

Occurrence of Filamentous Fungi in Human Milk, Infant Formula and Milk-Based Products for Young Children Nutrition

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Abstract: The safety of foods (human milk-HM; infant formula-IF; milk-based products-MBPs) aimed for children nutrition (from birth to 5 years old) through filamentous fungi & yeasts were investigated ($n = 158$). Their analysis followed the ISO 6611: 2004 recommended for total load (isolation & enumeration) in milk and dairy products. The occurrence of filamentous fungi & yeasts was observed in 29, 51 and 60% of the HM ($n = 98$), IF ($n = 45$) and MBP ($n = 15$) samples surveyed. Fungi isolated present counts above 10^2 CFU/g and yeasts higher ($> 10^4$ CFU/g). *Aspergillus*, *Penicillium* and *Thichoderma* genera were identified in all the three sample types at percentages of 100/13/9%, 78/11/22% and 32/39/25% for IF, MBP and HM samples, respectively, being *Aspergillus* the most isolated, especially in the IF samples. Despite deterioration that can cause, the presence of filamentous fungi in HM and other infant foods, can enable mycotoxins production as long as toxigenic species are present which are hazardous for babies.

Keywords: Fungi, Food Safety, Human Milk, Infant Formula

1. Introduction

Human milk (HM) is the best food for children from birth up to 6 months of age [1]. However, when breast feeding is not possible or desired, infant formulas (IF) are an adequate substitute [2-3]. Thus milk and milk-based products are the major nutrients for children. Quality and safety aspects of infant foods are of key importance for child health. Despite that, quite often they do not get much attention by the health care professionals whose interest tends to focus on functional benefits of early nutrition [4].

Infant foods are commonly based on cow's milk formulations and starchy gruels [3, 5]. Food products, including powdered IF, are not sterile and may contain viable microorganisms and their spores, including pathogens such

as *Salmonella enterica*, *Salmonella typhi*, *Shigella dysenteriae* or *Cronobacter* spp. (formerly *Enterobacter sakazakii*) which can cause serious infants infections [4, 6]. Every single IF ingredient must have an excellent quality and safety approach because (even in very small quantity in a single product) serious consequences may arise if they are not taken seriously by the manufacturer [7]. Despite that, the IF and milk powders are considered reasonably safe products for public health, however, any failure during processing may favor the occurrence of final product poor microbiological quality [8]. While most studies have focused on HM and infant foods bacteria contamination, just a few have focused on its fungal contamination, leaving a gap in the literature regarding this information.

Milk, both human and non-human, is an extremely

complex biological matrix that contains thousands of nutritional components [9]. Therefore, it is considered an ideal substrate for fungal growth [10]. Breast milk is not sterile, as the microorganisms can multiply when the milk is not handled properly. Additional exogenous contamination should be prevented. Strict hygiene and careful temperature and time control are important during the expression, collection, transport, storage, and feeding of maternal milk [11-12].

Filamentous fungi are extremely versatile; most species can assimilate any carbon source derived from food. Most of them also are indifferent regarding the nitrogen sources and can use nitrate, ammonium ions and organic nitrogen. In addition, fungi and yeasts are very resistant to adverse conditions such as low pH and water activity (aw). Most yeasts need at least aw of 0.88 and fungi 0.80 for growth [13-14]. Fungi of the genus *Aspergillus* are widely distributed in the world, however they mainly occur in subtropical and tropical regions. The *Penicillium* species are able to develop into a wider range of temperatures than *Aspergillus*; however, they are more abundant in temperate regions [15].

The major toxigenic fungi genera are *Aspergillus*, *Penicillium* and *Fusarium* and the main mycotoxins produced by them are aflatoxins - AFLs (AFB₁, AFB₂, AFG₁, AFG₂), ochratoxin A (OTA), fumonisins, zearalenone and trichothecenes (deoxynivalenol, nivalenol, T2 and HT2). AFLs can be produced mainly by four *Aspergillus* species (*A. flavus*, *A. parasiticus*, *A. nomius* and *A. pseudotamarii*). Two additional AFLs, the AFM₁ and AFM₂, are products of the AFB₁ hydroxylation in the liver [16]. They are excreted in

the lactating animals milk and are often found in dairy products. AFB₁ and AFM₁ are human carcinogens, classified in the Group 1 [17]. OTA is produced mainly by *A. carbonarius* and *P. verrucosum* and is considered a possible human carcinogen, classified in the Group 2B by IARC [18-19].

