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Evaluation of hygiene indicators in raw milk commercialized in Caruaru, Brazil

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Additional keywords

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SUMMARY

This study aimed to evaluate the raw milk commercialized in Caruaru, located in the Northeast of Brazil, by physical, chemical and bacteriological assays. Physical and chemical tests were based and interpreted by Brazilian Normative Instruction (NI) 69/2006 and the comparison of results was performed according to NI 62/2011 of Ministry of Agriculture, Livestock and Supply. Bacteriological investigations included research and estimation of the most probable number of total and fecal coliforms, *Pseudomonas aeruginosa* and counting of *Staphylococcus aureus*. Blood presence has been reported in 6.7% of the samples and 70% were positive for animal inflammatory exudate. The presence of total coliforms was reported in all the samples and 96.6% showed the presence of fecal coliforms. A total of 70% of the samples was inappropriate for the pasteurization process and 100% were positive for *S. aureus*, whose counting varied from 7×10^3 to 1.8×10^6 CFU/ml. The analyzed samples were also positive for *P. aeruginosa*, which 90% of those samples showed MPN/100 ml > 110. The raw milk commercialized in Caruaru has poor sanitary conditions and is not appropriate for human consumption. The easiness on acquisition of this food shows the necessity of a greater oversight by specific agencies.

Palavras chave adicionais

Microbiologia de alimentos.
Contaminação de alimentos.
Qualidade de alimentos.

Avaliação de indicadores higiênico-sanitários em leite in natura comercializado em Caruaru, Brasil

RESUMO

Esse estudo objetivou avaliar o leite in natura comercializado em Caruaru, localizada no Nordeste do Brasil, através de ensaios físico-químicos e bacteriológicos. Os testes físico-químicos foram baseados e interpretados de acordo com a Instrução Normativa 69/2006 e a comparação dos resultados foram realizadas de acordo com a Instrução Normativa 62/2011 do Ministério da Agricultura, Pecuária e Abastecimento. A investigação bacteriológica incluiu a pesquisa e estimativa do número mais provável de coliformes totais e fecais, *Pseudomonas aeruginosa*, e contagem de *Staphylococcus aureus*. A presença de sangue foi evidenciada em 6,7% das amostras e 70% apresentaram positividade para a presença de exsudato inflamatório. A presença de coliformes totais foi reportada em todas as amostras e 96,6% apresentaram positividade para coliformes fecais. Um total de 70% das amostras apresentou-se inapropriado para o processo de pasteurização e todas foram positivas para a presença de *S. aureus*, cuja contagem variou de 7×10^3 a $1,8 \times 10^6$ UFC/ml. As amostras analisadas foram também positivas para *P. aeruginosa*, das quais 90% destas apresentaram NMP/100ml >110. O leite in natura comercializado em Caruaru possui baixa qualidade sanitária e apresenta-se inapropriado para consumo humano. A facilidade de aquisição do produto demonstra a necessidade de maior vigilância pelas agências específicas.

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INTRODUCTION

Milk is a rich food, comprising water, lactose, proteins, fats, minerals and vitamins, making it an abundant source of nutrients (Pereira 2014). This composition makes it susceptible to physical and chemical variations and contamination by microorganisms (Santiago et al. 2011). *In natura* milk, also known as informal milk, is the product sold in its raw state, without any thermic treatment or decontamination procedures (Beloti et al. 1999). Despite legal prohibition (Brasil 1969), it is estimated that about 30% of Brazilian milk production is informally commercialized and with no inspection (Bassan et al. 2013).

Bovine milk provides to microorganisms most of necessary conditions to growing. The number of bacteria present in this element has decisive effect on safety and quality of dairy products (Mhone et al. 2011). Among the bacteria, which compromises hygienic milk quality, coliforms, *Pseudomonas aeruginosa* and *Staphylococcus aureus* can be highlighted.

Coliforms are the main hygienic quality indicator of foods. They can potentially indicate presence of fecal origin pathogens, since these can be found on mammal's intestinal tract (Hakalehto et al. 2013).

