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Methods for thermal inactivation of pathogens in mozzarella: a comparison between stretching and pasteurization

[*Métodos para inativação térmica de patógenos em mozarela: comparação entre filagem e pasteurização*]

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ABSTRACT

This study aimed to evaluate the efficiency of stretching in the reduction of pathogens when compared to milk pasteurization, the official method to ensure safe cheese production. Whole buffalo milk was contaminated with *Mycobacterium fortuitum*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus.* Part of the milk was used in mozzarella production and the other part was submitted to holder pasteurization. Pathogens were quantified before and after thermal processing (mozzarella stretching and milk pasteurization). Pasteurization and stretching led to the following reductions in log cycles, respectively: 4.0 and 6.3 for *Mycobacterium* sp.; 6.0 and 8.4 for *Listeria* sp.; >6.8 and 4.5 for *Staphylococcus* sp.; and >8.2 and 7.5 for *Salmonella* sp.

Keywords: mozzarella, stretching, pasteurization, pathogenic bacteria

RESUMO

Este estudo teve como objetivo avaliar a eficácia da filagem na redução de patógenos, em comparação com a pasteurização do leite, que é o método oficial para garantir a produção de queijos seguros. Leite de búfala integral foi contaminado com Mycobacterium fortuitum, Listeria monocytogenes, Salmonella typhimurium *e* Staphylococcus aureus. *Parte desse leite foi empregada na fabricação da mozarela e outra parte foi submetida à pasteurização lenta. Os patógenos foram quantificados antes e após os processos térmicos (filagem da mozarela e pasteurização do leite). As reduções, em ciclos logarítmicos, causadas pela pasteurização e pela filagem, respectivamente, foram: 4,0 e 6,3 de* Mycobacterium *sp., 6,0 e 8,4 de* Listeria *sp., ≥6,8 e 4,5 de* Staphylococcus *sp. e ≥8,2 e 7,5 de* Salmonella *sp.*

Palavras-chave: mozzarella, filagem, pasteurização, bactérias patogênicas

INTRODUCTION

Buffalo mozzarella is a typical *pasta filata* cheese, which means that ripened curd is coarsely milled, melted in hot water, and stretched. The result is an elastic and kneadable cheese mass with a filiform structure (Walstra *et al*., 1999). Although milk pasteurization is mandatory for the production of cheeses that are aged for less than a 60 days, such as buffalo mozzarella (Brazil, 1952), this is not always observed. Therefore, an evaluation of the microbicide potential of stretching has become relevant from the perspective of Microbiological Risk Assessment.

Under this perspective, this study aimed to evaluate the efficiency of buffalo mozzarella stretching in the inactivation of *Mycobacterium fortuitum*, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Staphylococcus aureus* in experimentally contaminate milk, compared with the effect of milk pasteurization on the same agents.

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MATERIALS AND METHODS

Milk samples were obtained from a buffalo farm located in the Sorocaba region, in São Paulo, Brazil, and maintained at 4°C. Two aliquots were taken to determine fat content (Brazil, 2006) and the presence of *Mycobacterium* spp., *Listeria monocytogenes; Staphylococcus aureus,* and *Salmonella* spp.

Buffalo mozzarella was manufactured with *M. fortuitum* (NCTN 8573), *Listeria monocytogenes* (ATCC 19115, 4b), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhimurium* (ATCC 14028). The decision to use *Mycobacterium fortuitum* instead of *Mycobacterium bovis* in this study was due to observations that both species show similar thermal inactivation kinetics (Grant *et al*., 1996), and to the fact that *M. fortuitum* grows faster and is less pathogenic to human beings than *M. bovis*, posing less risk to the handler (WHO, 1984).

In order to be able to isolate this single variable (the effect of stretching), only one batch of cheese was made. Curd was milled, homogenized, and then divided into five portions to be stretched. Samples of curd and stretched mass were collected for the enumeration of the bacteria.

The same procedure was done with pasteurization: one batch was contaminated and five portions were submitted to holder pasteurization (65°C/30 minutes) in a water bath.

Working culture was prepared by growing *M. fortuitum* for seven days in a Lowënstein-Jensen medium at 37°C. Approximately 0.600 g of the culture was vigorously homogenized with 1mL of 0.85% saline and 0.05% Tween 80 solution. After that, 24mL of 0.85% saline solution were added to the flask (containing glass beads to prevent cell clumping), yielding a 25mL inoculum.