The microbiological quality of foods intended for children consumption, especially for infants hospitalized in Neonatal Intensive Care Units (NICUs) is a subject of interest to public health, since these children have low resistance to neonatal infections [20]. In addition, those toxigenic fungi species can produce toxins that produce a wide variety of toxic effects [16, 21-24].

This study aimed to evaluate the presence of filamentous fungi and yeasts in foods (HM, IF and milk-based products-MBP) intended for children (from birth to 5 years old) consumption in order to contribute with information about their contamination occurrence.

2. Materials and Methods

2.1. Material

2.1.1. Samples

Foods for young children nutrition ($n = 158$) being (a) HM-bottles with 20-60 mL content ($n = 98$) from the Human Milk Bank of Blumenau (HMBB-SC), Santa Catarina State (SC), Southern Brazil and (b) IF and MBP- packs of 400 g ($n = 45$ & 15, respectively) from manufacturers and chemist stores, commercialized in Florianopolis city, SC (Table 1).

Table 1. Food for infants and young children samples characteristics evaluated.

Sample			Product			Total number*
Food	Type	Characteristics	Package	Brand	Amount (g or mL)	
FOR INFANTS						
	Human milk	<i>in natura</i>	<i>in natura</i>	NA	20	98
	Infant formula ^a	powder	cans	A, B, C	400	45
FOR YOUNG CHILDREN						
	Milk-based products ^b	powder	cans	D	400	15

^abovine protein-based products for infants from birth to a year old; ^bbovine milk-based product with addition of vegetable oils for children from 1 to 5 years old NA: not applicable * 158 samples

2.1.2. Reagents and Culture Media

Peptone, glucose, yeast extract, malt extract, agar and chloramphenicol, all from Vetec (Duque de Caxias, RJ, Brazil). They were used to prepare the yeast extract glucose chloramphenicol agar (YEGC) and the malt extract agar (MEA) culture media.

2.1.3. Equipment

Autoclave, Phoenix (Araraquara, SP, Brazil), laminar flow, Veco (Campinas, SP, Brazil), semi-analytical balance, Mettler (Barueri, SC, Brazil), stomacher, Marconi (Saint Nom, France), incubator, Fanen (Sao Paulo, SP, Brazil) colony counter, Phoenix (Sao Paulo, SP, Brazil) and optical microscope, Olympus (Tokyo, Japan) were used to the analysis.

Note: this study was approved by the Ethics Committee of the University of Blumenau. All lactating donors who provided the HM samples were informed about the content of this study and when agreed to participate, an informed written consent was signed by both parties before inclusion in the study.

2.2. Methods

2.2.1. Sample Collection

(a) HM - were collected (20-60 mL) after milking expression, in glass bottles, then frozen at the HMBB and stored frozen until the time of analysis at the LABMICO. (b) IF and MBP- were purchased randomly from manufacturers and pharmacies/chemists in Florianopolis city, SC and stored in their own sealed packs/bags at room temperature (+ / -

20°C) until analysis (Table 1).

2.2.2. Total Fungi Load

(a) *isolation* -the analysis of fungi and yeasts followed the ISO 6611: 2004 recommendations, directed to their enumeration in liquid milk, milk powder and other dairy products [25] as follows (b) *HM*- aliquots of HM(0.1 ml) and two decimal dilutions in peptone water 0.1% (10^{-1} and 10^{-2}) were plated in duplicate by the spread plating technique in YEGC and incubated at $25 \pm 1^\circ\text{C}$ for 5 days and (b.1.2) *IF & MBP*- portions of each sample (25g) were diluted in sterile peptone water (0.1%) and homogenized in stomacher for 1 min. From these solutions two decimal dilutions in peptone water 0.1% (10^{-1} and 10^{-2}) were prepared. Aliquots from each dilution (0.1 ml) were plated and incubated at $25 \pm 1^\circ\text{C}$ for 5 days. (c) *Enumeration of yeasts & fungi*: the total yeasts and fungi counts were performed as according to the IN62 regulation [26], ISO 7218: 2007 [27] and Silva *et al.* [13].

