International Food Research Journal 20(5): 2911-2915 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Microbiological quality of *Minas Frescal* cheese treated with ozonated water

[1]Cavalcante, D. A., [2]Leite Júnior, B. R. C., [2*]Tribst, A. A. L. and [2]Cristianini, M.

[1]Aeronautic Command, Aeronautic Farm of Pirassununga, Pirassununga, SP Brazil
[2]Food Technology Department, School of Food Engineering, University of Campinas, UNICAMP, Caixa Postal 6121, 13083-862, Campinas, SP Brazil

Article history

Received: 19 February 2013
Received in revised form: 18 March 2013
Accepted: 19 March 2013

Keywords

Minas Frescal cheese
Ozone
Shelf-life

Abstract

This work evaluated the microbiological and physicochemical quality of *Minas Frescal* cheese sanitized with ozonated water at concentration of 2 $mg.L^{-1}$ for 1 or 2 minutes. No counts of *Enterobacteriaceae*, *Staphylococcus* sp. and total and thermotolerants coliforms were found in the samples. Additionally, significant microbial reductions were observed for ozonated samples and the lowest counts were observed after 2 minutes of sanitation. The microbial reductions obtained at this process condition was 1.64, 1.91 and 2.14 log cycles for total aerobic mesophiles, lactic acid bacteria and yeasts and molds, respectively. Complementary, significant lower counts ($p < 0.05$) were observed for ozonated samples during their shelf-life. No significant changes ($p > 0.05$) were observed in physicochemical parameters of cheese after ozone sanitation. Therefore, the results highlighted the ozonation as a promising process to improve the cheese quality and stability.

Introduction

The *Minas Frescal* cheese is a typical Brazilian cheese produced since XVIII century (EPAMIG, 1987; Campos, 2001). It is a non-regular cheese with no pre-defined properties of consistency, texture, flavor, yield and centesimal composition. This is a consequence of the lack of standardization in the product and productive process (ABIQ, 2011).

According to Brazilian food law (Brasil, 1997), the *Minas Frescal* cheese is a fresh cheese obtained by enzymatic coagulation of cow milk using calf rennet or coagulants. This coagulation can be completed by other specific lactic acid bacteria. It is classified as a semi fat with very high moisture cheese (>55%) (Brasil, 2004) destined to the fresh consumption. The cheese is characterized by mild and soft consistency and can have mechanical holders. Its flavor is smooth or slight acid and can have a fine crust. It is marketed in cylindrical format with 0.3 to 5 kg.

The microbiological parameters of this cheese are established by Brazilian law (Brasil, 1996; Brasil, 2001), being acceptable to have $1x10^3$ $CFU.g^{-1}$ of total coliforms, $5x10^2$ $CFU.g^{-1}$ of thermotolerant coliforms and Staphylococcus positive coagulase and $5x10^3$ $CFU.g^{-1}$ yeasts and molds. Additionally, the absence of *Salmolella* sp and *Listeria monocytogenes* in a cheese sample of 25 g is mandatory.

The *Minas Frescal* cheese is not a high stable cheese, since is a fresh (not cured) product, with very high moisture (>55%) content and low (1.4-1.6%) salt concentration. These characteristics easily allow the microbial growth in the cheese (Carvalho, 2003). Additionally, this cheese is commonly manufactured by small produces with inadequate manufacturing practices and excessive manipulation (Bulhões and Rossi Junior, 2002).

Therefore, new technologies and process need to be considered for *Minas Frescal* cheese production, aiming to improve its quality and to increase its shelf-life.

The ozone has been studied in food application as a sanitizer (Kim *et al.*, 1999). It can be applied as gas or liquid (ozonated water) and is a powerful sanitizer with high reactivity and spontaneous decomposition in non-toxic products. The ozone is a high oxidative compound and has a broad antimicrobial spectrum, being able to inactivate vegetative and sporulated cells, yeast, molds and viruses, additionally is able to kill pests of grain storage (insets) and to degrade mycotoxins (Tiwari *et al*., 2010). Ozone destroys microorganisms by the progressive oxidation of vital cellular components.