Pseudomonas aeruginosa is a psychrotrophic microorganism and has the capability of remain viable even in low temperatures, such refrigerated foods (Chapaval et al. 2010). Although some species of *Pseudomonas* genera compose the milk microbiota (Meer et al. 1991), *P. aeruginosa* requires special attention. This bacterium is an opportunistic pathogen commonly found in poorly sanitized milking environments and can also synthesize endotoxins (Chapaval et al. 2010). *P. aeruginosa* as mastitis causer is unusual, however, in cases reported in the literature, milk reveals the presence of lumps, pus and occasionally presence of blood, as described by Fernandes et al. (2009).

S. aureus is a gram-positive bacteria present on mucosa and human skin (Hanson et al. 2011). This specie is identified as a major etiological agents of bovine mastitis (udder inflammation), compromising the final milk quality (França 2017). Therefore, besides being an indicator of hygienic conditions, *S. aureus* can also point to the possibility of infection of animal mammary glands. Standing out also as one of the major food pathogens, *S. aureus* has receiving greater attention due to health impacts caused by its ability of enterotoxin production, resistant even to pasteurization process, especially when in quantities higher than 10^5 colony-forming units/ml (CFU/ml) (Gustafson et al. 2015, Martins et al. 2013).

In addition to bacteriological characteristics, physical and chemical parameters (such as heat-protein stability, temperature and titrable acidity) and sensory properties (appearance and odor), are also important in the quality of milk as food (Brasil 2011). Other contaminants also worth mentioning, as foreign bodies (visible elements to naked eye), blood and inflammatory exudate (pus), the latter, indicative of bovine mastitis (Brasil 2006).

Despite health risks, *in natura* milk trading is an established practice in Brazil (Montanhini & Hein 2013), strengthened by low costs and the belief of non-industrialization keeps it purest.

Descriptive research conducted by Liro et al. (2011) about milk consumption habits of Petrolina (Pernambuco, Brazil) habitants revealed that 97.9% of interviewed consumes in direct form. A total of 95.8% of the participants answered that they heat the milk before consumption. However, heat application time might be insufficient and, besides do not ensure all microorganisms destruction, there is the possibility of thermo-stable toxins produced by bacteria present in the medium persists on the food (Gustafson et al. 2015).

In Caruaru (Brazil), unpasteurized milk trade happens by bicycles, motorcycles, trucks or commercial establishments, such as bakeries and grocery stores. The product is usually packed in transparent plastic bags or PET (polyethylene terephthalate) bottles, routinely with inappropriate refrigeration and sanitary control.

Given the public health impact and the lack of data on the physical, chemical and bacteriological profile of raw milk marketed in the Northeast of Brazil, this study aimed to verify the quality of this product in Caruaru.

MATERIAL AND METHODS

This is a descriptive cross-sectional study with quantitative approach. A total of 30 samples of 30 different establishments (bakeries, grocery stores, minimarkets, butchery), located in most populous neighborhoods, were collected by direct purchase of the product in retail establishments. This measure insures the same conditions and characteristics that are provided to the costumers. Convenience sampling was applied using the sampling techniques described by Codex Alimentarius (Jay 2005).

Physical, chemical and sensory tests and parameters evaluated were based and interpreted by Normative Instruction (NI) 68/2006 (Brasil 2006) from the Ministry of Agriculture, Livestock and Supply (MAPA), comparing results to NI 62/2011 from the same organ (Brasil 2011).

Organoleptic evaluation included aspect, color and odor. Temperature was measured by analog thermometer graduated in Celsius scale, immediately after acquisition and homogenization of samples.

Visible foreign bodies analysis was accomplished by filtering in qualitative filter paper (80 g), observing if there was any residues.

The heat-protein assessment was made by the milk stability test in the presence of 72% alizarol (1:1 v/v).

Acidity was determined by titration with sodium hydroxide (NaOH) 0.111 M in 10 ml of milk added with phenolphthalein alcohol solution 2% as a pH indicator. Results were expressed by means of Dornic scale (°D).

Blood presence was based on centrifugation (1500 x g for 5 minutes), separating red blood cells to the bottom of the tube, in case of positive result.