L. monocytogenes, *S. typhimurium*, *S. aureus* were inoculated individually into BHI (Brain Heart Infusion, *Difco*, USA) broth, incubated at 37°C for 24 hours and inoculated onto agar, as these microorganisms are grown as a culture on semisolid agar. *L. monocytogenes* was inoculated on Palcam, *S. typhimurium* on Brilliant Green Bile with lactose (2%), and *S. aureus* on a Baird-Parker agar base. All cultures were incubated at 37°C for 24 hours, and then two colonies of each agent were inoculated individually in BHI broth and incubated at 37°C for 24 hours.

Mozzarella was produced in the laboratory from 5 liters of milk, which was heated to 38°C and contaminated with 15.0mL of the *M. fortuitum* inoculum, and 7.5mL of each of the *L. monocytogenes, S. typhimurium,* and *S. aureus* inocula.

Contaminated milk received an addition of 5.0mL of starter culture (FDST-M7, *Chr. Hansen*, Denmark). After 30 minutes, an addition of 1.5mL of rennet (Estrella ,*Chr. Hansen*, Denmark) diluted 1:15, to induce coagulation. Forty-five minutes later, curd was cut, and whey was partially removed. Curd was ripened for about 4.5 hours. After that, curd was milled, melted in hot water (above 90°C), and stretched to form a smooth and shiny mass. Samples of curd and stretched mass were collected (25g each) for microbiological analysis.

To evaluate the efficiency of holder pasteurization, 100mL of the milk was contaminated with 4mL of the *M. fortuitum* inoculum and 0.2mL each of the *L.* monocytogenes, *S. typhimurium,* and *S. aureus* inocula. After homogenization, milk was distributed into 11 (16x160 mm) tubes (5mL in each tube). One of the tubes was used for microbiological counts in milk before pasteurization, and the other 10 were used for 5 pasteurization assays using 2 tubes for each repetition.

Pasteurization was performed in a water bath (Unitemp, *Fanen*, Brazil) at 65°C, with submersion of the entire tube surface, except for the plug. One of the tubes was removed when milk temperature reached 65°C (time 0) and the other, 30 minutes later. Once removed from the water bath, the tubes were immediately put in an ice bath and kept in the refrigerator until the moment of analysis.

To assess milk temperature, a thermometer was attached to a tube with 5mL of non-contaminated raw milk. To control the pasteurization process, three samples of non-contaminated raw milk (5mL each) were used in every repetition to evaluate the presence of the enzymes peroxidase

(Peroxidase leite- tiras, *Laborclin*, Brazil) (Brazil, 2006) and alkaline phosphatase (Fosfatase Alkalina leite- tiras, *Laborclin*, Brazil) at the end of the thermal treatment.

Samples were submitted to serial 10-fold dilutions in peptone water (0.1%), and inoculated into media specific for each agent.

Mycobacterium fortuitum: 0.1mL of each dilution was inoculated in duplicate on the surface of modified Lowenstein-Jensen medium (Centro Panamericano de Zoonosis, 1985). The modification by Donaghy *et al*. (2003) was adapted to our procedure. To suppress lactic bacteria, the following antibiotics were added to the medium before distribution and coagulation: amphotericin B (5µg/mL), nalidixic acid (60µg/mL), polymyxin B (50U/mL), and trimethoprim (5µg/mL). Inoculated plates were incubated at 37°C for 5 days (Koneman *et al.*, 2001). Dilutions that presented between 15 and 150 colonies were used for counts.

Listeria monocytogenes: The previously published methodology (Brazil, 2003) was adapted to obtain the MPN (Most Probable Number)/mL or g. UVM broth and Frazer broth were used as enrichment steps followed by streaking onto plates of Palcam. Every colony that had characteristics of the agent on the corresponding agar was assumed to be *Listeria*.

Staphylococcus aureus: The official methodology (Brazil, 2003) was used, and all colonies with characteristics of the agent in the medium were assumed to be *Staphylococcus.* The dilution chosen for counting was the one that presented between 25 and 250 colonies.

