Hygienic quality of raw and fermented cow milk in the local milk sector of the Liptako-Gourma area in Niger

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Abstract

Background and Aim: Milk is a food of high nutritional value, which occupies an undeniable place in the human food ration, but is an ideal medium for microbial growth. This study aims to assess the hygienic quality of local raw and fermented milk from the Liptako-Gourma region in Niger.

Materials and Methods: We performed physical and bacteriological analyses on 330 samples of bovine milk from local breeds, including 110 individual milk samples (per cow), 110 fermented milk samples, and 110 blended milk samples. The microbiological parameters were determined using standard methods.

Results: The physical analysis revealed temperatures during sample collection for all milk types between 35.2°C and 37.8°C. The average pH of fermented milk varied between 3.16 and 4.92, and those of individual and blended raw milks between 5.42 and 6.98. The titratable acidity varied from 15° to 18.1°D for raw milk and between 59° and 122°D for fermented milk. The average density of individual and blended milks ranged between 1.028 and 1.035. Regionally, milk from the Dosso and Niamey regions had high quality; 97.4% and 98.2%, respectively, were judged as Grade I. In contrast, milk from the Gourou region had low quality; 96.1% was judged as Grade I and 3.9% was Grade II. Salmonella spp. were 86.36%, 12.73%, and 20.91%, respectively, in fermented milk. Phenotypic identification pointed toward three genera: E. coli (30.76% ± 0.25%), S. aureus (20.58% ± 0.14%), and Salmonella spp. (2.74 ± 0.04%).

Conclusion: The present data suggest that milk samples collected from three regions in Liptako-Gourma had low quality; further, some of the bacteria identified (E. coli, S. aureus, and Salmonella spp.) could be potential foodborne pathogens.

Keywords: bovine, fermented milk, microbiological quality, milk, physical parameters.

Introduction

Milk and dairy products have long been a key element in the human diet because they provide a matrix of nutritional compounds, including fats, proteins, vitamins, antioxidants, and minerals [1, 2]. In addition to their nutritional value, the consumption of dairy products is also associated with health benefits [3]. Simultaneously, a recent report by the Center for Disease Control stated that consumption of raw dairy products is linked to foodborne illness; dairy products are the second main source of pathogenic microorganisms for humans, causing 14% of foodborne illnesses and 10% of deaths [4]. Indeed, milk is a fragile product, liable to be altered by numerous chemical, biochemical, and microbiological reactions if not well preserved [5]. Raw or processed milk is an excellent culture medium for several microorganisms [6] and can result in product spoilage or infections/poisoning among consumers [7].

In West Africa, sustained population growth and the emergence of a middle class have significantly increased the demand for dairy products [8]. In Niger, animal products, including milk, contribute to meeting the nutritional requirements of the population [9]. However, direct milk sale to the consumer, which is the most frequent form of acquisition, presents a risk due to the defective quality of milk and dairy products [10]. In fact, other developing countries are also paying a heavy price for unsafe food products. The World Bank estimates that food safety problems cost developing countries >$100 billion a year. Despite existing standards to preserve milk quality in Niger, including Law No. 2004-048 of June 30, 2004, on the framework law related to breeding [11] or the Nigerian standard on milk and fermented milk products of December 2006 [12], Decree No. 2011-616/PRN/MEL of November 25, 2011, regulating the hygiene inspection of animal and animal-related food...
products [13], there is still an issue with milk quality. Milk contamination is closely dependent on hygienic conditions [14]. Milk quality, considered with respect to bacterial contamination, largely depends on the observance of sanitary standards at all stages (milkking, processing, storage, and transportation) [15]. The previous studies [9, 15] in some regions in Niger reported poor hygiene in dairy farms. The lack of hygiene in milk production increases the risk of microorganism proliferation. Moreover, since the quality of raw milk influences that of dairy products [16], it would be important to know the quality of the local milk used to generate the country’s dairy products. This knowledge would also improve the market value of local milk production. However, data on the microbiological quality of milk are scarce in Niger [17, 18]. The most recent data relates to germs linked to mastitis and bovine tuberculosis [19, 20].

This study aimed to investigate the hygienic quality of local milk, which may support the country on its journey toward its Zero Hunger objective by 2030.

