

## Research about *Listeria* Sp., *Salmonella* Sp. And Others Contamination Indicators to Milk's and Cheese's Samples Sale in the South of Minas Gerais State

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**Abstract:** A study was done in 112 milk samples and milk derivative samples, being 46 homemade cheese (QC) and industrialized cheese (QI) and 66 raw milk (LC) and pasteurized milk (LPC), in order to quantify mesophilic aerobics (AM), positive coagulase Staphylococci (SCP), MPN of totals coliforms (CT) and fecals (CF) and a research was made on *E. coli*, *Salmonella* sp., *Pseudomonas aeruginosa* and *Listeria* sp. For *Listeria*, the FDA official methodology was adopted including cold enrichment stage (45 days /4°C) and microaerophilic system. Count averages of AM for QI (4.88x10<sup>9</sup> CFU g<sup>-1</sup>) were significantly different (P <0.05) from QC. SCP counting showed 47.83% (QC) and 34.78% (QI) which exceeded default value. Averages (MPN g<sup>-1</sup>) for CT were 2.39x10<sup>5</sup> (QC) and 2.48x10<sup>5</sup> (QI); and for CF was 1.67x10<sup>5</sup> (QI). *E. coli* was detected in QC: 78.26% and in QI: 60.87%, *Salmonella* sp. was isolated in QI: 8.70% and *P. aeruginosa* was isolated in 100% of QC and QI. *Listeria* sp. was isolated before and after of cold enrichment and it was identified *L. innocua* 6a, 6b, 4ab and untypable. In the milk sampling the positivity for *E. coli* was 75.76% (LC), 39.39% (LPC) and 12.12% with isolation of *Salmonella* sp. in LPC. It was concluded by the results that both milk and cheese were disqualified for consumption for offering potential risks to public health.

**Key words:** Cold enrichment stage, *Listeria*, *Salmonella*, milk, cheese, microbiological quality.

### INTRODUCTION

The world's food supply chain have become increasingly complex, resulting in difficulty in obtaining a food standard under appropriate security conditions alimentary (Forsythe, 2002). Based on the standards of food safety, food considered safe for consumption shall contain, according to the HACCP / HACCP (Hazard Analysis Critical Control Point / Hazard Analysis and Critical Control Point), control in continuous production line. The traceability from the production of milk, pasteurization, test control and monitoring of microbial recontamination desirable since the processes involved during the stages of production to marketing, should be provided in order to guarantee the microbiological quality of food as the patterns of alimentary security (Silva *et al.*, 2003; 1998; 2001).

The health and microbiological standards for cheese type "Minas Frescal" are determined by the Technical Regulation on the Microbiological Standards for Foods of the Ministry of Health (RDC n° 12/01) recommends that the tolerable limits of Coliforms 45°C and absence of *Salmonella* and *Listeria monocytogenes* in cheese "Minas Frescal" The Technical Rules of Practice for Food Services of the Ministry of Health (RDC n°. 216/04) establishes standards for the regulation of handling of food, explaining the need for constant improvement of the shares of health surveillance in the area of food (Brasil, 2004; 2001). The milk, traditionally, is considered essential to the initial phase of growth as a complete food, balanced in nutrients and with high nutritional value for human and animal development.

The various steps involving the collection and production of milk makes it susceptible to contamination, since it can be considered optimal means of culture for the growth of microorganisms, including some potentially pathogenic, the predisposing susceptible to infectious and contagious diseases and food toxic infections (Catão and Ceballos, 2001; Guimaraes, 2002; Olival *et al.*, 2002; Ponsano *et al.*, 2001). Thus, the use of contrasting high and low temperature by the technique of pasteurization is to ensure greater microbiological safety of the milk food, since this procedure is based on the deleterious effects of heat on pathogenic microorganisms known (Brasil, 2002).

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However, we should not discard the possibility of mechanical failure in the pasteurization or possible recontaminations after processing, as raw material and product are susceptible to proliferation of microorganisms, especially those which support wide range of temperatures, including mesophilic, thermophilous and psychrotrophic. In the past, we can highlight the bacteria of the genus *Listeria* (Bemrah *et al.*, 1998; D'angelis *et al.*, 2001; Feniman *et al.*, 2003).

Thus, the processes of production and reception of the milk industry should follow the platform to the standards established by Normative Instruction n°. 51 Ministry of Agriculture, Livestock and Supply (IN n° 51/02) in its legislation establishing the standards and conduct of federal inspection, including targets for improvement, modernization and industrialization of the production of milk (Brasil, 2002).