Salmonella typhimurium: Colonies were counted using the adapted MPN method (Brazil, 2003), as follows: pre-enrichment was carried out in peptone water (1%); selenite cystine broth was used as selective enrichment and, after that, enriched cultures were streaked on Brilliant Green Lactose Bile (BGB) plates. The presence of at least one characteristic colony of *Salmonella* in the BGB plates was considered to be positive for the bacteria.

All results were transformed into a CFU or MPN log per milliliter or gram.

RESULTS AND DISCUSSION

Listeria spp. (in 25mL), *Salmonella* spp. (in 25mL), *Mycobacterium* spp. (in 1mL) and *Staphylococcus* spp. (in 1mL) were absent in raw milk with 6.0% fat.

The enzymatic analysis of holder pasteurization showed that the process caused inactivation of the alkaline phosphatase in milk but did not have a significant effect on peroxidase, which is in accordance to the Brazilian official regulations.

Table 1, shows the results of the microbiological counts (in log of MPN or CFU per g or mL) for both holder pasteurization and stretching, and Tab. 2, summarizes the lethal effects of both processes on the inoculated agents. Considering that water activity (a_w) and salt content are not hurdles for these agents in this product (mozzarella is a fresh, soft cheese, and no salt was added), they did not influence the survival of the microorganisms, but the acidity in the curd could negatively affect their thermal resistance (Jay, 2000).

Pasteurization caused a 4-log cycle reduction in *Mycobacterium* sp., 6-log cycles in *Listeria* sp. and complete destruction of the inocula of *Staphylococcus* sp. (reduction $\geq$ 6.8 cycles) and *Salmonella* sp. (reduction $\geq$8.2 cycles).

It is interesting to observe that the heating phase of pasteurization (until milk reached 65ºC) produced an important lethal effect on *Listeria* sp., *Staphylococcus* sp. and especially *Salmonella* sp (Table 2), but not on *Mycobacterium* sp.

Table 1. *Listeria monocytogenes. Mycobacterium fortuitum*, *Staphylococcus aureus*, and *Salmonella typhimurium* counts in milk before holder pasteurization, in the curd before stretching, and average counts of the agents in milk (beginning and end of pasteurization) and in the mass after stretching.

	Pasteurization				Stretching	
	Repetition	Before	Beginning (T0)*	End (T30)**	Before	After
Listeria monocytogenes log MPN/mL or g¶	1	6.6	3.0	0.0§	8.4	0.0
	2		3.0	0.7		0.0
	3		2.6	1.4		0.15
	4		3.0	0.7		0.0
	5		3.0	0.0		0.0
	average		2.9	0.6		0.03
Mycobacterium fortuitum log CFU/mL or g†	1	7.7	5.4	3.3	7.7	0.0
	2		6.2	3.4		4.7
	3		7.9	3.9		2.3
	4		7.9	4.1		0.0
	5		6.4	4.0		0.0
	average		6.8	3.7		1.4
Staphylococcus aureus log CFU/mL or g	1	6.8	1.0	0.0	6.7	1.7
	2		3.4	0.0		3.0
	3		3,4	0.0		0.0
	4		3.4	0.0		3.3
	5		4.6	0.0		2.9
	average		3.2	0.0		2.2
Salmonella typhimurium log MPN/mL or g	1	8.2	0.7	0.0	7.6	0.0
	2		2.6	0.0		0.7
	3		1.2	0.0		0.0
	4		1.4	0.0		0.0
	5		2.0	0.0		0.0
	average		1.6	0.0		0.14

* when milk reached 65°C. ** at the end of pasteurization.
¶ most probable number per milliliter or gram
† colony forming unit per milliliter or gram
§ indicates that the contamination was lower than the limit of detection for the method used, i.e.: <0.3 MPN/mL or g or <0.5 CFU/mL or g.