Materials and Methods

Ethical approval

Ethical approval was not required for this study; however, milk samples were collected as per the standard collection procedure without any harm to the animals.

Study period and location

The study was conducted from March to September 2015 in the Liptako-Gourma area in Niger. This area is made up of 3 regions of the country, including Dosso, Niamey, and Tillabery, in which 11 municipalities were selected, each corresponding to a dairy basin. In the area of Liptako-Gourma, breeding represents the second economic activity of the country. The Azawak breed is the most exploited [21], and produces an average of 10 L of milk/day in 6 months of lactation [22]. However, its average production does not exceed 3 L/day [23]. Indeed, the choice of this area is justified by its vast water network, which is a fundamental factor for animal production, the genetic potential of the Azawak (one of the most exploited breeds in this area but also one of the best dairy of the sub-region) and the presence of the country’s capital (Niamey) where the demand for dairy products is the highest. For the study, 11 dairy basins were selected namely, Hamdallaye, Baleyara, Karma, Torodi, Tillaberi, Say, Sansane-Haoussa, Kollo, Niamey, Dosso, and Birni N’Gaouré.

Sample collection

A cross-sectional study was carried out to assess the microbiological quality of raw and fermented milk samples from the three major players (producers, collectors, and sellers) in the local milk sector in the Liptako-Gourma area. Samples were collected aseptically at a rate of 500 mL of raw milk directly from the cow’s udder (individual), 500 mL of mixed milk (from collection points), and 500 mL of fermented milk (from markets). The samples were packaged in sterile Pirex bottles® (Thermo Fisher Scientific, France) and transported in coolers fitted with frozen carbo glass to the laboratory. The samples were analyzed within 24 h.

Sampling sites

Sampling concerned individual milk at the farm level (producers), blending milk at the farm and collector level, and fermented milk at the vendor level (Table-1). Each site was visited only once. These products are made from local milk and are the most consumed in target localities. In each of the three regions, the farms were selected by iterative sampling guided by resource persons (snowball sampling), where the first was selected at random from a list provided by the local authorities [24]. The farm selection was based on the following criteria:

- Farms primarily used for milk production
- Farm with ≥10 lactating cows
- Cooperation and acceptance of the breeder to participate in the study and allow the collection of milk samples

To select collectors, the collection points in each dairy basin were identified with the help of local authorities. The first collectors to arrive and who have given their consent for the collection of milk samples were selected. Fermented milk was acquired from the points of sale in the localities of the selected farms. A total of 330 samples were taken from the 11 regions selected at a rate of 30 samples per region.

Physical analysis

The temperature and pH of raw and fermented milk samples were measured using a pH meter branded EXTECH/Exstik™ pH Meter thermometer (PH105-PH Module; CL205-Chlorine Module; RE305-ORP Module). A sufficient milk quantity to cover the immersed electrode was transferred into beakers for temperature and pH measurements. The density was determined using a thermolacto-densimeter (PAAR DMA 35) at 20°C. The acidity was measured by titration with NaOH solution and expressed in Dormic degrees (°D).

Microbiological analysis

The isolation of various microorganisms in all raw and fermented milk samples was carried out according to International Organization for Standardization (ISO) 7218 standards in food microbiology [25]. In this study, we investigated the total aerobic flora at 30°C, thermotolerant coliforms and Escherichia coli at 44°C, Staphylococcus aureus, sulfite-reducing anaerobic germs at 37°C, Clostridium perfringens at 46°C, Salmonella spp., Listeria monocytogenes, yeasts, and molds. Suspected pathogenic strains were isolated and biochemically identified using analytical profile index galleries which are a biochemical panel for identification and differentiation of members of the family of bacteria and yeasts (Table-2).