Therefore, this work evaluated the effects of sanitation using ozonated water in the shelf life of *Minas Frescal* cheese, considering microbiological

*Corresponding author.
Email: *alline.lima.tribst@gmail.com*
Tel: +55 19 3521 4021

quality of the cheese.

Materials and Methods

Cheese production

The assays were performed using samples of Minas Frescal cheese of 250 g. A volume of 800 liters of whole pasteurized cow milk was obtained from a Brazilian Farm (Brazilian Aeronautic Farm, Pirassununga, Brazil). The cheese was manufactured according to Furtado (1994): milk was heated to 36ºC ± 1°C and then added by 500 g of potassium sorbate, 500 mL of calcium chloride solution at 40%, 6 kg of sodium chloride and 500 mL of recombinant coagulant from *Aspergillus niger* var. awamori (activity of 1:10.000 or 98 IMCU/mL) solvated in 500 mL of water. The mixture was stirred for 2-3 minutes and then the coagulation occurred during 40 minutes. After coagulation, the cheese mass was cut and slowly stirred during 25 minutes for separation of the protein mass and 2/3 of the milk whey. After, the cheeses were shaped in cylindrical forms and then stored in cold chamber at 4ºC± 1ºC for 24 h. After this period, the cheese was turned upside down and then stored for more 24 h. Finally, the cheeses were removed from the forms, submitted to the ozonation process and then stored under vacuum at 4ºC ± 1ºC. The cheese was manufactured in duplicate for each treatment.

Ozonated water

The ozonated water was produced using an ozone generator (model PNZ 714®, Panozon, Brazil) linked to an oxygen concentrator (90-95% of purity). The ozonated water was obtained by bubbling the ozone gas in the water, by coupling the ozone generator in a hose with a porous plate made of ore sand linked at the end (aerate volume: 0.6 $L.min^{-1}$; size: 12 x 20 mm). This plate was placed at the bottom of a stainless steel tank containing 50 L of water. The water was ozonated at the concentration of 2 $mg.L^{-1}$ (maximum concentration able to be reached in the system). Additionally, the system had a gasification vat to separate the ozone produced as gas, which posteriorly was degraded in oxygen.

The ozone concentration for each process was controlled by colorimetric test, which is based on the ozone reaction with N-dietil-P-Fenilenodiamin. The reaction produces a pink color that has intensity proportional to the ozone concentration. The final color was measured using a colorimetric test (Brand HACH, model OZ-2) able to evaluate the ozone concentration at the range of 0-2.3 $mg.L^{-1}$.

Sanitation of Minas Frescal cheese using ozonated water

The cheeses were immersed in ozonated water (2 $mg.L^{-1}$) for one or two minutes before being packaged. The ozone concentration was chosen considering the maximum concentration able to be solvated in water and the contact times were chosen considering the stability of the ozone after its generation (about 2 minutes). After sanitation, the cheeses were removed from the tank using a sterile stainless sieve. Then, to remove the excess of water, the samples rested for 1 minute in a surface previously sanitized, avoiding posterior contamination. Each cheese unit was packaged in low density polyethylene (LDPE) under vacuum (Mastervac, Brazil, model 200) and stored at 4ºC ± 1ºC. A control sample was prepared by immersion of the cheeses in distilled water for 2 minutes.

Microbiological Analysis

The samples were evaluated by counts of total mesophilic aerobic (TMA), *Enterobactereaceae*, total and thermotolerant coliforms, lactic acid bacteria (LAB), yeasts and molds (YM) and *Staphylococcus* sp. The counts were performed at days 0, 1, 5, 10, 15, 20, 25 and 30 of shelf-life. The table 1 shows the growth media and incubation time and temperature used for each evaluated microorganism (except for coliforms). The microbiological experiments were performed using the grounded cheese, aiming to evaluate the cheese according to law requirements.