2.2.3. Identification of Filamentous Fungi Genera

The fungi grown on YEGC plates were isolated in MEA for the slide cultures preparation, according to Riddell [28] and Weber and Pitt [29]. The fungigenera identification was carried out microscopically according to Pitt and Hocking [30].

3. Results and Discussion

From the total food samples (HM, IF and MBP) aimed for children nutrition (in their early ages), it was possible to register a variation on the filamentous fungi & yeasts

presence and so among the products type. Tables 2 to 4 show data including differences on fungi genera isolated per product surveyed and their frequency distribution.

3.1. Total Filamentous Fungi Load Versus Different Foods for Children Nutrition

As far as the young children nutrition food samples (HM, IF and MBP) filamentous fungi total load are concerned, it was observed that their occurrence was most detected in the MPB samples, followed by IF and HM at 60% ($n = 9$), 51% ($n = 23$) and 28% ($n = 28$), respectively (Tables 2 and 3).

HM: data also showed that, apart from the fungi load in the HM samples, it was also possible to observe & separate filamentous fungi counts from the yeasts, i.e., only filamentous fungi total ($> 10^2 \leq 10^4 \text{CFU/mL}$ $n = 5+1$). The others samples were just yeasts contaminated or not contaminated at all (NG = 30%) (Table 2). On the other hand, a higher percentage (42%) of samples was registered containing only yeasts reaching rather high counts above 10^4CFU/mL ($n = 3+7$). It should be emphasized that high occurrence of yeasts in the HM samples may come from the mother's skin and often produce maternal infections such as mammary candidiasis by *Candida* including *C. albicans* and *C. parapsilosis* yeasts [31-32].

IF & MBPs - although IF&MBP percentage of positive samples was higher than HM, the occurrence of filamentous fungi isolated from them (dry/powder products) reached similar counts at the range: $> 10^2 \leq 10^4 \text{CFU/mL}$ with $n = 4$ and $n = 1$ samples, respectively and no yeasts were detected in those dry samples as expected (Table 3).

Table 2. Enumeration of filamentous fungi and yeasts in HUMAN MILK samples for young children nutrition from HMBB**.

Plate content	HM contamination				
	Positive	Range (CFU/mL)			
	n (%) ^a	< 1CFU/mL (est)	$> 10^0 \leq 10^2$	$> 10^2 \leq 10^4$	$> 10^4 \leq 10^5$
Filamentous fungi + yeasts (%) ^b	22 (22)	NA	NA	NA	NA
(a) yeasts		NA	10	9	3
(b) filamentous fungi ^c		NA	17	5	NG
Only filamentous fungi (%) ^c	6 (6)	NA	5	1	NG
Only yeasts n (%)	41 (42)	NA	16	18	7
No fungal growth	29 (30)	29	NG	NG	NG

CFU: colony forming units; ^a percentages from the total samples ($n = 98$); ^b yeasts and filamentous fungi were enumerated separately; ^c estimated enumeration due to the lower number of colonies than the accuracy and repeatability range of the method (15-150 colonies); NA: not applicable; NG: no growth*HMBB Human Milk Bank of Blumenau

Table 3. Occurrence of filamentous fungi in INFANT FORMULA AND MILK-BASED PRODUCTS for young children nutrition from HMBB**.