Preparation of the stock suspension and dilutions

Sample dilutions were performed according to ISO 8261 [26]. Of the 500 mL of milk, 25 mL from each
Statistical analysis

The data collected were entered into Microsoft Excel (Microsoft, USA), and then analyzed with Statistical Analysis System (SAS) 9.4. software (SAS Institute Inc., NC, USA). For physical parameters (temperature, density, acidity, and pH), a single-factor analysis of variance was used with collection region and type of milk collected as a source of variation. The Proc General Linear Model (GLM) was used for the analysis of variance and the F test was used to determine the importance of region and type of milk on the variables. The means were calculated and compared in pairs by Student’s t-test. For microbiological parameters (Mesophilic aerobic flora, Lactic bacteria, E. coli, S. aureus, Yeasts/Molds, Sulfite reducing anaerobes, and L. monocytogenes), microbial counts were converted to log base 10 of the number of Colony-forming units (CFUs) per mL of raw cow’s milk (log CFU/mL). Means and standard deviations were calculated. Data were analyzed with ANOVA using the GLM procedure of the SAS software; the least significant differences were used to separate the averages at p < 0.05. P indicates the relative frequency and N the sample size. Frequencies were calculated by the SAS Proc freq procedure and compared by the Chi-square test (α = 0.05) and bilateral Z test (α = 0.05).

Results

Physical analysis

The average temperature ranged between 35.2°C and 37.8°C for all types of milk. The average pH of fermented milks ranged between 3.16 and 4.92; 57.27% (62.99/110) complied with the pH standard (4–4.5). The pH of individual and blended milks ranged between 5.81 and 6.96 and between 5.42 and 6.98, respectively. However, only 21.81% (24.06/110) of these individual milks and 16.36% (17.99/110) of blended milks were within the standard pH range of normal raw milk (6.6 < pH < 6.8). The titratable acidity ranged between 15–17.9°D, 15–18.1°D, and 59°–122°D for individual milk, blended milk, and fermented milk, respectively. However, there were no significant differences (p > 0.05) between milk types. The average density of individual and blended milks ranged between 1.028 and 1.033 and between 1.030 and 1.035, and showed significant differences (p ≤ 0.0001). Regarding density, 96.36% of individual milks and 91.81% of mixed milks were not wet, being within the normal density standard for raw milk (1.030–1.034). All physical parameters are reported in Table-3.

Microbiological analyses

Microbial loads of milk within regions

All parameters investigated in the milk samples from the three regions varied (Table-4). For germs indicative of overall hygiene (Mesophilic aerobic germs), milk in the Tillaberi region was significantly more contaminated (p = 0.0025). Lactic acid bacteria, coliforms, and Staphylococci were also found in greater proportion in milk from Tillaberi, although the differences between regions were not significant. Milk samples from the Niamey region were the most contaminated with yeasts and molds. Salmonella was absent from milk samples taken in the Niamey region. However, it was present in 20% (22/110) of milk samples from Dosso and in 15% (16.5/110) from Tillaberi.

Microbial loads

The average total flora ranged between 7.36 ± 0.49 and 7.39 ± 0.51 × 10³ CFU/mL. Fermented milks exhibited the highest concentrations, followed by blended milks and individual milks (Table-5). They also had the highest average load of lactic acid bacteria (average between 6.51 ± 0.33 and 6.55 ± 0.29 × 10⁴ CFU/mL), yeast (average between 6.51 ± 0.33 and 6.52 ± 0.37 × 10⁴ CFU/mL), and molds average (6.40 ± 0.34 and 6.44 ± 0.40 × 10⁴ CFU/mL). Blended milks were the most contaminated, with coliforms with an average load between 5.94 ± 0.70 and 6.02 ± 0.62 × 10³ CFU/mL and average S. aureus loads from 3.54 ± 1.89 to 3.92 ± 1.57 × 10³ CFU/mL. However,

Table-1: Distribution of the number of samples taken by dairy basins.

| Regions | Dairy basins | Individual milk | Blending milk | Fermented milk | Total |
|---------|-------------|-----------------|---------------|----------------|-------|
| Niamey  | Niamey      | 10              | 10            | 10             | 30    |
| Dosso   | Dosso       | 10              | 10            | 10             | 30    |
| BirniN’Gaoure | 10        | 10              | 10            | 30    |
| Tillaberi | Hamdallaye  | 10              | 10            | 10             | 30    |
|         | Baleyara    | 10              | 10            | 10             | 30    |
|         | Karma       | 10              | 10            | 10             | 30    |
|         | Torodi      | 10              | 10            | 10             | 30    |
|         | Say         | 10              | 10            | 10             | 30    |
|         | Sansane-Haoussa | 10        | 10            | 10             | 30    |
|         | Tillaberi   | 10              | 10            | 10             | 30    |
|         | Kollo       | 10              | 10            | 10             | 30    |
| Total   |             | 110             | 110           | 110            | 330   |