Despite the numerous and significant advances in science and technology, food-borne diseases continue to be problem in the modern world. The Center for Disease Control U.S. considers microorganisms as *Listeria monocytogenes*, *Salmonella* sp and pathogenics strains of *Escherichia coli* (EC O157:H7) as emergent pathogens implicated in serious cases of diseases transmitted by food and serious concerns for public health (Figueiredo, 2000).

This study aimed to find *Listeria* sp, *Salmonella* sp and other indicators of contamination in samples of cow's milk and cheese. Thus, there was the analysis of the microbiological quality of samples of cheese like "Minas Frescal" craft manufacturing (QC) and industrial (QI) of raw milk (LC) and pasteurized type C (LPC) marketed in Alfenas-MG and region, thereby assessing the standard of hygiene and health products as existing laws.

## MATERIAL AND METHODS

Were analyzed in duplicate, 46 samples of cheese distributed in 23 samples of homemade cheese (QC) of artisanal and 23 samples industrial preparation cheese (QI) of four different brands, with the postage stamp of the SIF (Federal Inspection Service).

As for milk, 66 samples were analyzed, with 33 of raw cow's milk (LC) and 33 of pasteurized milk type C (LPC) of different brands A, B, C, X, Y and Z.

The collection of samples was done randomly in trade in Alfenas-MG and region. The analysis is carried out in the Laboratory of Public Health and Food Microbiology, University Federal of Alfenas (UNIFAL-MG), covering the period september 2005 to february 2007.

The microbiological analysis was performed on representative sampling, quantifying it aerobic mesophilic (AM), coagulase positive *Staphylococci* (SPC), total Coliform (CT) and fecal (CF) and through research of *Escherichia coli*, *Salmonella* sp (Silva *et al.*, 2001; Tortora *et al.*, 2000) and search for *Pseudomonas aeruginosa* (Silva *et al.*, 2005), as called RDC ANVISA Resolution n°. 12 of 2 January 2001 (Brasil, 2001) and IN n° 51/2002 (Brasil, 2002), which establish the microbiological standards for foods intended human consumption in retail and industrial platform, respectively.

For the detection of *Listeria* sp adopted is the official method of the Food and Drug Administration (Silva *et al.*, 2001; Tortora *et al.*, 2000), adding up step of cold enrichment (Farber and Peterkin, 1991) and microaerophilic system (Silva *et al.*, 2003; 1998). Confirmation of antigen serogroups and serovars (Seeliger and Hohne, 1979) from *Listeria* was performed in the IOC/Fiocruz/RJ.

Microbiological tests were performed on 46 samples of cheese for determination of total coliforms, fecal coliforms, *Escherichia coli*, *Listeria* sp, coagulase positive *Staphylococci* and *Salmonella* and 10 samples for aerobic mesophilic and *Pseudomonas* sp. The analysis of cow's milk consisted of 66 samples for determination of total coliforms, fecal coliforms, *Escherichia coli*, *Listeria* sp and *Salmonella* sp and *Pseudomonas* sp and determination of aerobic mesophilic, in twenty and thirty samples, respectively.

## RESULTS AND DISCUSSION

### *Analysis of the Sampled Cheeses:*

Count of AM has revealed that the QI difference ( $P < 0.05$ ) significant in relation to QC, to display higher average ( $4.88 \times 10^9$  UFC  $g^{-1}$ ) (Table 1). These results shows more contamination in samples of QI, despite carrying the same stamp of Federal Inspection Service (CFU). Agreeing with the results of this research, with values for aerobic mesophilic counts of  $1.87 \times 10^8$  CFU  $g^{-1}$  were reported by Aygun *et al.* (2005) to conduct studies on the microbiological quality of 50 samples of "Carra" cheese, traditional cheese in Turkey.

In the analysis of coagulase positive *Staphylococci* (SCP) 47.83% (QC) and 34.78% (QI) of the samples of cheese, were in disagreement with the RDC n° 12/01 (Figures 1 and 2). Loguercio and Aleixo (2001) were

coagulase positive *Staphylococcus* count of over  $10^3$  CFU  $g^{-1}$  in 29 of 30 samples of cheese produced Minas Frescal type craftsmen in Cuiabá-MT. Similarly, Little *et al* (2008) performed analysis of the quality of cheeses from raw milk and pasteurized in the United Kingdom. According to the authors, both types of cheeses showed unsatisfactory microbiological results. In the case of cheese from pasteurized milk, the counts for coagulase positive *Staphylococcus* were  $10^3$  CFU  $g^{-1}$  for cheeses from raw milk,  $10^4$  CFU  $g^{-1}$ .