Sample			Children nutrition products contamination				
Type	Brand	n	Positives n (%)	Range (CFU/mL)			
				< 1CFU/mL (est)	$> 10^0 \leq 10^2$	$> 10^2 \leq 10^4$	$> 10^4 \leq 10^5$
IF	Total	45 ^a	23 (51)	27	14	4	NG
	A	24	10 (42)	16	8	NG	NG
	B	13	7 (54)	9	4	NG	NG
	C	8	6 (75)	2	2	4	NG
MBP	D	15 ^a	9 (60)	8	6	1	NG

MPs: milk products CFU: colony forming units; IF: infant formula; MBP: milk-based products; ^a total samples ($n = 45$ & 15); NG: no growth *HMBB Human Milk Bank of Blumenau.

3.2. Filamentous Fungi Genera Isolated Versus Children Food Types

Regarding the main filamentous fungi isolated and identified in the different samples types (HM, IF and MBP), they were those belonging to the *Aspergillus*, *Penicillium*, *Mucor*, *Paecilomyces*, *Trichoderma* and *Geotrichum* genera. Some samples 6% ($n = 6$) showed growth of more than one fungus genus. Table 4 presents the frequency of occurrence of each genus isolated from the young children food positive samples. Important to emphasize that, as HM has a quite high humidity (aw & moisture content-mc) when compared to those processed IF&MBP food (low humidity), other filamentous fungi were also isolated and identified. They were from the *Alternaria*, *Botrytis* and *Cladosporium* genera which need high humidity to grow and are called field fungi, as they can grow under high moist in open environments.

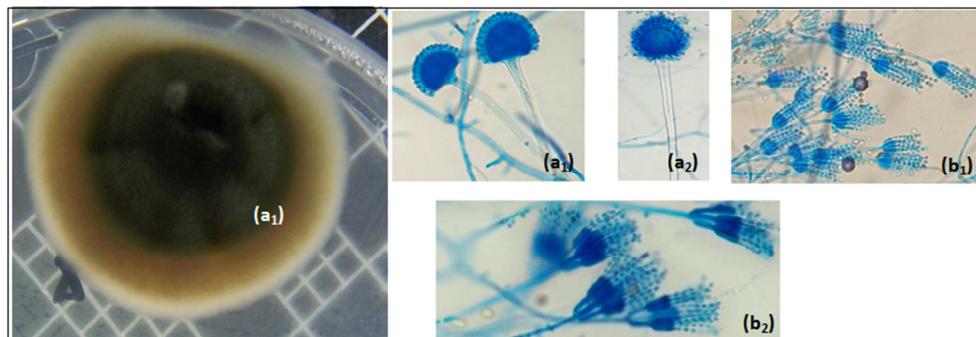


Figure 1. Fungi colony and reproductive structures of: (a) *Aspergillus* - (a.1) macroscopic & (a.2) microscopic [a₁; a₂] and (b) *Penicillium*- (b.1) microscopic [b₁; b₂] genera isolated from the human milk samples, infant formula and milk-based products for young children nutrition.

3.3. HMFungi Contamination, Hygiene and Temperature Treatment

The HM data of filamentous fungi enumerated (load), isolated and identified in the current work showed that some samples were exposed to inadequate conditions either, of handling, storage or feeding environment leading to certain contamination. That could be either, the HM temperature treatment, containers hygiene and/or substrate exposure conditions. Regarding HM data reported in the literature on HM hygiene and/or treatments, Almeida [33], registered filamentous fungi prevalence in 69.4% of the samples surveyed (with counts reaching 10^6 CFU/mL). After employing the mammary gland hygiene with soap and water, authors reported that contamination declined to 16.7% (counts up to 3.0×10^2 CFU/mL). On the other hand, Novak et al. [10] observed the occurrence of fungi & yeasts in 5.2% (43) of the samples surveyed with counts reaching 10^3 CFU/mL. From those positive samples, authors identified mainly *Penicillium* (60.4%), followed by *Syncephalastrum* (14.5%), *Paecilomyces* (12.6%), *Aspergillus* (4.2%) and *Rhizopus* (2.0%) generagroup. Authors were able also to isolate and identify the ochratoxigenic species of *Aspergillus niger* (6.3%). Regarding temperature treatment, Serafini et al. [20] reported rather similar contamination being in 22% (43) of crude HM samples, and in 25.7% (37) of pasteurized HM -