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no significant difference was observed between milk types regarding microbial loads (Table-5). With respect to the prevalence of the pathogenic microorganisms (Table-6), fermented milks were the most contaminated, with 86.36% ± 0.06% S. aureus, 12.73% ± 0.06% E. coli, and 20.91% ± 0.08% Salmonella spp. Sulfite-reducing anaerobes equally predominated in fermented and individual milks, while L. monocytogenes was found most commonly in blending and individual milks. Biochemical identification of suspected pathogenic strains (Table-7) confirmed the presence of 30.76% (4/13) E. coli, 20.58% (7/34) S. aureus, and 2.74% (2/73) Salmonella spp.

**Table-2:** Culture media used for the isolation of microorganisms in milk.

| Microorganisms                  | Identification media/ References | Incubation (Temperature °C/ Time Hours) | Standards | Methods |
|---------------------------------|----------------------------------|-----------------------------------------|-----------|---------|
| Mesophilic aerobic flora        | PCA/Biokar BK144HA - Solabia Group France | 30°C/72 h | NF EN ISO 4833-1: 2013 | Enumeration on PCA |
| Total coliforms                 | Crystal VRBL/Biokar BK152HA - Solabia Group France | 30°C/24 h | NF ISO 4832, 2006 | Enumeration on VRBL, biochemical confirmation by API 20 E gallery |
| Escherichia coli                | Rapid’ E. coli/Bio-Rad 355 5299 - Bio-Rad Laboratories | 37°C/24 h | EN ISO 16140, 2003 | Isolation of strains on Rapid’ E. coli, biochemical confirmation by API 20 E gallery |
| Sulfite-reducing anaerobes      | TSC/Biokar BK031HA - Solabia Group France | 37°C/24 h | NF EN ISO 13401, 2003 | Enumeration on TSC medium, biochemical confirmation by API 20A gallery |
| Staphylococcus aureus           | Baird Parker/Biokar BK055HA - Solabia Group France | 37°C/24 h | NF EN ISO 6888-1, 1999 | Enumeration on Baird Parker agar medium, confirmation by coagulase test, biochemical confirmation by Galerie API Staph |
| Listeria monocytogenes          | *Fraser-half* broth/ OxoidCM 1053B - Oxoid Group Thermo Fisher Europe | 30°C/24 h | NF EN ISO 11290-2 (1998) | Selective enrichment with "Fraser - demi," Enrichment with Fraser Broth, isolation on Palcam |
|                                | *Fraser broth/Oxoid CM 0895 - Oxoid Group Thermo Fisher Europe | 37°C/24 h | ISO 6579 2002 | Selective enrichment with Rappaport-Vassiliadis |
|                                | *Palcam/Oxoid CM 0877 - Oxoid Group Thermo Fisher Europe | 37°C/48 h | ISO 6611:2004 (IDF 94: 2004) | Isolation on XLD and SS medium, biochemical confirmation by API 20 E |
|                                | *TSYE/Conda 1398 - Conda Laboratories Europe | 37°C/24 h | ISO 7889/IDF, 2003 | Selective enrichment with Sabouraud Chloramphenicol Agar |
| Salmonella spp.                 | Buffered peptone water (Eur Pharm) Conda - Conda Laboratories Europe | 37°C/24 h | NF EN ISO 6887-5: 2010 | Pre-enrichment in buffered peptone water |
|                                | Rappaport-Vassiliadis/ Conda 1240.00 - Conda Laboratories Europe | 37°C/24 h | ISO 6579 2002 | Selective enrichment with Rappaport-Vassiliadis |
|                                | SS (Conda 1064.00)/ Xylose-Lysine-Deoxycholate (Conda 610060) - Conda Laboratories Europe | 37°C/24 h | ISO 6611: 2004 (IDF 94: 2004) | Isolation on XLD and SS medium, biochemical confirmation by API 20 E |
| Yeasts and molds                | Sabouraud with chloramphenicol/ Conda 1090.00 - Conda Laboratories Europe | 30°C/72 h | ISO 6611: 2004 (IDF 94: 2004) | Isolation on Sabouraud Chloramphenicol Agar |
| Lactic acid bacteria            | MRS agar/Oxoid CM 361-Oxoid Group Thermo Fisher Europe | 37°C/24 h | NF ISO 15214, 1998 | Enumeration on MRS agar, biochemical confirmation by API 50CH |
|                                | MRS broth/Oxoid Group Thermo Fisher Europe | 37°C/24 h | NF ISO 15214, 1998 | Enumeration on MRS agar, biochemical confirmation by API 50CH |
|                                | M17 agar/Conda 1318.00 - Conda Laboratories Europe | 37°C/24 h | ISO 7889/IDF, 2003 | Enumeration on M17 agar, biochemical confirmation by API 50CH |
|                                | M17 broth/Oxoid CM 0817 - Oxoid Group Thermo Fisher Europe | 37°C/24 h | ISO 7889/IDF, 2003 | Enumeration on M17 agar, biochemical confirmation by API 50CH |