Although not established by Brazilian legislation, in determining the MPN of TC 30/35 °C, high values were obtained for cheeses: QC: $> 1.1 \times 10^5$  MPN  $g^{-1}$  and QI: $> 1.1 \times 10^6$  MPN  $g^{-1}$ . The values of minimum, maximum and average CT of the MPN  $g^{-1}$  for the QC and QI are presented in Table 2.

The results presented in Table 2 showed similar microbiological quality ( $P>0.05$ ) between the sampling of QC ( $2.39 \times 10^5$  MPN  $g^{-1}$ ) and IQ ( $2.48 \times 10^5$  MPN  $g^{-1}$ ), even if the QI has been obtained, theoretically of pasteurized milk.

In the analysis of CF, the values of Table 2 showed statistical similarity ( $P>0.05$ ) between average sampling QC ( $8.19 \times 10^4$  MPN  $g^{-1}$ ) and QI ( $1.67 \times 10^5$  MPN  $g^{-1}$ ), indicating possible failures pasteurization of milk used in the manufacture of the QI or recontamination of the final product. According to RDC n° 12/01, in representative sample ( $n> 5$ ,  $c> 2$ ,  $m> 1.0 \times 10^3$ ;  $M> 5.0 \times 10^3$ ), up to two samples may contain between  $1.0 \times 10^3$  and  $5.0 \times 10^3$  MPN FC  $g^{-1}$  (Table 2). In this study, 16 samples of QI and 18 QC samples had counts between  $1.1 \times 10^4$  and  $1.1 \times 10^6$  MPN  $g^{-1}$ , showing itself in disagreement with the RDC 12/01.

Agreeing with the results presented in this research in relation to contamination by fecal coliforms, Pereira *et al.* (1999a) also found in samples of similar quality cheese "Minas Frescal" with or without registration record in the SIF. García Dangla *et al.*(1981) and Kivanç (1989) also found unsatisfactory microbiological quality in relation to contamination by fecal coliforms in homemade cheese produced in Costa Rica and typical cheeses in Turkey, respectively.

**Table 1:** Quantification of aerobic mesophilic in Minas cheese type Frescal home (QC) and industrial (QI) and study the statistical t-test means

	QC	QI
Minimum value	$5.05 \times 10^7$	$2.72 \times 10^9$
Maximum value	$4.44 \times 10^9$	$8.00 \times 10^9$
Mean*	$1.54 \times 10^{9A}$	$4.88 \times 10^{9B}$
t- test		2.63
Probability t		0.0302

\* Means with different letters (A, B) in rows differ significantly ( $P<0.05$ ) at 5% level by the test t.

**Table 2:** Most Probable Number of total coliform (TC) and fecal (FC) in Minas cheese type Frescal home (QC) and industrial (IQ) and statistical analysis by t test of means.

	CT (MPN/g)		CF (MPN/g)	
	QC	QI	QC	QI
Minimum value	$1.10 \times 10^3$	$1.10 \times 10^4$	0	73
Maximum value	$1.10 \times 10^6$	$1.10 \times 10^6$	$1.10 \times 10^6$	$1.10 \times 10^6$
Mean*	$2.39 \times 10^{5A}$	$2.48 \times 10^{5A}$	$8.19 \times 10^{4A}$	$1.67 \times 10^{5A}$
t- test		-0.0722		-0.93
Probability t		0.9428		0.3585
Standard RDC n° 12/01**	-	$n>5$ ; $c>2$ ; $m>1,0 \times 10^3$ ; $M>5,0 \times 10^3$		

\* Means with same letters in the lines do not differ ( $P>0.05$ ) at 5% level by the test t.

\*\* n: number of units to be picked randomly from the same batch and analyzed individually; c: maximum number of sample units with counts between the limits m M, m: separating the lot or batch of the acceptable product with acceptable intermediate quality, M: separating the acceptable lot from the unacceptable one. Values above M are unacceptable.

The presence of *Escherichia coli* was found in 78.26% of QC sampling and 60.87% of QI (Figures 1 and 2). Similar results were obtained by Feitosa *et al.* (2003) also reported the presence of *E. coli* in cheese produced in Rio Grande do Norte. In the United Kingdom, Little *et al.* (2008) were the presence of *E. coli* in cheeses made with raw milk ( $10^3$  CFU  $g^{-1}$ ) and cheese from pasteurized milk ( $10^5$  CFU  $g^{-1}$ ). In Turkey, "Carra" cheese, according Aygun *et al.* (2005), also revealed the presence of *E. coli* in the order of  $4.27 \times 10^3$  CFU  $g^{-1}$ .