Fortunately, the clinically important fungi, such as those from genera *Microsporium*, *Trichophyton* and *Epidermophyton* cited by Novak et al. [10], which cause skin infections, were not isolated in the current HM study. Therefore, the data obtained indicate that the fungi observed in the samples either, came from the environmental and/or from the HM handled by the donors [30]. Figure 1 shows the macroscopic and microscopic aspects of isolated colonies of *Aspergillus* and *Penicillium*. On the other hand, the data on processed food IF & MBP for children nutrition showed that the IF positive samples were 100% ($n = 23$) of *Aspergillus*, a quite high contamination when compared to those for *Penicillium* and *Trichoderma* with only 39.3% ($n = 11$) and 8.7% ($n = 3$), respectively. As those products are dry, the problem could rise after their dissolution (in water), if kept longer from feeding the child (spores proliferation).

a worrying result indicating possible environment contamination after pasteurization or the processing ineffectiveness. The presence of pathogenic fungi in pasteurized HM suggests that this could be a source of infection to newborns during lactation and also to early exposure to mycotoxins [21-23].

Table 4. Frequency distribution of the FILAMENTOUS FUNGI GENERA isolated from foods for infants and young children.

Fungi genera isolated	Children nutrition products positive samples		
	HM ^b	IF ^c	MBP ^d
<i>Aspergillus sp</i>	9 (32.1)	23 (100)	7 (77.7)
<i>Penicillium sp</i>	11 (39.3)	3 (13)	1 (11.1)
<i>Trichoderma sp</i>	7 (25)	2 (8.7)	2 (22.2)
<i>Mucor sp</i>	2 (7.1)	NI ^e	NI
<i>Alternaria sp</i>	1 (3.6)	NI	NI
<i>Botrytis sp</i>	1 (3.6)	NI	NI
<i>Paecilomyces sp</i>	1 (3.6)	NI	NI
<i>Cladosporium sp</i>	1 (3.6)	NI	NI
Other*	2 (7.1)	NI	NI

^a positive samples; ^b human milk ($n = 28$) ^c infant formula ($n = 23$) ^d milk-based products ($n = 9$) ^e not isolated/identified * *Geotrichum*.

3.4. HM Versus IF and MBP Contamination

Despite of filamentous fungi HM contamination detected in the current work at reasonable lower counts [$\leq 10^2$ ($n = 17$)

& 5); $< 10^4$ ($n = 1$)], the results showed that their fungi presence in IF&MBP were also similar [$IF: \leq 10^2$ ($n = 14$); $> 10^2 < 10^4$ ($n = 4$)] and $MBP: > 10^0 \leq 10^2$ ($n = 6$) / $> 10^2 \leq 10^4$ ($n = 1$)] and do not represent a public health problem. The low occurrence of fungi in these products is explained by its low aw and mc, which hinders the fungi development [34], different of HM (quite high moist conditions), thus optimum substrate for microorganisms development. Apart from the known HM bacteria optimal environment, also yeasts may grow, different from fungi that have difficulty to grow at too high aw & mc. However, when contamination occurs, it is usually related to the microorganisms presence in the processing environment, such as external parts of equipment and surroundings of the processing lines (their entrance possibility into the lines) [35]. Despite that, when comes on fungi genera identification, *Aspergillus* was detected in all filamentous fungi positive IF samples at 23 (100%) followed by MBP with 7 (78%) (Table 4). It can be observed that HM showed the greatest diversity of fungi genera, while the fungi isolated from IF and MBP were limited to the *Aspergillus*, *Penicillium* and *Trichoderma* genera. Two samples of IF (4%) showed co-occurrence of *Aspergillus* and *Trichoderma*. While the co-occurrence of *Aspergillus* and *Penicillium* was observed in 3 (6%) samples of IF and in one (6%) MBP sample. Most studies on HM and other infant foods have focused on bacteria contamination, just a few evaluated its fungal & yeast contamination, being the cause of the scarcity of such information.