Table 2. Reduction (in log cycles) of the population of *Listeria monocytogenes*, *Mycobacterium fortuitum*, *Staphylococcus aureus*, and *Salmonella typhimurium* for holder pasteurization (heating phase and complete process) and stretching

	Pasteurization		Stretching
	Heating phase (up to 65°C)	Complete process (heating phase + 30 min/65°C)	
Listeria monocytogenes (log MPN/mL or g) ¶	3.7	6	8.4
Mycobacterium fortuitum (log CFU/mL or g) †	0.9	4	6.3
Staphylococcus aureus (log CFU/mL or g)	3.6	$\geq$ 6.8	4.5
Salmonella typhimurium (log MPN/mL or g)	6.6	$\geq$ 8.2	7.5

¶ most probable number per milliliter or gram
† colony forming unit per milliliter or gram

Stretching was more efficient than pasteurization in the inactivation of *Mycobacterium* sp. and *Listeria* sp., but curiously, the opposite occurred with *Salmonella* and especially with *Staphylococcus* sp. Stretching inactivated 6.3 logarithmic cycles of *Mycobacterium* sp., 8.37 cycles of *Listeria* sp., 7.46 cycles of *Salmonella* sp., and only 4.5 cycles of *Staphylococcus* sp. The greater efficiency of stretching could be explained by the fact that microorganisms were already injured by curd acidity (Jay, 2000), but this explanation does not elucidate the behavior of *Salmonella* sp. and *Staphylococcus* sp. under the same conditions. It is possible that these microorganisms are less sensitive to acid injury than the others.

The maximum natural contamination of milk by *M. bovis* is 10^4 CFU/mL (Ball, 1943), and it ranges from 10^3 to 10^6 CFU/mL for *L. monocytogenes* (Bemrah *et al.*, 1998). In these conditions, milk submitted to holder pasteurization would end up with 1 CFU/mL of *M. bovis* and from 10^{-3} to 1 CFU/mL of *L. monocytogenes* (depending on the initial load) after the thermal process. Curd mass with this level of contamination would present, after stretching, $<10^{-2}$ CFU of *M. bovis* per gram and, in the case of *L. monocytogenes*, $<10^{-5}$ to $<10^{-2}$ CFU/g.

It is worth pointing out that national regulations do not define acceptable counts for *M. bovis*, but require absence of *Listeria monocytogenes* and *Salmonella* spp. in 25 grams of cheeses like mozzarella, which mean $<4x10^{-2}$ CFU per gram. The same regulations allow up to 10^3 CFU of *Staphylococcus aureus* per gram of product (Brazil, 2001).

Regardless of our results, *M. bovis* and *M. fortuitum* were completely inactivated by holder pasteurization in the studies published by Kells and Lear (1960), Harrington and Karlson (1965), and Grant *et al.* (1996). Data on the reduction of *Mycobacterium avium* subsp. *paratuberculosis* vary from 2 to 5 log cycles, when submitted to pasteurization at 63°C for 30 minutes (Paolicchi, 2008).

Holder pasteurization of cow and goat whole-fat milk contaminated with *M. fortuitum* (NCTN 8573) caused an average reduction of 3.9 log cycles and 4.4 log cycles of CFU/mL, respectively (Nishimoto, 2006; Starikoff, 2006). Both authors observed that the heating phase (until milk reaches 65°C) caused an average reduction of 2.0 log cycles and 0.3 log cycles of the agent in cow and goat milk, respectively. These data are similar to those of this study, also showing that the substrate has an important effect on thermal destruction characteristics of this agent.

Reductions of *Listeria monocytogenes* and *Staphylococcus aureus* caused by pasteurization that were lower than those observed in the present study were described in literature. Reductions of 4 to 5 logarithmic cycles of *Listeria* sp. (MacDonald and Sutherland, 1993) and of 2 cycles of *Staphylococcus* at 63°C for 20 minutes (Ambrosili, 1991) were reported. However, results similar to those obtained in this study were observed with *Salmonella javiana*, with reductions of more than 9 logarithmic cycles (Eckner *et al.*, 1990).

Stretching has already been evaluated as a thermal treatment to improve the sanitary quality of cheese (Caserio *et al.*, 1977; Eckner *et al.*, 1990; Villani *et al.*, 1996; Kim *et al.*, 1998; Silva *et al.*, 1999; Spano *et al.*, 2003) but there are few reports, and none of them studied the behavior of *Mycobacterium* after this treatment.

Eckner *et al.* (1990) showed full inactivation of *S. javiana* by stretching, starting from an inoculum of 10^5 CFU/mL in milk, an efficiency that was similar to the one obtained for *Salmonella* in this study.

