicators in cheese can be related to contamination of fecal origin, probably due to poor hygienic practices of milking storing, transporting and manufacturing (Viljoen, 2001). There is limited published information on outbreaks of human illness linked to consumption of the raw milk cheeses made with acid curds, but there have been reports of foodborn infections associated with *Staphylococcus aureus* in cheeses (Jorgensen *et al.*, 2005; Jelastopulu *et al.*, 2006). The presence and high numbers of *S. aureus* is indicative of potential risk to public health and questionable sanitation measures in industry, since this microorganism is part of the normal human microbiota of skin. *Staphylococcus aureus* food poisoning is caused by ingestion of food containing preformed enterotoxins (SE) (Zouharova and Rysanek, 2008). Pietrowski *et al.* (2008) found *S. aureus* in some mozarella samples from markets in Paraná. Buzy *et al.* (2009) showed that mozarella cheese marketed in São Paulo present good hygienic conditions, and only a few samples presented significant coliform counts. Although *Salmonella* spp. in dairy products caused several outbreaks of foodborne disease in Mozzarella and other cheese (D'oust, 1994), there is not a report of *Salmonella* isolation in muzzarella in Brazil (Olivieri, 2004).

The aim of this work was to describe the yeast species associated with water buffalo mozzarella commercialized in Belo Horizonte, in the state of Minas Gerais, Brazil, and their occurrence during the manufacturing process, as well as to determine the main hygienic-sanitary microbial indicators in these cheeses.

Material and Methods

Sample collection

From September 2005 to May 2006, 18 samples of trademark A and 12 samples each of two other trademarks (B and C), all vacuum packed without water or whey, were collected from retail outlets in the state of Minas Gerais, Brazil. Five samples of starter cultures, pasteurized milk, water and cheese after salting, and four samples of cheese curd before and after acidification were aseptically collected during five different visits to the industry belonging to trademark A, also in Minas Gerais, from March to May 2006. The samples were transported under refrigeration in a ice bath (≤ 8 °C) to the laboratory for microbiological analyses.

Microbiological procedures

All microbiological analyses were performed in triplicate. Detection of total and fecal coliforms was determined in all samples by the Most Probable Number (MPN) technique using a three-tube series. Total coliforms were enumerated in 2% bile brilliant green broth (Difco), incubated at 37 °C for 24-48 h; fecal coliforms were determined in EC broth (Difco) incubated at 44 °C for 24 h (Downes and Ito, 2001). For curdle and cheese, 25 g portions were homogenized with 225 mL of 0.1% buffered peptone water in a Stomacher 400 Lab Blender (London, UK) for 1 min and decimal dilutions were prepared therefore using the same diluent.

Water samples were also screened for the presence of heterotrophic bacteria determined on plate count agar (Difco Laboratories, Detroit, USA) and the plates were incubated at 30 °C for 48 h. Milk samples were analyzed for the presence *Salmonella* spp. after pre-enrichment in buffered peptone-water and enrichment in selenite cystine broth (Biobrás, MG, Brazil) and Rapapport-Vassiliads broth (Biobrás), incubated at 35 °C and 42 °C for 24 h. Enrichment cultures were streaked on *Salmonella-Shigella* agar (Biobrás) and Hektoen enteric agar (Biobrás). The plates were incubated at 35 °C for 48 h (Downes and Ito, 2001). The typical colonies were identified by Triple Sugar Iron (TSI) agar (Oxoid, Ltd. Basingstoke, Hampshire, England), Lysine Iron Agar (LIA) (Oxoid) fermentation tests and urease test (Urea Broth, Oxoid).

Starter cultures, curdle and cheese samples were screened for yeasts and *Staphylococcus* spp. For the determination of yeast populations, aliquots of 0.1 mL of the appropriate decimal dilutions from each sample were spread, in triplicate, on yeast extract-malt extract agar plates (YMA- 1% glucose, 0.5% peptone, 0.3% malt extract, 0.3% yeast extract and 2% agar) supplemented with