For the evaluation of total and thermotolerant coliforms, it was used the most probable number (MPN) technique (APHA, 1992). The presumptive test was carried out using lauryl sulphate broth and incubation at 36ºC/48 h. For positive growth, the confirmation test were performed in Brilliant green lactose bile incubated at 35ºC/48 h (total coliforms) and in *E. coli* broth incubated at 45.5ºC/24 h (thermotolerant coliforms)

Physicochemical analysis

The pH, acidity, fat and protein content (AOAC, 1995) of the cheeses were evaluated at days 0, 5, 10, 15 and 30 after manufacturing. These analyses were performed to evaluate the cheese accordance to the law.

Experimental design

For microbiological evaluation, the experiments were fully randomized and factorial 3 x 8, with 3 levels of time exposure to ozone (0, 1 and 2 minutes) and 8 days of cheese evaluation (0, 1, 5, 10, 15, 20, 25 and

30 days after cheese manufacturing). Each treatment was evaluated in triplicate, totalizing 72 experimental cheese units for microbiological assays.

For physicochemical evaluation, the experiments were fully randomized and factorial 3 x 5, using the same times of ozone exposure described for microbiological test and 5 days of cheese evaluation (0, 5, 10, 15 and 30 days after cheese manufacturing). The physicochemical assays were also carried out in triplicate, totalizing 45 experimental cheese units for physicochemical assays.

Data were statistically evaluated by ANOVA using the software SAS® to determine significant differences at 95% of confidence level ($p < 0.05$) (SAS Institute, 2004).

Results and Discussion

The results of the cheese microbiological evaluation showed absence of *Enterobacteriaceae*, total and thermotolerant coliforms and *Staphylococcus* sp. during the cheese shelf-life. This indicates the adequate sanitary condition of the cheese manufacturing whereas this kind of cheese (with high humidity and low salt content) is easily contaminated by microorganisms if the good manufacturing practices are not correctly adopted.

Other previous work reported counts of coliforms above to the limit established by the law ($1x10^3$ CFU.g^{-1} of total coliforms and $5x10^2$ CFU.g^{-1} of thermotolerant coliforms) for *Minas Frescal* cheese.

Oliveira *et al.* (1998) evaluated 36 *Minas Frescal* cheese and observed that 47% of samples had high counts of total coliforms and more than 9% had high counts of thermotolerant coliforms. Similarly, Figueiredo *et al.* (2004) found that 100% and 40% of 20 samples of *Minas Frescal* cheese had higher counts of total and thermotolerant coliforms, respectively. Pereira *et al.* (1999) evaluated 44 samples of the same cheese and found that 90% of them had higher counts of thermotolerant coliforms and the results obtained by Almeida Filho and Nader Filho (2002) showed that 37.5% of 80 samples had higher counts of thermotolerant coliforms.

Moreover, Pedro (2003) evaluated the counts of *Staphylococcus* positive coagulase in 60 samples of *Minas Frescal* cheese and observed that more than 27% of these cheese have counts above the limit ($5x10^2$ CFU.g^{-1}).

The other evaluated microorganisms were viable immediately after the cheese manufacture and their counts level were monitored during the product shelf-life. These results are shown in the Figure 1.

The control sample showed initial counts of 2.27 x 10^2, 8.10 x 10^1 and 1.37 x 10^2 CFU.g^{-1} of TMA, LAB and YM, confirming the adequate sanitary quality of the cheese, in accordance with the microbial limits determined by Brazilian law. The results of samples sanitized with ozonated water for 1 or 2 minutes showed significant reductions ($p > 0.05$) of the initial load for all studied microorganisms. Additionally, lower counts were obtained after 2 minutes of contact time with ozone.