However, literature presents different data on the behavior of *Salmonella typhimurium* and *Staphylococcus aureus*, with reductions of about 2 and 5 logarithmic cycles, respectively (Caserio *et al.*, 1977), while *Listeria monocytogenes* showed about 2-log cycle reduction (Villani *et al.*, 1996). A reduction of about 3 logarithmic cycles of coagulase-positive *Staphylococcus* was reported by Silva (1997).

In this study, *Mycobacterium* sp. showed more resistance to pasteurization than *Listeria* sp., *Salmonella* sp. or *Staphylococcus* sp., an expected finding, once *Mycobacterium* is considered to be the most resistant nonspore-forming bacteria that may contaminate milk (Behmer, 1991). The surprising fact was

Staphylococcus aureus showing more resistance to stretching than the other microorganisms analyzed.

A first analysis of the results obtained in this study might suggest that mozzarella production from raw milk is safe. However, when the results of stretching are analyzed in relation to *Mycobacterium* sp. and *Staphylococcus* sp. separately, we observed that the efficiency of the process varied.

This variation becomes more relevant when we take into consideration that in real continuous production, other variables may influence the efficiency of the process. Microbiological characteristics of the milk, curd ripening point, water temperature, water: mass ratio during stretching, as well as the contact period between mass and water, examples of variables that may interfere with the final result. Caserio *et al.* (1977) and Villani *et al.* (1996) also emphasized that stretching presents an irregular time/temperature profile that might compromise its efficiency in microbial load reduction.

CONCLUSION

Under the conditions of this study, mozzarella stretching was more efficient than milk pasteurization in reducing the inocula of *Listeria monocytogenes*, *Mycobacterium fortuitum*, and *Salmonella typhimurium*, but it was less efficient in the elimination of *Staphylococcus aureus*. Data suggest that a mozzarella production flowchart based on stretching as the only phase for elimination of pathogenic microorganisms might not ensure the necessary safety of the product.

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REFERENCES

AMBROSILI, R. Effetto della pastorizzazione in caldaia su batteri di interesse igienico-sanitario in alcuni caseifici artigianali. *Annali di microb. ed enzimol.*, v.41, p.237-242, 1991.

BALL, C.O. Short-time pasteurization of milk. *Ind. Eng. Chem.*, v.35, p.71-84, 1943.

BEHMER, M.L.A. *Tecnologia do leite*. Brasil: NOBEL, 1991. 320p.

BEMRAH, N.; SANAA, M.; CASSIN, M.H.; *et al.* Quantitative risk assessment of human listeriosis from consumption of cheese made from raw milk. *Prev. Vet. Med.*, v.37, p.129-145, 1998.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. *Aprova o Regulamento da Inspeção Industrial e Sanitária dos Produtos de Origem Animal*. Diário Oficial da União, Brasília, 07 de julho de 1952. Seção 1.

BRASIL. Agência Nacional de Vigilância Sanitária – ANVISA. Resolução nº 12 de 02 de janeiro de 2001. *Aprova o Regulamento Técnico sobre padrões microbiológicos para alimentos*. Diário Oficial da União, Brasília, 10 de janeiro, 2001. Seção 1.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa nº62, de 26 agosto de 2003. *Oficializa os Métodos Analíticos Oficiais para Análises Microbiológicas para Controle de Produtos de Origem Animal e Água*. Diário Oficial da União, Brasília, 26 de agosto de 2003. Seção 1.

BRASIL. Ministério da Agricultura Pecuária e Abastecimento. Instrução Normativa nº68, de 12 de dezembro de 2006. *Oficializa os Métodos Analíticos Oficiais Físico-Químicos para o controle de leite e produtos lácteos*. Diário Oficial da União, Brasília, 14 de dezembro de 2006, Seção 1.

CASERIO, G.; SENESI, E.; FORLANI, M.; EMALDI, G. Condizioni igieniche delle Mozzarelle in rapporto alla tecnologia di produzione. *L'Industria del latte*, v.2, p.19-39, 1977.

CENTRO PANAMERICANO DE ZOONOSIS. Manual de normas y procedimentos técnicos para la bacteriología de la tuberculosis. Buenos Aires: OPA/OMS, 1985. 25p.

DONAGHY, J.A.; TOTTON, N.L; ROWE, M.T. Evaluation of culture media for the recovery of Mycobacterium avium subsp. paratuberculosis from Cheddar cheese. *Lett. Appl. Microbiol*., v.37, p.285-291, 2003.