In the QC sampling, there was absence of Salmonella sp, agreeing with the reported by Pereira *et al.* (1999a) when analyzed 20 samples of cheese like "Minas Frescal" in the city of Belo Horizonte-MG. Agreeing with the above authors, Tortora *et al.* (2000) and considered that the lack of detection of the microorganism can be justified by the health of livestock and handlers.

Analyzing a sample of QI, there was positivity of 8.70% (2/23 samples) for *Samonella* sp (Figure 2). Similar results were presented by Feitosa *et al.* (2003) when analyzed 11 samples of cheese rennet type with

9% of positive samples and 13 types of cheese butter with 15% positive for *Salmonella* in products marketed in Rio Grande do Norte, describing them as unfit for consumption and Colak *et al.* (2007), who after analyzing 250 samples of “Tulum” cheese made from raw milk, the bacteria detected in 6 samples (2.4%). However, results with no bacteria in this sample of cheese was made by Little *et al.* (1998) and Aygun *et al.* (2005) in the United Kingdom and Costa Rica, respectively.

The presence of *Pseudomonas aeruginosa* occurred in 100% of samples of QC and QI (Figures 1 and 2). Regarding the high percentage of contamination, Carvalho (2001) has considered that *Pseudomonas* sp is a possible indicator of contamination, showing the use of non-potable water or improper handling in the production of food.

Considering the positivity for the pathogen *Listeria* sp (Figure 2), it is important to emphasize that this was 4.35% (QC) and 21.74% (QI) before and 34.70% (QI) after cold enrichment (45 days in refrigerator/4°C).

The species *L. innocua* was isolated in samples from both QC and QI. These results are consistent with findings of Pereira and Roccourt (1994), Farber and Peterkin (1991) and Pereira *et al.* (1999b) when describing that the temperature of cooling promotes the growth of *Listeria* sp, probably by inhibiting the competing microflora.

According to Laciari *et al.* (1999) the characteristic psychrotrophic of *Listeria* sp constituting problem is the conservation of food for long periods under low temperatures, as it would favor the growth of the microorganisms, also resulting in economic losses for the food industry.

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According to Laciari *et al.* (1999) the characteristic psychrotrophic of *Listeria* sp constitute problem is the conservation of food for long periods under low temperatures, as would the growth of the microorganism, resulting also in economic losses for the food industry.

In this research, the strains of *Listeria* sp with their serovars identified were: QC: *L. innocua* serovar 6a; QI: *L. innocua* serovar 6a, 6b, 4ab and not before characterized (AEF) and 4ab and not characterized after cold enrichment (DEF).

Figures 1 and 2 are presented the percentages of isolated microorganisms and in Figure 3, the percentages of serovars of *Listeria innocua* concerns in the FIOCRUZ-RJ, QI-R sampling of isolates before (AEF) and after (DEF) by cold enrichment.

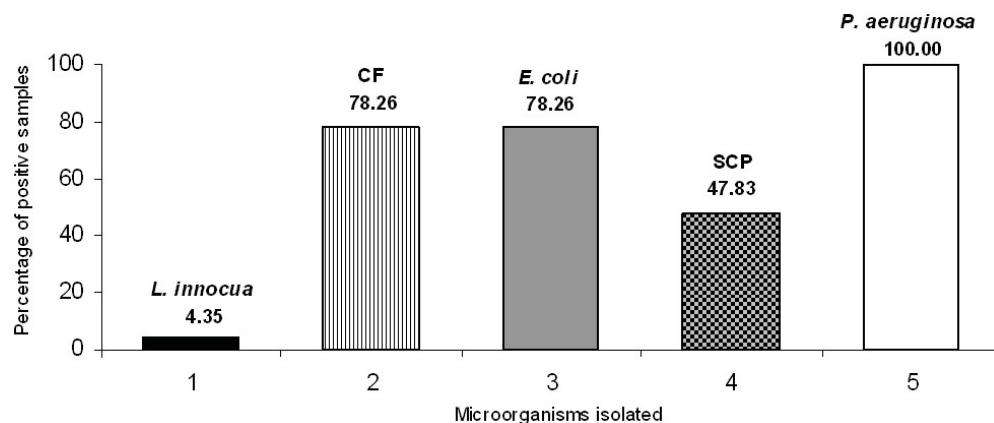


Fig. 1: Percentage of microorganisms isolated from samples of homemade cheese (QC)