3.5. Other Fungi Genera Isolated, Substrates Diversity and Food for Children Nutrition Growth Conditions

As far as those fungi isolated in the 3 types of children food are concerned, they can grow in a wide range of food substrates and may contaminate the current study samples, as long as they are handled during children feeding. Regarding the isolated storage fungi (*Aspergillus*, *Penicillium*, *Trichoderma* and *Paecilomyces*), there are just a few foods from which *Aspergillus* cannot be isolated consistently (its species are related to a variety of food spoilage, from processed food (milk, cheese) to grains and oilseeds (rice, maize, wheat, peanuts- low humidity) as well as stored fruits (tomatoes, grapes-high humidity)[30]. On the other hand, *Penicillium* species have as preferred substrates also processed food, cereals and stored fruits (citrus and pome) [36]. *Trichoderma* species which are considered soil fungi has been isolated from different stored food (cassava, apples, peas, maize and nuts) [30]. *Paecilomyces* is a ubiquitous foods contaminant either for raw materials and/or those high in oil content (margarine, peanuts and cocoa) [37]. Regarding the field fungi (need high humidity substrates-aw &mc) *Alternaria* and *Botrytis* species can cause spoilage of stone fruits (peaches, apricots, cherries) also onions and peas. *Cladosporium*, occurs mostly in fresh fruits and vegetables [30]. It should be mentioned that fungi spores, which are aerially dispersed, when bottles are opened, make a serious source of contamination and so those on the food (fruits, grains) present in the child's room environment. Therefore,

the occurrence of filamentous fungi in the low aw samples such as IF and MBP (aw 0.2), can also be derived from environmental contamination [30].

3.6. Regulation for Filamentous Fungi

One of the difficulties of the current work was to find other data on their occurrence in IF and MBP for infant feeding and also the lack of regulated microbiological limits to fungi and yeasts in these products, and so for HM. Some microorganisms presence is indicative that the food has been exposed to conditions that pose an increased risk of pathogens contamination or having been held under conditions that would allow their proliferation.

The Brazilian Health Ministry (RDC 12), established standards for IF and MBP just for coliforms, coagulase positive *Staphylococci*, *Bacillus cereus* and *Salmonella* sp. Breast milk from HM banks, has standards established for Mesophilic Aerobic Bacteria, coliforms, coagulase positive *Staphylococci* and *Salmonella* sp [26, 38]. Also international regulations such as from Australia, China, EU, Japan, USA established similar standards with some differentiations though [39].

4. Conclusions

Data obtained on HM showed filamentous fungi contamination. It is likely that the fungi spores present in the HM (handled by milk donors) were the source of fungi contamination detected, as their characteristics were quite similar to those of food deterioration. When HM is exposed to microbiologic contaminants, usually it is related to (a) mothers skin or hands / breast pump components / milk containers and/or (b) the environment where the milk is expressed and exposed).

It is assumed that HM pasteurization (62.5°C / 30 min) at the HM Banks inactivates filamentous fungi. However when it comes to the product (HM) transfer to hospitalized premature babies in NICUs, fungi spores can get into and proliferate. Therefore, it is essential to comply with proper conditions (during collection / storage / transportation) to avoid the presence and multiplication of such contaminants.

Regarding the IF and MBP filamentous fungi presence, the results showed that did not represent a serious public health problem. The low occurrence of fungi in these products is explained by their low aw (± 0.2) and mc, which hinders the development of fungi. Care should be taken after their dissolution in water prior children feeding.

This is the first study reporting IF and MBP filamentous fungi and yeasts enumeration and identification.

References

- [1] WHO - World Health Organization, "Infant and young child feeding. Model chapter for textbooks for medical students and allied health professionals," Geneva, Switzerland: WHO Press, 2009.