ECKNER, K.F.; ROBERTS, R.F.; STRANTZ, A.A.; ZOTTOLA, E.A. Characterization and behavior of *Salmonella javiana* during Manufacture of mozzarella type cheese. *J. Food .Prot*., v.53, p.461-464,1990.

GRANT, I.R.; BALL, H.J.; ROWE, M.T. Thermal inactivation of several *Mycobacterium* spp. in milk by pasteurization. *Lett. Appl. Microbiol*., v.22, p.253-256, 1996.

HARRINGTON, R.; KARLSON, A.G. A destruction of various kinds of mycobacteria in milk by pasteurization. *Appl. Microbiol*., v.13, p.494-495, 1965.

JAY, J.M. *Modern Food Microbiology*. 2.Ed. Nova York: CHAPMAN & HALL, 1996. 661p.

KELLS, H.R.; LEAR, S.A. Thermal death time curve of *Mycobacterium tuberculosis* var. Bovis in artificially infected milk. *Appl. Microbiol.*, v.8, p.234-236, 1960.

KIM, J.; SCHMIDT, K.A.; PHEBUS, R.K.; JEON, I.J. Time and temperature of stretching as critical control points for *Listeria monocytogenes* during production of mozzarella cheese. *J. Food Prot*., v.61, p.116-118, 1998.

KONEMAN, E.W.; ALLEN, S.D.; JANDA, W.M. *et al*. Micobactérias. In: KONEMAN, E.W.; ALLEN, S.D.; JANDA, W.M. *et al*. *Diagnóstico microbiológico*. 5ed. Rio de Janeiro: MEDSI, 2001. p.903-946.

MACDONALD, F.; SUTHERLAND, A.D. Effect of heat treatment on *Listeria monocytogenes* and Gram negative bacteria sheep, cow and goat milks. *J. Appl. Microbiol*., v.4, p.336-343, 1993.

NISHIMOTO, E.J. *Efeito da gordura do leite de vaca sobre o valor D65ºC do Mycobacterium fortuitum (NCTN 8573).* 2006. 81f. Dissertação (Mestrado em Medicina Veterinária) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

PAOLICCHI, F.A. Paratuberculosis: implicancia zoonótica com la enfermedad de Crohn em humanos. In: CACCHIONE, R.A.; DURLACH, R.; MARTINO, P. *Temas de Zoonosis IV*. Buenos Aires: ASSOCICIÓN ARGENTINA DE ZOONOSIS, 2008. p.273-284.

SILVA, E.O.T.R.. *Fabricação artesanal de mozzarella elaborada com leite cru de búfala. Estudo da contaminação microbiológica associada à manipulação em produção manual ou parcialmente mecanizada*. 1997. 47f. Dissertação (Mestrado em Medicina Veterinária) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

SILVA, E.O.T.R.; PANETTA, J.C.; ISHIZUKA, M.M. Efeito microbiocida da fase de filagem durante a fabricação de "mozzarella" elaborada com leite cru de búfala. *Rev. Hig. Aliment.*, v.13, p.28-34, 1999.

SPANO, G.; GOFFREDO, E.; BENEDUCE, L. *et al*. Fate of *Escherichia coli* O157:H7 during the manufacture of mozzarella cheese. *Lett. Appl. Microbiol*., v.36, p.73-76, 2003.

STARIKOFF, K.R. *Efeito da gordura do leite de cabra sobre o valor D65°C do Mycobacterium fortuitum (NCTN 8573).* 2006. 83f. Dissertação (Mestrado em Medicina Veterinária) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo.

VILLANI, F.; PEPE, O.; MAURIELLO, G.; MOSCHETTI, G. *et al*. Behavior of *Listeria monocytogenes* during the traditional manufacturing of water buffalo Mozzarella cheese. *Lett. Appl. Microbiol*., v.22, p.357-360, 1996.

WALSTRA, P.; GEURTS, T.J.; NOOMEN, A.; *et al*. *Dairy technology*: principles on milk properties and processes. New York: Marcel Dekker Inc, 1999. 727p.

WHO - World Health Organization. Guidelines on disinfection in animal husbandry for prevention and control of zoonotic diseases. Geneva: WHO, 1984. p.49.