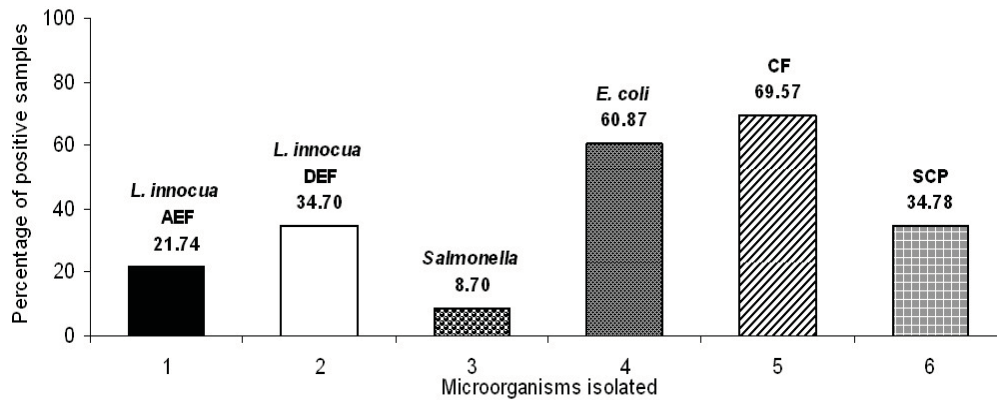


Fig. 2: Percentage of microorganisms isolated from samples of cheese industrialized (QI).

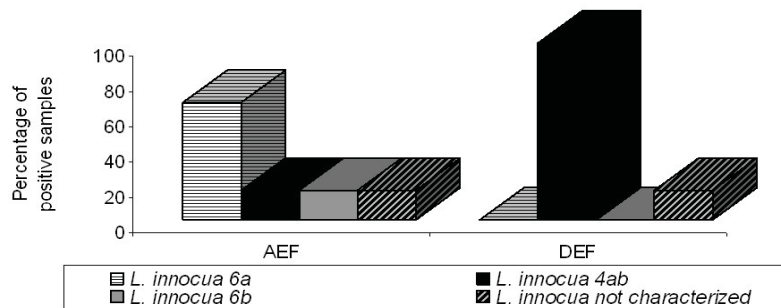


Fig. 3: Percentage of serovars of *L. innocua* isolated before (AEF) and after (DEF) by cold enrichment in samples of cheese industrialized mark R (QI-R)

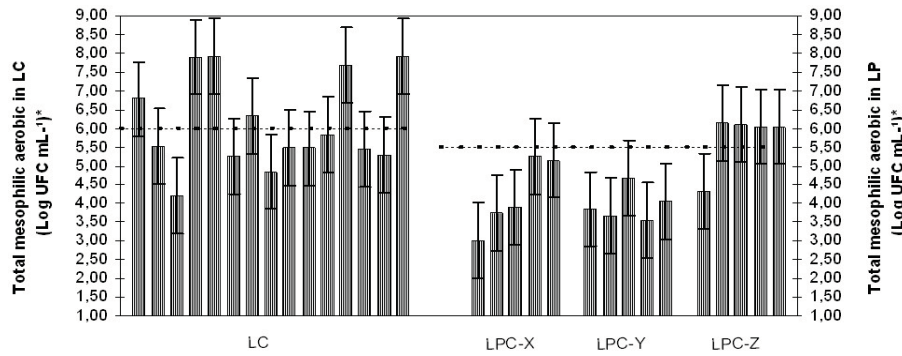
As studies and analysis conducted in 3112 samples by Hofer (2001) during the years of 1971 at 1997, the identification of *L. innocua* prevailed on the other species, and 80.9% (1889 samples) of positivity for this species among the isolates. The serovars most incidents in milk and derivatives were L1/2a, L4b and L1/2b. In regions of Colombia, Aygun and Pehlivanlar (2006) analyzed the total of 157 samples of raw milk and dairy, among them, the "Turkish White" cheese. The result showed isolation of *L. monocytogenes* in 8.23% of Turkish white cheese. Beckers *et al.* (1987) detected the presence of 10.14% (7 in 69 samples) of *Listeria monocytogenes* in samples of cheese prepared with raw milk.

Results similar to those of this study were reported by Santos *et al.* (2001), Guerra and Bernardo (2001) to mention that the formation of biofilm on the surface of piping materials and factory closed because of difficult removal, tend to make the environment conducive to the development of microorganisms potentially pathogens, including the genus *Listeria*. Thus, under the circumstances described, can be explained in part, the incidence of *Listeria* sp in cheeses of industrial origin. In research conducted by Silva *et al.* (1998) occurred and isolation of 17.65% of *L. innocua* and 41.17% of *L. monocytogenes* in 103 samples of cheese, of which 17 samples were "Minas Frescal" homemade.