- [2] M. Alles, "Current trends in the composition of infant milk formulas," *Current Pediatrics*, 14 (1), 51-63, 2004.
- [3] M. Guo, "Chemical composition of human milk," in *Human milk and infant formula manufacturing and technology*, M. Guo, Ed. Woodhead Series in Food Science, Technology and Nutrition, chap 2, Woodhead Publishing, 2014a, pp. 19-31.
- [4] B. Koletzko, R. Shamir, and M. Ashwell, "Quality and safety aspects of infant nutrition," *Annals of nutrition & metabolism*, 60 (3), 79-84, 2012.
- [5] G. Weisstaub, and R. Uauy, "Non-breast milk feeding in developing countries: challenge from microbial and chemical contaminants," *Annals of nutrition & metabolism*, 60 (3), 215-219, 2012.
- [6] Turck, D, "Safety aspects in preparation and handling of infant food," *Annals of nutrition & metabolism*, 60 (3), 211-214, 2012.
- [7] P. Hamrin, and B. Hoefft, "Quality control throughout the production process of infant food," *Annals of nutrition & metabolism*, 60 (3), 208-210, 2012.
- [8] T. Krey, and C. F. V. Souza, "Avaliação da qualidade microbiológica e físico-química do leite em pó integral produzido numa indústria da região do vale do taquari - RS," *Interbio*, 3, 2009.
- [9] M. C. Neville, and R. G. Jensen, "The Physical Properties of Human and Bovine Milks," *Handbook of milk composition*: Academic Press, 1995.
- [10] F. R. Novak, J. A. G. Almeida, M. J. S. Santos, and B. Wanke, "Human milk fungi mycelia contamination," *Journal of Pediatrics*, 78, 197-201, 2002.
- [11] V. Cossey, A. Jeurissen, M. J. Thelissen, C. Vanhole, and A. Schuermans, "Expressed breast milk on a neonatal unit: a hazard analysis and critical control points approach," *American Journal of Infection Control*, 39 (10), 832-838, 2011.
- [12] M. Guo, "Human milk banking," in *Human milk and infant formula manufacturing and technology*, M. Guo, Ed. Woodhead Series in Food Science, Technology and Nutrition, chap 5, Woodhead Publishing, 2014b, pp. 112-134.
- [13] N. Da Silva, V. C. A. Junqueira, N. F. A. Silveira, M. H. Taniwaki, R. F. S. Santos, R. A. R. Gomes, "Manual de Métodos de análise microbiológica de alimentos e água," 4th ed, Sao Paulo, Varela, 2010, pp. 624.
- [14] V. M. Scussel, M. A. Kluczkowski, and G. D. Savi, "Factors for development of fungi and toxin production," in *Grains Storage*, I. Lorini, L. Miike, L. D'a. Faroni, V. M. Scussel, Section 6. chap 3 - BioGeneziz Ed., Campinas SP, Brazil, 2017, pp. 548.
- [15] F. C. O. Freire, I. G. P. Vieira, M. I. F. Guedes, and F. N. P. Mendes, "Mycotoxins: their food importance to human and animal health," *Fortaleza: Embrapa Agroindústria Tropical*, 2007, pp. 48.
- [16] S. A. Kiliç, S. Gürbüz, and E. Ayağ, "Aflatoxin M1 in human breast milk in Southeastern Turkey," *Mycotoxin Research*, 33 (2), 103-107, 2017.
- [17] IARC - International Agency for Research on Cancer. Monographs on the evaluation of carcinogenic risks to humans, some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. International Agency for Research on Cancer, 56, 489-521, 1993.
- [18] Cast. *Mycotoxins: Risks in plant, animal, and human systems*. Ames Iowa: Council for Agricultural Science and Technology, 2003, pp. 217.
- [19] IARC - International Agency for Research on Cancer. Some traditional herbal medicines: some mycotoxins, naphthalene and styrene. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. WHO: Lyon, France, 82, 1-556, 2002.
- [20] A. B. Serafini, M. C. Andre, M. A. Rodrigues, A. Kipnis, C. O. Carvalho, M. R. Campos, E. C. Monteiro, F. Martins, and T. F. Jubé, "Microbiological quality of human milk from a Brazilian milk bank," *Revista de Saude Publica*, 37 (6), 775-779, 2003.
- [21] N. Magan, and M. Olsen, "Mycotoxins in Food: detection and control," Boca Raton, Woodhead Publishing, 2004, pp. 471.
- [22] K. M. Tonon, "Mycoflora evaluation and mycotoxins in Human Milk and Food for Children and Infants by Tandem Mass Spectrometry," Thesis, Food Science and Technology Department, CCA, UFSC, Florianopolis, SC, Brazil, 2013. pp. 129.
- [23] K. M. Tonon, M. G. R. Reiter, and V. M. Scussel, "Dietary intake of human milk donors and LC-MS mycotoxin occurrence," in XIX National Nutrology Congress, 2015, Sao Paulo, International Journal of Nutrology, Brazilian Association of Nutrology, 8, 69, 2015.
- [24] K. M. Tonon, M. G. R. Reiter, G. D. Savi, and V. M. Scussel, "LC-MS/MS multi-mycotoxin analysis of human milk and dietary intake of Brazilian nursing mothers," *Journal of Human Lactation*, *in press*, 2017.
- [25] ISO - International Organization for Standardization. ISO 6611: 2004 (IDF 94: 2004) Milk and milk products: enumeration of colony-forming units of yeasts and/or moulds - colony-count technique at 25 degrees C, ISO, 2 ed, 2004, p. 8.
- [26] MAPA - Ministry Agriculture, IN62 of 26/09/2003. Official microbiological analytical methods for for animal food products and water control. DOU, Brasilia, 2003.
- [27] ISO - International Organization for Standardization. ISO 7218: 2007. Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations. International Organization for Standardization, 2007, p. 66.
- [28] R. W. Riddell, "Permanent stained mycological preparations obtained by slide culture," *Mycologia*, 42, 265-270, 1950.
- [29] R. W. S. Weber, and D. Pitt, "Teaching techniques for mycology Riddell's slide cultures," *Mycologist*, 14 (3), 118-20, 2000.
- [30] J. I. Pitt, and A. D. Hocking, "Fungi and Food Spoilage." New York: Springer, 2009, pp. 519.
- [31] E. Jiménez, L. Fernández, A. Maldonado, R. Martín, M. Olivares, and J. Xaus, "Oral administration of *Lactobacillus* strains isolated from breast milk as an alternative for the treatment of infectious mastitis during lactation," *Applied and Environmental Microbiology*, 74 (15), 4650-5, 2008.