100 mg/L chloramphenicol. Plates were incubated at 25 °C for five days. Yeast colonies were counted, isolated and stored in liquid nitrogen for further identification. *Staphylococcus* spp. were counted on Baird-Parker agar (Biobrás) with added egg yolk tellurite, incubated at 37 °C for 48 h. After growth, *Staphylococcus* colonies were counted and classified as typical for *S. aureus* (jet black to dark gray, smooth, convex, entire margins with an opaque zone, clear halo beyond the opaque zone) and atypical (jet black to dark gray colonies, entire margin without a halo). Ten colonies from each sample (5 typical and 5 atypical) were selected and transferred to individual tubes with nutrient agar (stock culture), and tested for coagulase, thermonuclease (TNase), anaerobic fermentation of glucose and mannitol, production of hemolysin on sheep's blood agar, and furazolidone sensibility(Downes and Ito, 2001). Curdles and cheeses were also searched for *Salmonella* spp. and *Listeria monocytogenes* as described in Downes and Ito (2001).

Yeast identification

Yeast colonies were characterized phenotypically according to standard methods currently used in yeast taxonomy (Kurtzman *et al.*, 2011). Yeast identities were verified using the taxonomic keys published in Kurtzman *et al.* (2011). Yeast identities were also confirmed by PCR using the primer EI1 (5'-CTGGCTTGGTGTATG-3') that contains sequences complementary to intron splice sites that target mutable regions of the yeast genome (De Barros-Lopes *et al.*, 1998). DNA was purified as described by de Barros Lopes *et al.* (1998). Each PCR assay was performed according to Pataro *et al.* (2000). PCR products were analyzed by electrophoresis on a 1.5% agarose gel in TBE buffer (90 mmol/L Tris base, 89 mmol/L boric acid and 2 mmol/L EDTA) at 100 V for 3.5 h (Pataro *et al.*, 2000). The gel was stained with ethidium bromide and photographed under UV light. Reference and authentic cultures, confirmed by sequencing of the D1/D2 region of the large subunit of the rRNA gene, were used as standards for comparison in this PCR assay. These control cultures included *Zygosaccharomyces bailii* UFMG-L10, *Kluyveromyces lactis* UFMG- PJ01B, *Debaryomyces hansenii* UFMG-QA12-02, *Torulaspora delbrueckii* UFMG-PJ01A and UFMG-QJ11-02, *Candida catenulata* UFMG-QP10-2, *Candida albicans* ATCC18804, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750 and *Candida lusitaniae* CBS 6936. Yeast isolates of uncertain identity were identified by sequencing the D1/D2 variable domains of the large subunit of the rRNA gene. Genomic DNA was prepared from yeast cultures after two days of incubation on YMA using the methodology described by de Barros Lopes *et al.* (1998). The D1/D2 variable domains of the large subunit of the rRNA gene were amplified by PCR using primers NL1 (5'-GCATATCAATAAGCGGAGGAA AAG-3') and NL4 (5'- GGTCCGTGTTTCAAGACG G-3') as described by Lachance *et al.* (1999). The amplified DNA was concentrated, cleaned (Kit Wizard Plus SV Minipreps DNA Purification System-Promega, USA) and sequenced using the MegaBACE^{TM} 1000 automated sequencing system (Amersham Biosciences, USA). The sequences were analyzed using the DNAMAN program, version 4.1 (Lynnon BioSoft, Vaudreuil, QC, Canada). Existing sequences for other yeast were retrieved from GenBank.

Results and Discussion

Curd before and after acidification and mozzarella cheese had yeast counts that ranged from ND (not detected) to 4.81 log cfu/g of sample (Table 1). In mozzarella obtained from the markets, yeast counts ranged from < 3 to 6.08 log cfu/g in trademark A, 4.75 to 6.28 log cfu/g in trademark B, and < 0.3 to 4.36 log cfu/g in trademark C (Table 2).

Among samples from the industry, the highest number of yeast species was detected in the curd after acidification (Table 3). The acidification time can last 4 to 4.5 h in this industry, and it can explain the yeast contamination found in the curd; this contamination might originate from the hands of the manipulators or from contaminated utensils that may have come in contact with the cheese. Yeast is able to grow in milk and dairy products due to their ability to assimilate and ferment lactose, assimilate citric and lactic acids, produce lipases and extracellular proteases, and tolerate a high salt content (Fadda *et al.*, 2004; Aponte *et al.*, 2010). Aponte *et al.* (2010) suggest that yeasts contribute to the organoleptic definition of the water buffalo Mozzarella. According to these authors the recorded dominance of fermenting yeasts such as the lactose-fermenting *Kluyveromyces marxianus* (38.3% of the total isolates) and the galactose-fermenting *Saccharomyces cerevisiae* (21.6% of the total isolates) suggests that these yeasts contribute to the organoleptic definition of the water buffalo Mozzarella produced in the provinces of Salerno, Caserta, and Frosinone (Italy). In the present work, yeast species found in curds such as *Debaryomyces hansenii* and *Kluveromyces lactis* can modify the organoleptic characteristics of the cheese, since first species can ferment galactose, and the second is able to ferment lactose. Species such as *Candida catenulata*, *C. krusei*, *C. parapsilosis* and *C. rugosa* found in the curd samples can be considered contaminants of the production process.