According to literature, Kamat and Nair (1996) reported that *L. innocua* could be a safe and ideal body as a marker of *L. monocytogenes* in food industries.

**Analysis of the Milk Sampled:**

Regarding the count of aerobic mesophilic in CFU mL<sup>-1</sup>, the values of means and maximum (max) allowed by law for each type of milk studied, the second representative samples, were: LC: 2.02 x10<sup>7</sup> (max: 1.0 x10<sup>6</sup>); LPC-X: 6.63 x10<sup>4</sup> (max: 3.0 x10<sup>5</sup>); LPC-Y: 1.44 x10<sup>4</sup> (max: 3.0 x10<sup>5</sup>) and LPC-Z: 9.84 x10<sup>5</sup> (max: 3.0 x10<sup>5</sup>) CFU mL<sup>-1</sup>. It was found that 40% of samples of LC (6 of 15) and 80% of samples of LPC-Z (4 of 5) were in disagreement with the standard IN 51/2002 (Brasil, 2002), differing (P<0.05) significantly from the standard t test of means (Figure 4).



\* - Mean power of the base 10 log. Dashed line represents the maximum permitted by IN 51/02. In the sampling, 40.0% for LC and 26.67% of LPC appeared to be in disagreement with the legislation ( $P < 0.05$ )

**Fig. 4:** Quantification of mesophilic aerobes in samples of raw milk (LC) and pasteurized type C (LPC) of brand X, Y and Z traded in Alfenas-MG and region.

Gonçalves and Franco (1998) analyzed 30 samples of milk pasteurized type C, eight different brands, acquired the retail business in Rio de Janeiro, and obtained five samples (16.66%) with counts above the established by legislation.

The results of determination of total and fecal coliforms ( $\text{MPN mL}^{-1}$ ) in the sample of LC and LPC and their differences are presented in Table 3. The microorganisms evaluated in counts showed differences in mean and variance with the RDC n° 12/01 (Brasil, 2001) and IN n° 51/02 (Brasil, 2002).

For the means counts of total coliforms (CT, Table 3), pasteurized milk ( $34 \text{ MPN mL}^{-1}$ ) showed better ( $P < 0.0001$ ) compared to raw milk ( $338.84 \text{ MPN mL}^{-1}$ ). In a study performed by Oliveira (2005), was also found that the values found for the total coliform  $\text{MPN mL}^{-1}$  of raw milk were higher than those found for samples of pasteurized milk, reinforcing the importance of pasteurization of milk. The author also points out that there are no microbiological parameters for total coliforms in raw milk.

Taking into account a representative sample of the LPC for total coliforms, the marks A, C, X, Y and Z, is 75.76% (25 samples of total 33) were shown to be at odds with the law (Brasil, 2002)

The values found by researchers (Freitas *et al.*, 2002; Goncalves and Franco, 1998) ranged from 8.00 to  $1.70 \times 10^2 \text{ MPN mL}^{-1}$ , close to the range found in this study: 0.00 to  $1.10 \times 10^2 \text{ MPN mL}^{-1}$ , as the mean value presented in table 3. However, for the rule, the average quality revealed in disagreement when examined representative sample ( $n > 5$ ,  $c > 1$ ,  $m > 2$ ,  $M > 2$ ).

Whereas the count of fecal coliform (CF, Table 3), pasteurized milk ( $20.16 \text{ MPN mL}^{-1}$ ) quality had significantly ( $P < 0.0001$ ) better in relation to raw milk ( $89.16 \text{ MPN mL}^{-1}$ ).

For raw milk, the range found during the tests was 0.00 to  $2.39 \times 10^2 \text{ MPN mL}^{-1}$  (Table 3). In analysis of samples of raw milk, in Piracicaba-SP, Oliveira (2005) found fecal coliform values of 4.6 to  $9.2 \times 10^2 \text{ MPN mL}^{-1}$ . At work, the author mentions the benefits of treatment in reducing bacterial load. Viganò *et al.* (2007) examined a total of 300 foods, among them, 115 samples of raw milk and fecal coliforms detected in 98% of samples, and the count in  $\text{CFU mL}^{-1}$  of  $3.0 \times 10^4$ .

**Table 3:** Most Probable Number of total coliform (CT) and fecal (CF) in samples of raw milk (LC) and milk (LPC) and statistical analysis by t test of means.