After sanitation with ozone during 2 minutes, the counts were 4.95 CFU.g^{-1} (TMA) and <1 CFU.g^{-1} (LAB and YM), resulting in 1.64, >1.91 and >2.14 decimal reductions of total mesophilic aerobic, lactic

Table 1. Growth media and incubation time and temperature used for each evaluated microorganism

Microorganism	Growth media	Time and temperature of incubation
TMA counts	PCA	35°C /48 hours
LAB counts	MRS	30°C /72 hours
YM counts	PDA	22°C /48 hours
Enterobacteriaceae	VRBA	35°C /48 hours
Staphylococcus sp	BP	35°C /48 hours

PCA (plate count agar - DIFCO®), MRS (Lactobacillus MRS agar - HIMEDIA®) PDA (potato dextrose agar - DIFCO®), VRBA (violet red bile dextrose agar - DIFCO®), BP (Baird Parker agar - DIFCO®). Analyses were performed according to American Public Health Association (APHA, 1992).

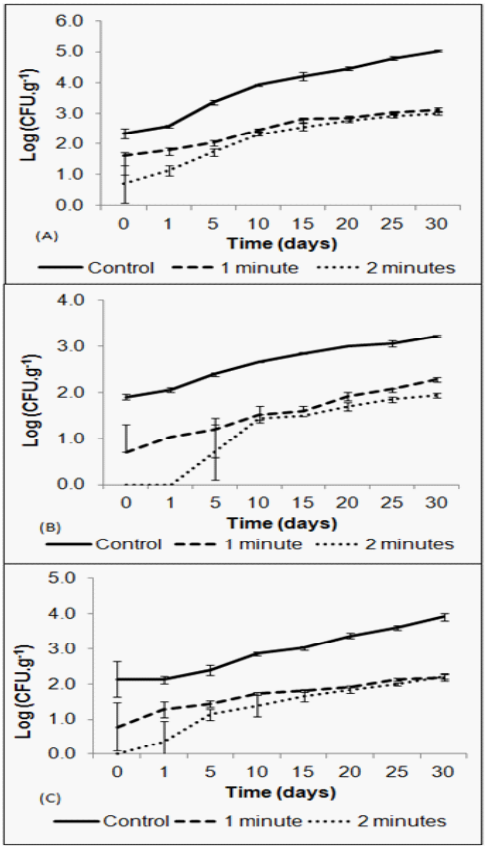

Figure 1. Average counts (Log10 CFU.g^{-1}) of total mesophilic aerobic (A), lactic acid bacteria (B) and yeasts and molds (C) in *Minas Frescal* cheese after sanitation with 2.0 mg L^{-1} at different contact time

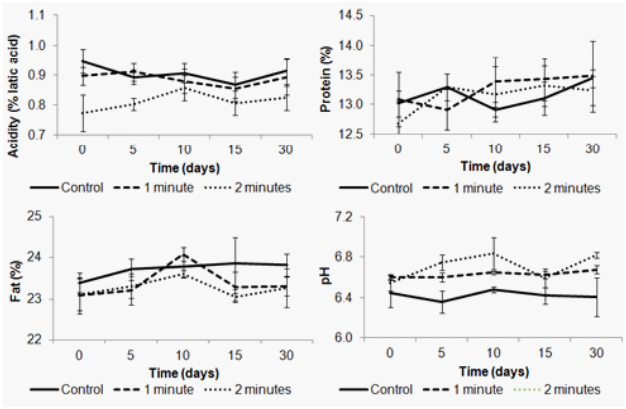

Figure 2. Effects of ozone sanitation in the physicochemical parameters of *Minas Frescal* cheese after sanitation with 2.0 mg L^{-1} at different contact time

acid bacteria and yeasts and molds, respectively. These reductions were statistically significant ($p < 0.05$), therefore, the ozone sanitation improves the cheese microbial quality, increasing the product shelf-life.

The evaluation of microbial growth on cheese during its expected shelf life (30 days) demonstrated that samples ozonated for 1 minute took 10 (TMA), 20 (LAB) and 30 days (YM) to reaches the count obtained for the control sample at day 0. For samples sanitized for 2 minutes, these times increased to 15 (TMA) and 30 days (LAB and YM).