The number of yeast species was lower in mozzarella than in curd after acidification. This result can be due to the stretching of the cheese in hot water (90 to 95 °C) which may have reduced the presence of these microorganisms. Silva *et al.* (1999) demonstrated that exposure of the cheese to hot water can reduce the level of yeast and fecal contamination up to 1,000 times. Yeasts were not isolated from the starter culture samples collected from the industry.

The number of species present was similar in all cheese trademarks (Table 3). Several studies have shown

Table 1 - Hygienic-sanitary indicators in water, starter culture, milk, curd and cheese collected in a mozzarella industry of Oliveira, Minas Gerais (the microbial counts were expressed in log values).

Sample[1]	Total coliforms (MPN[2]/g or /mL)	Fecal oliforms (MPN/g or /mL)	*Escherichia coli* (MPN/g or /mL)	Heterotrophic bacteria (cfu[c]/mL)	*Staphylococcus* spp. (cfu/mL or g)	Coagulase-positive strains (cfu/mL or g)	Yeast (cfu/ g or mL)
1st collection							
Water	0.3	< 0.3	< 0.3	2.176			
Starter culture	1.54	1.54	< 0.477		< 3	< 3	ND[4]
Milk	> 3.04	> 3.04	0.477				
Curd A[5]	> 3.04	1.43	1.63		4.68	4.68	ND
Cheese	2.66	2.66	1.97		4.36	ND	4.28
2nd collection							
Water	1.08	< 0.3	0.3	< 0			
Starter culture	0.95	0.95	0.6		1.48	ND	ND
Milk	> 3.04	> 3.04	> 3.04				
Curd B[6]	> 3.04	> 3.04	> 3.04		5.415	5.32	4.81
Cheese	2.32	2.32	1.18		2.48	ND	ND
3rd collection							
Water	< 0.3	< 0.3	< 0.3	0.11			
Starter culture	0.84	0.84	< 0.48		< 2	< 2	ND
Milk	> 3.04	> 3.04	2.38				
Curd A	> 3.04	> 3.04	> 3.04		6.11	ND	3.28
Curd B	> 3.04	> 3.04	> 3.04		6.23	6.15	4.15
Cheese	> 3.04	> 3.04	> 3.04		5.46	5.39	ND
4th collection							
Water	1.52	< 0.3	0.3	0.75			
Starter culture	0.48	0.48	< 0.48		2.11	ND	ND
Milk	0.6	< 0.48	< 0.48				
Curd A	> 3.04	> 3.04	3.04		5.95	ND	3.2
Curd B	> 3.04	> 3.04	1.56		4.99	4.69	3.9
Cheese	2.38	2.38	0.84		4.2	3.8	ND
5th collection							
Water	1.23	< 0.3	< 0.3	0.50			
Starter culture	1.18	1.18	< 0.48		< 2	< 2	ND
Milk	> 3.04	> 3.04	1.63				
Curd A	> 3.04	> 3.04	3.04		6.255	6.255	ND
Curd B	> 3.04	> 3.04	2.66		5.23	5.15	3
Cheese	3.04	3.04	0.95		3.78	ND	2.78

[1]Indicative sample; [2]MPN = Most Probable Number; [3]cfu = Colony forming units; [4]ND = Not detected; [5]Curd A = Curd before acidification; [6]Curd B = Curd after acidification.