	CT ( $\text{MPN mL}^{-1}$ )		CF ( $\text{MPN mL}^{-1}$ )	
	LC	LPC	LC	LPC
Minimum value	1	0	0	0
Maximum value	738	110	239	110
Mean*	338.84 <sup>A</sup>	34.00 <sup>B</sup>	89.16 <sup>A</sup>	20.16 <sup>B</sup>
t- test		5.64		4.31
Probability t		0.0001		0.0001
Standard IN n°51/02	-	$n > 5$ ; $c > 2$ ; $m > 2$ ; $M > 4$	-	$n > 5$ ; $c > 1$ ; $m > 1$ ; $M > 2$
Standard RDC n°12/01	-	-	-	$n > 5$ ; $c > 1$ ; $m > 2$ ; $M > 4$

\* Means with different letters (A, B) in rows differ significantly ( $P < 0.0001$ ) by t test

Pasteurized milk, according to table 3, vary from 0.00 to 1.10 x10<sup>2</sup> MPN of fecal coliforms mL<sup>-1</sup>. Oliveira (2005) found values of faecal coliforms of up to 2 MPN mL<sup>-1</sup> in the analysis of samples of pasteurized milk type C. Catão and Ceballos (2001) and also showed high contamination by fecal and total coliform in samples of raw milk and pasteurized. Of the 30 samples analyzed, 10 (33.3%) were contaminated by total coliforms and 3 (10%) for faecal coliforms above the current standards.

The percentage of *Pseudomonas* sp and *Pseudomonas aeruginosa* isolated in LC and LPC analysis are presented, respectively, Figures 5 and 6.

The positivity for *Escherichia coli* was 75.76% for the sampling of LC (Figure 5) and 39.39% for LPC (Figure 6), reflecting the relative influence, if not effective, the pasteurization of the total microorganisms present in sampling.

There was absence of *Salmonella* in samples of LC. However, the LPC showed 12.12% of positivity for this microorganism (4 samples analyzed in 33). Corroborating the results of this research, Avilla and Gallo (1996) analysis of quality in raw milk and pasteurized type C commercialized in Piracicaba-SP, depending on the detection of *Salmonella*, which was not found in any of 19 samples of each type of milk. Rather, Padilha *et al.* (2001) performed isolation of *Salmonella* in a sample of pasteurized milk type C (total of 250 samples) and a raw milk (total of 50 samples). Viganò *et al.* (2007) also isolated the bacteria in 11% of raw milk analyzed in Tanzania. According to the Center for control and prevention of diseases (CDC, 2007), the Ministry of Health and the Pennsylvania Department of Agriculture confirmed that 29 cases of diarrheal diseases were caused by *Salmonella* enteric serotype *Typhimurim*. The confirmation of these outbreaks was associated with the consumption of raw milk and derivatives.

Figures 5 and 6 shows the percentage of *Pseudomonas* sp and *Pseudomonas aeruginosa* isolates analyzed in LC and LPC, respectively.

The microorganism *Pseudomonas* sp considered opportunist, was isolated in 73.33% of samples of LC (CDC, 2007; Farber, and 1991), and the distribution of positivity for *P. aeruginosa* from 46.47% (7 in 15).

In LPC, 26.67% of the sample (4 in 15 samples) contained *Pseudomonas* sp., and in 20.0% (3 of 15 samples) was isolated *P. aeruginosa*. Fagundes *et al.* (2006) found high counts of *Pseudomonas* sp in both milk newly obtained as in cold milk on dairy farms, where there was a greater concentration of *Pseudomonas* sp in refrigerated raw milk, milk when compared with the newly obtained. Possibly, this fact is the current recommendation for cooling milk in natura, it favors the development of psychrotrophic microorganisms. Avilla and Gallo (1996) found positive for *Pseudomonas* sp in 10 samples of raw milk for sampling total of 30 milk analyzed.

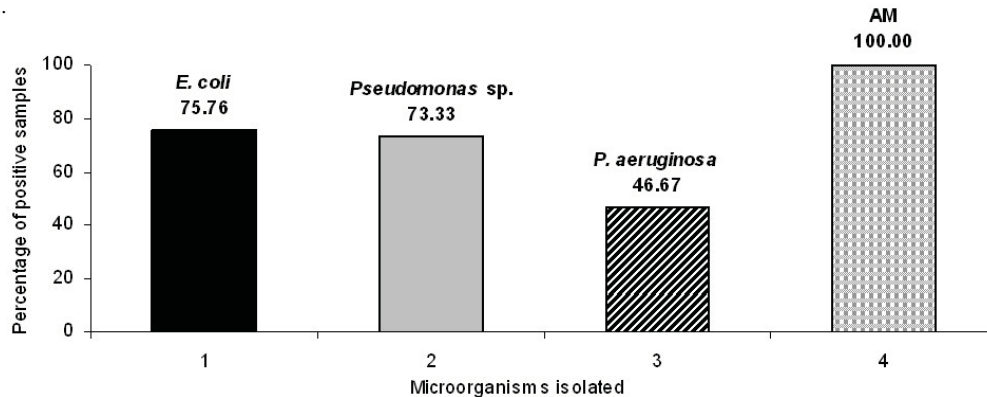


Fig. 5: Microorganisms isolated (%) in the samples of raw milk collected in Alfenas, MG.