PCA=Plate Count Agar, VRBL=Violet and Neutral Red Bile Lactose Agar, TSC=Tryptone Sulfite Cycloserine, TAYEA=Tryptose Soy and Yeast Extract, MRS=Man Rogosa Sharpe, XLD=Xylose-Lysine-Deoxycholate, SS=Salmonella-Shigella, ISO=International Organization for Standardization
In the present study, we found that the temperature of raw milk (individual and mixed) and even that of fermented milk was close to body temperature (37°C), which is the average temperature during the study period (March to September). This shows that after milking, milk does not immediately arrive at the collection center having time to acclimate. Fermented milk (the most widely consumed dairy product) mostly drinks alone or mixed with processed cereal (porridge, scoop, and dengue) as a midday or evening meal. The times of high demand coincide with the maximum temperature, which accentuates
Table-6: Level of contamination of milk by pathogenic microorganisms.

| Microorganisms               | Level of pathogen presence in the types of milk (% ± IC) | Significance |
|------------------------------|----------------------------------------------------------|--------------|
|                              | Individual milk  | Blending milk | Fermented milk |
| Staphylococcus aureus        | 81.82 ± 0.07a    | 81.82 ± 0.07a | 86.36 ± 0.06a  | NS           |
| Listeria monocytogenes       | 4.55 ± 0.04a     | 13.64 ± 0.06a | 13.64 ± 0.06a  | NS           |
| Sulfite-reducing anaerobes   | 10 ± 0.06a       | 9.09 ± 0.05a  | 10 ± 0.06a     | NS           |
| Escherichia coli             | 3.64 ± 0.03a     | 7.27 ± 0.05a  | 12.73 ± 0.06a  | NS           |
| Salmonella spp.              | 10.91 ± 0.06a    | 13.64 ± 0.06a | 20.91 ± 0.09a  | NS           |

IC = Correlation index, NS = Not significant, a = no significant difference for means followed by the same letter

Table-7: Percentage of positive for pathogenic microorganisms.

| Pathogenic microorganisms | Number of isolates | Suspected | Percentage of positives | IC   |
|---------------------------|---------------------|-----------|-------------------------|------|
| Staphylococcus aureus     | 215                 | 34        | 20.58% (7)              | 0.14 |
| Salmonella spp.           | 73                  | 73        | 2.73% (2)               | 0.04 |
| Escherichia coli          | 26                  | 13        | 30.76% (4)              | 0.25 |

IC = Correlation index

milk acidification, explaining the low pH observed. The lack of freshness of certain milk samples might be attributable to a long time after milking and storage at an inappropriate temperature, conditions conducive to the transformation of lactose into lactic acid by lactic acid bacteria [27, 28]. However, the amount of acid produced by bacteria is a determining factor in milk quality, as is tampering. Indeed, pH and acidity depend on hygienic conditions during milking, the initial total microbial flora and its metabolic activity and milk handling [29, 30]. The fact that we found standard densities is proof that dairy producers do not adulterate milk intended for sale.

Microbiological analysis revealed that microbial loads depend on the nature of the product analyzed and the microorganism investigated.