that the prevalent yeast species typically associated with cheese are *D. hansenii, K. marxianus, Yarrowia lipolytica* and several species of *Candida* (Westall and Filtenborg, 1998; Fadda *et al.*, 2004; Borelli *et al.*, 2006a; Aponte *et al.*, 2010). Aponte *et al.* (2010) report the dominance of fermenting yeasts such as the lactose-fermenting *Kluyveromyces marxianus* (38.3% of the total isolates) and the galactose-fermenting *Saccharomyces cerevisiae* (21.6% of the total isolates) in traditional water buffalo Mozarella in Italy. In our study, the most prevalent species was *D. hansenii*, which occurred in 34 out of 42 samples of market cheese with population counts ranging from 2.5 to 7.2 log cfu/g and also in all samples from industry. *D. hansenii* has previously been isolated from different types of cheese produced in different countries (Westall and Filtenborg, 1998; Fadda *et al.*, 2004; Borelli *et al.*, 2006a; Álvarez-Martín *et al.*, 2007). This halophilic yeast has the ability to assimilate/ferment different carbon compounds,

Table 2 - Hygienic-sanitary microbial indicators present in cheese samples of three trademarks collected from markets of Belo Horizonte, Minas Gerais.

Sample	Microorganisms					
	Total coliforms (MPN[1]/g)	Fecal coliforms (MPN/g)	*Escherichia coli* (MPN/g)	*Staphylococcus* (cfu[2]/g)		Yeasts cfu/g
				Staphylococcus spp.	Coagulase-positive	
Trademark A (n = 18) [3]	< 0.48- > 3.04[d]	< 0.48-2.66	< 0.48-2.38	< 3-5.34	0- < 3	< 3-6.08
Trademark B (n = 12)	< 0.48-2.66	< 0.48-2.38	< 0.48-0.95	< 3-2.82	ND- < 3	4.75-6.28
Trademark C (n = 12)	< 0.48-3.04	< 0.48-3.04	< 0.48-1.60	2.48-5.28	ND-4.98	< 3-4.36

[1]MPN = Most Probable Number; [2]MPN = Most Probable Number; [3]cfu = Colony forming units.

Table 3 - Frequency (log cfu/g) of yeast species found in samples of three Brazilian water buffalo mozzarella trademarks.

Yeast species	Curd A[1] (n = 4)[2]	Curd B (n = 4)[3]	Cheese (n = 5)	Trademark A (n = 18)[1]	Trademark B (n = 12)	Trademark C (n = 12)
Aureobasidium pullulans						1 (3.4)
Candida catenulata	1 (3.2)	3 (3.0-3.9)[4]		1 (3.3)	1 (5.0)	
C. guilliermondii				1 (6.5)		3 (2.5-3.6)[2]
C. krusei		2 (2.5-3.3)		1 (2.5)	1 (4.5)	
C. lusitaniae		1 (3.2)		5 (3.8-6.5)	6 (4.0-5.2)	1 (3.5)
C. parapsilosis		1 (3.3)	2 (2.8-4.1)	9 (2.5-4.8)	1 (3.9)	2 (3.6-3.7)
C. pararugosa						1 (2.8)
C. rugosa	1 (2.5)					1 (2.8)
C. tropicalis						1 (3.2)
C. zeylanoides				2 (3.5-5.7)	1 (4.8)	1 (3.4)
Debaryomyces hansenii	1 (3.2)	1 (4.5)	1 (3.8)	16 (2.8-7.2)	12 (4.4-6.7)	6 (2.5-3.7)
Galactomyces geotrichum				3 (2.5-4.2)		
Kluyveromyces lactis		2 (4.0-4.5)		1 (5.1)	1 (6.0)	
Metschinikowia pulcherrima						1 (2.5)
Pichia fermentans				1 (3.8)		
P. membranifaciens				1 (6.5)		
Rhodotorula glutinis						2 (3.3-3.5)
Rh. minuta						1 (2.5)
Rh. mucilaginosa				3 (2.5-5.0)	1 (3.4)	2 (2.6-3.4)
Rh. slooffiae						2 (2.8-2.9)
Trichosporon asahii					1 (4.5)	
Yarrowia lipolytica				8 (3.6-7.0)		1 (3.7)

[1] n = number of samples; [2]Curd before acidification; [3]Curd after acidification; [4]Numbers in parenthesis indicate the lowest and/or highest counts (log cfu/g) of yeast in the sample.

and some strains possess lipase and protease activity. Its growth in dairy products may influence the flavor, aroma and texture of the product (Romano *et al.*, 2001; Fadda *et al.*, 2004; Borelli *et al.*, 2006a). Other yeast species frequently found in the mozzarella cheese studied were *C. parapsilosis, C. lusitaniae* and *Y. lipolytica.* Ferreira and Viljoen (2003) suggested the incorporation of *D. hansenii* and *Y. lipolytica* into the starter culture to make cheddar cheese. However, *D. hansenii* has been associated with spoilage of cheese, and high counts of this yeast result in a strong yeasty smell (Westall and Filtenborg, 1998; Stratford, 2006).