The method for determining the presence of animal inflammatory exudate (pus) consisted in adding 0.1 ml of milk on a microscope slide and the same volume of ammonium hydroxide solution, homogenizing slightly. After 30 seconds, 0.1 ml of Ziehl fuchsin solution was added slowly to the slide underwent washing with running water. The filament formation, lumps or reddish veil indicated the positivity. Bacteriological investigations included research and estimation of the most probable number of total coliforms with the presumptive test using assay tubes containing upside down Durham tubes and Lactose Broth (37 °C for 24-48 h), being confirmed by Brilliant Green Bile 2% Broth (37 °C for 24-48 h). The investigation of thermotolerant

coliforms was made using E.C. broth (44.5±0.5 °C, 24 h), as recommended by the American Public Health Association - APHA (2005). The same tube scheme was used to the research and estimation of the most probable number of *Pseudomonas aeruginosa*, using Asparagine Broth and Acetamide Broth and incubating at bacteriological stove at 37 °C for 24-48 h.

For the research of *Staphylococcus aureus*, the samples was separately diluted to 10^{-1} to 10^{-5} in sterile ultrapure water and 0.1ml of each dilution was inoculated in Baird Parker agar and spread using Drigalski spatels, at 37 °C for 24-48h.

RESULTS AND DISCUSSION

The physical, chemical and organoleptic analysis of the 30 samples of fresh milk studied revealed that 100% (30/30) samples presented characteristic coloration, which, according to IN n° 68/2006 of MAPA, should be white. As regards the aspect, 10% (3/30) of the samples were altered by the presence of lumps possibly originating from the beginning of milk coagulation process.

Considering the odor parameter, 10% (3/30) of analyzed milks were classified as sour. The presence of foreign bodies was observed in 56.7% (16/30) of the samples, composed by bovine and tissue fragments constituents of the strainers commonly used to filter the product before distribution to commercial establishments. The milk commercialization temperature ranged of 2 °C to 32 °C (**Table I**). A total of 43.3% (13;30) of samples were identified in the acceptable temperature range, that is, close to 10 °C (Brasil 2011). However, refrigeration by itself is not capable of im-

Table I. Physical-chemical and bacteriological parameters of evaluated raw milk (Parâmetros físicos-químicos e bacteriológicas de leite cru avaliada).

Sample number	Inflamatory exudate	Latic acid (g/100mL)	Foreign bodies	Temperature (°C)	Thermotolerant coliforms (MPN/100mL)	*P. aeruginosa* (MPN/100mL)	*S. aureus* (CFU/mL)
L01	+	0.20	+	32.0	>110	>110	+; ND
L02	+	0.45	+	29.0	>110	>110	3×10^4
L03	-	0.36	-	11.5	>110	6.4	1.8×10^6
L04	+	0.37	+	11.0	>110	>110	2.8×10^4
L05	+	0.27	-	2.0	>110	>110	7×10^3
L06	-	0.14	-	10.0	>110	6.4	1.7×10^4
L07	-	0.16	-	6.5	>110	4.3	4×10^4
L08	-	0.23	-	4.0	>110	>110	3.2×10^4
L09	+	0.28	+	19.0	>110	>110	2.8×10^4
L10	+	0.24	-	15.0	>110	>110	2.4×10^4
L11	+	0.30	+	5.0	>110	>110	7.5×10^3
L12	+	0.24	+	11.0	>110	>110	1.4×10^4
L13	+	0.32	+	6.0	>110	>110	1×10^4
L14	+	0.28	+	3.0	>110	>110	2.2×10^4
L15	+	0.28	-	7.0	>110	>110	1.1×10^4
L16	+	0.21	+	9.0	>110	>110	1.2×10^4
L17	+	0.31	+	31.0	>110	>110	1.7×10^4
L18	+	0.32	+	5.0	>110	>110	7.5×10^4
L19	-	0.21	+	5.2	>110	>110	4.4×10^4
L20	+	0.30	-	22.0	<0.3	>110	4×10^4
L21	+	0.21	+	2.0	>110	>110	1.5×10^4
L22	+	0.31	+	3.2	>110	>110	2.8×10^4
L23	-	0.24	-	8.2	>110	>110	3.1×10^4
L24	+	0.29	-	30.0	>110	>110	2×10^4
L25	-	0.35	-	30.5	>110	>110	9×10^4
L26	+	0.29	-	17.0	>110	>110	1.3×10^4
L27	+	0.16	+	14.0	>110	>110	2×10^4
L28	+	0.23	-	16.5	>110	>110	2×10^4
L29	-	0.37	+	23.0	>110	>110	1.4×10^4
L30	-	0.37	+	23.0	>110	>110	1.2×10^4