- [32] G. Dos S. Oliveira, R. H. Luchese, F. R. Novak, D. P. B. Abreu, and A. M. D. Martins, "Fungal contamination in human milk and in the anatomic sites of breastfeeding mothers and infants," *Revista do Instituto Adolfo Lutz*, 71 (3), 450-5, 2012.
- [33] J. A. G. Almeida, "Human Milk quality collected and processed in HN Banks," Thesis, Universidade Federal de Viçosa, Viçosa, 1986.
- [34] P. Breeuwer, A. Lardeau, M. Peterz, and H. M. Joosten, "Desiccation and heat tolerance of *Enterobacter sakazakii*," *Journal of Applied Microbiology*, 95 (5), 967-73, 2003.
- [35] CAC - Codex Alimentarius Commission. Code of hygienic practice for powdered formulae for infants and young children CAC/RCP 66, (2008). [http://www.codexalimentarius.org/input/download/standards/..CXP_066e.pdf].
- [36] O. Filtenborg, J. C. Frisvad, and U. Thrane, "Moulds in food spoilage," *International Journal of Food Microbiology*, 33, 85-102, 1996.
- [37] H. Loiveke, E. Ilumae, and H. Laitamm, "Microfungi in grain and grain feeds and their potential toxicity," *Agronomy Research*, 2, 195-205, 2004.
- [38] Anvisa- National Agency of Sanitary Surveillance. Health Ministry, RDC12, of 02/01/2001. RTformicrobiological standards in foods. Brasilia, 02/01/2001.
- [39] Y. J. Jiang, "Infant formula products regulation," in *Human milk and infant formula manufacturing and technology*, M. Guo, Ed. Woodhead Series in Food Science, Technology and Nutrition, chap 11, Woodhead Publishing, 2014. pp. 273-308.