Some species of *Candida* are considered opportunistic pathogens and among these *C. guilliermondii, C. tropicalis, C. parapsilosis, C. lusitaniae, C. catenulata, C. rugosa* and *C. krusei* occurred in the mozzarella cheese samples analyzed, and the population counts ranged between 2.5 to 6.5 log CFU/g. The presence of these yeasts in cheese is probably caused by contamination of milk, air, clothes, hands, apparatus and equipment with which the cheese comes into contact during the production process (Suzzi *et al.*, 2000). In the industry responsible by the production of the trademark A, *C. parapsilosis, C. lusitaniae,* and *C. rugosa* were isolated from the manufacturing pro-

cess. The occurrence of limited yeast species confirms that the cheese ecosystem is characterized by specific environmental conditions and composition, which promotes selection of a uniform and well-defined mycobiota (Jakobsen and Narvhus, 1996). Among twenty-three yeast species, only 10 were isolated solely once, indicating they are transient contaminants, and the majority (13 species) was isolated from two or three samples. Trademark C cheese showed the highest frequency of transient species isolated only once (eight samples) including the opportunistic species *C. rugosa* and *C. tropicalis*, and the ubiquitous yeast *Aureobasidium pullulans*. *Trichosporon asahii* was isolated only from Trademark B cheese, and *Galactomyces geotrichum, Pichia fermentans* and *P. membranifaciens* were isolated only from Trademark A cheese.

Two out of five samples of water and one sample of milk (Table 1) were not in conformity with the Brazilian standards, that state the absence of fecal coliforms or *E. coli* in water (Brasil, 2004). Also, two samples of cheese were not in conformity with the Brazilian standards for cheese with high humidity (Brasil, 2001). Out of 72 *Staphylococcus* spp. isolates from industry samples, 32 were coagulase positive and 18 were identified as *S. aureus. Staphylococcus aureus* is salt-tolerant and has the ability to grow under very different conditions; it is often found in raw milk and in the cheese-making environment (Bergdoll, 1989; Borelli *et al.*, 2006b).

Salmonella spp. and *Listeria* spp. were not found in any samples of raw milk, curd or in cheese collected in this study. Although some samples were not appropriate for consumption, the results indicate a cheese with better quality than other Brazilian cheeses (Quintana and Carneiro, 2007; Lima *et al.*, 2009; Borelli *et al.*, 2011), and similar to the results obtained for mozzarella cheese collected in São Paulo State (Buzi *et al.*, 2009), and in Paraná State (Pietrowski *et al.*, 2008).

Table 2 shows the counts of hygienic-sanitary indicators found in cheese samples collected from retail outlets. All samples of trademark A and B were in accordance with Brazilian standards for cheese with high humidity (Brasil, 2001). Only one sample of trademark C exceeded the limit for coagulase-positive *Staphylococcus*. Out of the 121 *Staphylococcus* spp. isolates from market samples, seven were coagulase-positive and five were identified as *S. aureus.* Eleven samples showed counts of thermotolerant coliforms higher than 5×10^3 UFC.g^{-1}, but still lower than the maximum permitted by the Brazilian laws. *Salmonella* spp. and *Listeria* spp. were not found in any samples of cheeses from retail outlets.

Mozzarella cheese harbors yeasts typical of cheese environment, and the occurrence of possibly opportunistic yeasts may derive from milk contamination, air, clothes, hands, apparatus and equipment with which the cheese comes into contact during the production process and storage. Nevertheless low levels of contamination indicate a satisfactory hygienic standard in the industry, and it is reflected in the low levels of microbial contaminants in cheese from retail outlets. Although it is difficult to evaluate the risk associated with consumption of this cheese, the presence of opportunistic yeasts provides a ready source of inoculum to those people who may be susceptible to infection. The isolation of these yeasts in food presenting satisfactory sanitary conditions deserves a more extensive investigation on the possible sources of these possible pathogens in the Mozzarella cheese.

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