+ = presence; - = absence; ND = not determined; MPN/100mL = most probable number in 100 milliliters of milk; CFU/mL = colony-forming units in 1 milliliter of milk.

proving the overall quality of milk that has already been compromised. The largest number of samples (56.7%; 17/30) was marketed in the "danger zone", which comprises temperatures from 5 °C to 60 °C, where microorganisms are more easily multiplied (Silva 1995). Of the total analyzed, 26.6% (8/30) of samples were not under any type of refrigeration and were exposed to sun and heat.

In the present study, the presence of total coliforms was observed in all analyzed samples, which reached MPN > 110/100 ml of milk, and 96.6% (29/30) of them showed positive results for thermotolerant coliforms presence (**Table I**). The numbers described here were higher than those described by Quintana and Carneiro (2006) in study conducted in the city of Morrinhos (Goiás, Brazil), where only 5 of 21 samples had fecal coliforms. As well as some deteriorating and pathogenic microorganisms (Silva et al. 2008), coliforms have the property of fermenting the carbohydrates present in milk, with production of acidic substances that are incorporated into the product, acidifying it.

An appropriate *in natura* milk has acidity between 14 and 18 °D, corresponding to 0.14 to 0.18 g of lactic acid/100 ml of milk. An amount of 90% (27/30) of the analyzed samples presented high acidity, whose values varied between 0.2 and 0.45 g of lactic acid/100 ml, similar to the studies developed by Silveira and Bertagnolli (2014) and Molina et al. (2015). Only three samples (L6, L7 and L27) showed characteristic acidity for *in natura* milk. **Table I** lists the physical and chemical results of inflammatory exudate, acidity, presence of foreign bodies and the temperature of commercialization of the evaluated milks.

All samples analyzed showed positivity for *S. aureus* (**Table I**). Of these, one (L3) reached a count of 1.8×10^6 CFU/ml, revealing difficulties for producers and distributors in complying with hygiene standards. Considering food safety parameters, this is an alarming value, since this number of microorganisms favors the production of toxins in the food (Martins 2013). The levels of contamination in this study were higher than those described by Martins et al. (2013), whose values ranged from $<1.0 \times 10^1$ to 1.6×10^4 CFU/ml in raw milks of the Rio Pomba (Brazil). Microbiological values are described in **Table I**.

In addition to indicating the sanitary conditions of the food, these bacteria have a direct influence on health status of the herd and are often the main cause of inflammation of the animal's udders causing mastitis (Chapaval 2010), presumably detected by the alizarol 72% stability test. Using this method, it was observed that 73.3% (22/30) of samples evaluated presented suspicion for this type of occurrence, also indicated by the high percentage (70%; 21/30) of samples containing animal inflammatory exudate. The presence of blood was detected in 6.7% (2/30) of the analyzed samples.

In this study, all the analyzed samples presented positivity for *P. aeruginosa*. The MPN/100ml of milk from *Pseudomonas* sp. was > 110 in all the samples, whereas this measurement was repeated in 90% of the samples in relation to *P. aeruginosa*, with the lowest count in MPN/100ml equivalent to 4.3. The L27 and L28 sample suggests animal mastitis caused by *P. aeruginosa*, considering the presence of pus, blood and alizarol 72% stability tests, featuring the respective animals as unfit for milking.

Taking these findings together and considering the ease of access and acquisition of raw milk with low hygienic quality, a strict inspection should be incorporated by the specific governmental organs in order to prevent the illegal commercialization of the product. Alternatively, since the practice is already widespread, the possibility of drafting legislation specific to the region should be considered, regulating the quality of this food.

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