Comparing the counts obtained at the end of shelf life for control sample (that simulate a commercial process) and the ozonated samples, it was observed that the ones of the sanitized samples were, at least, 1 log cycle lower than the control, being statistically significant ($p < 0.05$). However, no differences were found between the counts of samples sanitized for 1 or 2 minutes of contact time, indicating that 1 minute of ozonation was enough to improve the cheese quality during its storage. Moreover, the evaluation of the growth curves for TMA, LAB and YM showed similar slope for control and sanitized samples, possibly demonstrating that ozonation just reduces the initial count of microorganisms but do not reduces its growth velocity.

The growth of mesophilic aerobic microorganisms in the control sample was 2.68 log cycles, being lower that the results previously reported by Sangaletti (2007) in 6 brands of Minas Frescal cheese stored at 4ºC, produced with no ozone sanitation. The small increment in TMA observed in the present work probably guarantees that cheese kept its physicochemical characteristics during the storage time. Sangaletti (2007) reported physicochemical changes and shelf life reduction of Minas Frescal cheese caused by 9 log cycles growth of spoilage microorganisms during the cheese storage. The high TMA counts observed by Sangaletti (2007) can be associated with undesirable transport and storage conditions (high temperature).

The results of the physicochemical parameters are shown in the Figure 2. The results demonstrated that ozone sanitation did not change the cheese pH immediately after the product manufacture. During the storage period, small but not significant pH variation was observed (differences were lower than the expected accuracy (± 0.1) of the pHmeter). However, previous studies showed pH reductions around 0.8 during the Minas Frescal cheeses storage (Buriti *et al.*, 2005; Sangaletti, 2007). The pH reduction is commonly associated with conversion of lactose to lactic acid by undesirable microorganisms. Thus, the pH results obtained in the present work, including the control ones, are in agreement with the results of microbiological evaluation.

A smaller acidity was observed for the samples treated with ozone during 2 minutes, which may is attributed to protein oxidation with liberation of amino groups. However, this small difference of acidity (~0.1% of lactic acid) probably did not alter the sensory profile of the cheese (Ribeiro, 2009). Additionally, no significant acidity increase ($p < 0.05$) were observed in the samples during its shelf life, which corroborates with low microbial growth during the storage period that was not enough to change the characteristics of the cheese.

The results obtained for cheese fat and protein content showed no changes after ozonation or during their shelf-life. The Minas Frescal cheese was characterized by lipid content of 23.1 - 24.1% and protein content of 12.7-13.5%, being in accordance with Brazilian law. These results were expected, since the process would not alter the cheese centesimal composition.

Conclusion

The results showed that the *Minas Frescal* cheese had an adequate microbial quality even with no sanitation with ozone. However, the ozonation process was effective for microbial reduction in the cheese, improving the cheese shelf-life in, at least, 10 and 15 days after treatment with ozonated water (2 $mg.L^{-1}$) for 1 and 2 minutes, respectively.

Acknowledgments

The authors would like to thank the Aeronautic Farm for using its dairy industry to process and evaluate the *Minas Frescal* cheese.

References

Internet: ABIQ - Associação Brasileira das Indústrias de Queijo 2011. Information on types of cheese in Brazil. Downloaded from *http: www.abiq.com.br* on 15/10/2011.

Almeida Filho, E. S. and Nader Filho, A. 2000. Ocorrência de Staphylococcus aureus em queijo tipo frescal. Revista de Saúde Pública 34(6): 578-580.

Association of Official Analytical Chemists - AOAC. 1995. Official Methods of Analysis. 16 ed. AOAC, Washington, DC.

APHA. 1992. Standard methods for the examination of water and wastewater. 18 ed. American Public Health Association, Washington, DC.

Brasil. 1996. Ministério da Agricultura, Pecuária e Abastecimento. Regulamento técnico de identidade e qualidade de queijos. Portaria n° 146. Diário Oficial da União n° 172, Brasília, 11 mar.