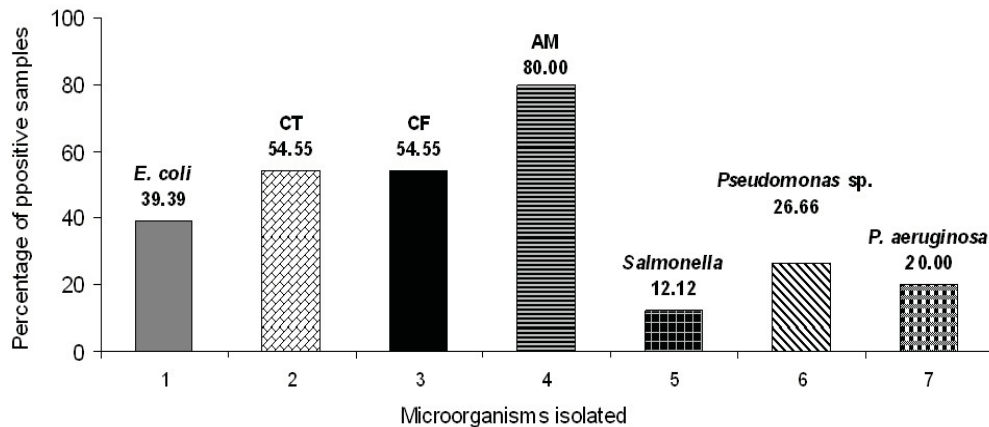


Fig. 6: Microorganisms isolated (%) from samples of milk collected in Alfenas, MG.

All samples of raw milk and pasteurized type C analyzed in this study, showed absence of *Listeria sp.* Similar results were described by D'Angelis *et al.* (2001) to analyze 48 samples of milk type C according to the detection of *Listeria monocytogenes* and found absence of *Listeria sp* in 100% of the tests. However, in contrast with the above information, Catão and Ceballos (2001) reported positive results for organisms of the genus *Listeria* to analyze 75 samples of milk (45 from raw milk, 15 fresh pasteurized milk and 15 pasteurized milk bagged) and reported that 33 (73.3%) samples of raw milk and 9 (30%) of pasteurized milk were contaminated with *Listeria sp*, and identified *L. monocytogenes* in 17 samples of raw milk and 9 milk received. Regarding the diversity of species reported by the authors mentioned above, in samples of raw milk were isolated: *L. monocytogenes* (66.6%), *L. innocua* (25.3%), *L. ivanovii* (3.9%), *L. welshimeri* (2.5%) and *L. grayi* (1.5%). Samples of milk were isolated: *L. monocytogenes* and *L. innocua*.

Still, results similar to those found in this research were also described in Turkey by Uraz and Yücel (1999) analyzed 211 samples of raw milk from different areas and detected the presence of *L. monocytogenes* in 2 samples (0.94%). Likewise, Moura *et al.* (1993) evaluated the incidence of *Listeria sp* in raw milk and pasteurized type C in the state of São Paulo. In total of 440 samples, the percentage of samples positive for *L. innocua* in milk was 0.9% and in raw milk were 9.5% for *L. innocua*, 0.9% for *L. welshimeri* and 0.4% for *L. grayi*.

### Conclusions:

Based on the results obtained in the analysis of samples of milk and cheese, it was concluded that:

The microorganism *L. innocua* was isolated from samples of homemade cheese and industrialized, before and after the enrichment step of the cold, identifying serovars are *L. innocua* 6a, 6b, 4ab and not typed serovar.

Both types of cheese appeared to be in disagreement with the law.

The cheese industrialized, even bearing the seal SIF did not present a better microbiological point of view on the homemade cheese.

The sampling of pasteurized milk revealed the presence of potentially pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Salmonella* and *Escherichia coli*, therefore, also in disagreement with the current legislation.

The presence of fecal coliforms above the limits set by law, in the products tested, reaffirms potential for the presence of intestinal pathogens.

The statistical similarity between the microbiological quality of sampling of raw and pasteurized milk cheeses and homemade and industrialized in relation to indicators of microbial contamination, showed that both products do not meet the standards of food safety.

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