The mesophilic aerobic germ load was very high in all milk samples from the three regions being over the 10^6 CFU/mL acceptable threshold. The total aerobic mesophilic flora provides information on milk’s hygienic quality [31], due to its relationship with non-compliance with good milk production and storage practices [32]. Non-compliance with the standard may render the product unfit for human consumption even in the absence of pathogenic flora [33]. Our result confirms the faulty hygiene observed in the practices of dairy farmers in the Liptako-Gourma region in Niger [9]. However, it should be noted that hygienic practices differ from region to region. The greatest overall microbial loads recorded in the Tillaberi region would result from the long time elapsed before the milk reaches the consumer. Due to the vastness of the region, production areas are not only far from each other but also from consumption areas, which increases the delivery time. The lowest contamination levels in Niamey would comply with hygienic rules. The hot weather does not help with milk conservation [34]. In fact, in Niger, high temperatures at certain times of the year contribute to the rapid deterioration of food in general and dairy products in particular and can increase the presence of pathogenic germs [34]. While temperature control is a big issue, adhering to hygienic production practices could drastically lower the microbial load in milk. Considered a flora of technological interest [35], but also of alteration [36], fungal flora can degrade product marketability by altering taste, odor, and appearance. The results of our study show that this flora is present in all types of milk but more so in fermented milk, probably due to fungi’s acidophilic nature and their low sensitivity to antagonistic lactic acid bacteria [37]. The massive presence of fungal flora is also the expression of strong external contamination and poor tool hygiene [38]. The presence of coliforms in all dairy products reflects poor hygienic conditions during production and an unhealthy environment because coliforms are usual hosts in mammal intestines and their presence in milk indicates fecal contamination [39]. Coliforms of the genus *Escherichia* contaminate milk directly (through the udder), or multiply as a result of improper cleaning of utensils [40], which could explain the higher microbial load in raw blended milks. The highest coliform load in blended milk would be due to the numerous manipulations by milk collectors together with the various unhygienic manipulations of dairy producers (washing of hands, teats, and utensils with water of questionable quality), which, instead of guaranteeing hygiene contaminate the hands, udder, and equipment. However, if fermentation can cause sufficient milk acidification (pH < 4.5) would impair their growth since these bacteria are not acidophilic. An exception is *E. coli* O157: H7, which can resist the acidic environment [41] and survive for up to about 4 weeks [42]. Another possibility might be insufficient acidification during fermentation [43]. Most *E. coli* strains are harmless, coexist with the host in the intestinal tract, and may benefit the host by protecting it against infection by pathogenic bacteria [44] and by synthesizing vitamin K in the host’s intestine. However, some can cause disease by influencing the
in the region unveiling the real risk incurred by consumers and also investigates the acidic level that inhibits pathogen growth. To guarantee food safety and improve the hygienic quality of milk and fermented milk, good hygiene practices and periodic microbiological control are an absolute necessity. Indeed, the absence of adequate regulations and product control to ensure sufficient hygienic quality of the dairy products marketed results in unsafe products. It is essential to raise awareness and train, individually or collectively, all players in the sector in hygienic rules and transformation processes. The concerted intervention of involved players in the sector considering their respective needs combined with incentive measures would also be desirable.

Authors’ Contributions

MHG, PS, and SF: Conceptualization and experimental design. MHG and FSPD: Collected milk samples, provided laboratory technical support, conducted the experiments, analyzed data, and drafted the manuscript. PS, PA, IAKY, SAG, and SF: Supervised the study and reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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Analyzing the water used in the farms and for dairy processing would have made allowed verifying the role of water quality in contamination. It would also be interesting to establish the link between risky practices and product quality while identifying the critical points to ensure the sanitary quality of these products. In addition, milk quality would be improved by respecting good production practices, good hygiene practices, respecting the heat treatment process, washing utensils with soap or bleach and good quality water, reducing transport time, use of lactoperoxidase, and mastery of fermentation processes. The finding of certain pathogenic species in milk represents a real threat to public health. Given the lacking process, environmental, and material hygiene, it is imperative to investigate the incidence and effects of the presence of these pathogenic microorganisms in milk.

Conclusion

The results of physical and microbiological analyses of raw and fermented cow’s milk samples demonstrated the presence of microorganisms such as E. coli, Salmonella spp., and Staphylococci, showing unsatisfactory hygienic quality. High and variable bacterial loads result from variability in production, collection, and processing practices. These products imply risks to consumers’ health. This is the first study...
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