Brasil. 1997. Ministério da Agricultura, Pecuária e Abastecimento. Regulamento técnico para fixação de identidade e qualidade de queijo *Minas Frescal.* Portaria n°352. Diário Oficial da União, Brasília, 08 set.1997. Seção I, p.13-68.

Brasil. 2001. Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução – RDC n° 12 de 2 de janeiro de 2001. Regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial da União n° 172, Brasília, 02 jan.

Brasil. 2004. Ministério da Agricultura, Pecuária e Abastecimento. Instrução Normativa n°4 de 1 de março de 2004. Diário Oficial da União, Brasília, 5 mar. 2004. Seção I, p.5.

Bulhões, C.C.C. and Rossi Junior, O.D. 2002. Ocorrência de bactérias do gênero *Aeromonas* em queijo-de-minas frescal artesanal. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 54(3): 320-24.

Buriti, F.C.A., Rocha, J.S. and Saad, S.M.I. 2005. Incorporation of *Lactobacillus acidochilus* in Minas fresh cheese and implications for textural and sensorial properties during storage. International Dairy Journal 15(12): 1279-1288.

Campos, D.C. 2001. Queijo: breve histórico e principais características. Núcleo de Apoio à Pesquisa em Microbiologia Agrícola. Piracicaba: ESALQ; NAPMA 11: 59.

Carvalho, J.D.G. 2003. Avaliação da Qualidade de Queijos Tipo Minas Frescal Elaborados por Diferentes Processos Tecnológicos e Comercializados em Campinas-SP. Campinas, São Paulo: State University of Campinas, MSc thesis.

Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG). 1987. Os queijos na Fazenda. Rio de Janeiro: Globo, 219p. (Coleção do Agricultor-Laticínios).

Figueiredo, H.M., Pinto, C.P., Mascarenhas, M. O. and Teshima, E. 2004. QUEIJO MINAS FRESCAL: AVALIAÇÃO DA QUALIDADE MICROBIOLÓGICA. Revista do Instituto de Laticínios Cândido Tostes 399(59): 198-200.

Furtado, M. M. 1994. Tecnologia de queijos: manual técnico para produção industrial de queijos. São Paulo: Dipemar, 118p

Kim, J.G., Yousef, A.E. and Dave, S. 1999. Application of ozone for enhancing the microbiological safety and quality of foods: a review. Journal of Food Protection, Des Moines 62(9): 1071-1087.

Oliveira, C. A. F., Moreno, J. F. G. and Mestieri, L. 1998. Características físico-químicas e microbiológicas de queijos Minas Frescal e mussarela, produzidos em algumas fábricas de laticínios do Estado de São Paulo. Higiene Alimentar 12: 31-35.

Pedro, S.C.M. 2003. Avaliação da qualidade higiênico sanitária dos locais de venda e de amostras de queijo minas meia cura, comercializado na cidade de São Paulo. São Paulo, São Paulo: University of São Paulo MSc thesis.

Pereira, M. L., Gastelois, M. C. A. and Bastos, E. M. A. F. 1999. Enumeração de coliformes fecais e presença de *Salmonella* sp. em queijo-de-minas. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 51(5): 427-431.

Ribeiro, E. P., Simões, L. G. and Jurkiewicz, C. H. 2009. Desenvolvimento de queijo minas frescal adicionado de *Lactobacillus acidophilus* produzido a partir de retentados de ultrafiltração. Ciência e Tecnologia Alimentos 29(1): 19-23.

Sangaletti, N. 2007. Estudo da vida útil do queijo Minas Frescal disponível no mercado. Piracicaba, São Paulo: University of São Paulo, MSc thesis.

Sas Institute 9.1. 2004. User.sguide. Carey: SAS Institute Inc., 1040p.

Tiwari, B. K., Brennan, C. S., Curran, T., Gallagher, E., Cullen, P. J. and Donnell, C. P. O. 2010. Application of ozone in grain processing, Journal of Cereal Science 51(3): 248-255.