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Hygienic quality of "Rayeb", a traditional Tunisian fermented cow's milk

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Abstract

A total number of 60 samples from traditional fermented milk "Rayeb" was randomly collected from different localities in Sfax governorate, Tunisia and subjected to determination of pH, sensory evaluation and determination of total yeast and mold count, Coliforms count, total staphylococcal count, *Staphylococcus aureus* count and detection of *Salmonella*. The obtained results revealed that pH was ranged from 3.8 to 4.7, 40 out of 60 samples (66.67 %) had the grade good in sensory evaluation, the mean yeast and mold count was $1.34\times10^{7}\pm$ 2262054 CFU/mL, the mean coliforms count was 3.707×10^{3} ± 1510 CFU/mL. the mean count of total staphylococci was 5.10×10^{3} ± 1257 CFU/mL, the mean *S. aureus* count was 68.4 ± 13 CFU/mL. The incidence of pathogens in fermented foods, as highlighted in this study suggests that measures that minimize the risk of food-borne illnesses should be taken. The application of Hazard Analysis and Critical Control Point (HACCP) as advocated by the WHO should be applied to a wide range of fermented foods in addition to other measures such as use of starter cultures, legislation and educating handlers who prepare such food.

Introduction

Traditional fermented milk products are widely consumed in the entire world. These products are an important supplement to the local diet and provide vital elements for growth, good health (Al-Otaibi, 2009; Uccello *et al*., 2012) and an appreciate flavor. Microorganisms of lactic acid starter cultures used for the conversion and preservation of milk by-products are unique bio-converters of energy. When they are used appropriately, these cultures elaborate specific metabolites during fermentation, these metabolites, in conjunction with partial hydrolysis of milk constituents (proteins, fat and lactose) contribute to better digestibility of the fermented food and their nutritional and therapeutic qualities also are enhanced (Sellar, 1981; Iannitti and Palmieri, 2010). Fortunately, the dominant bacteria in fermented milks are progressive type, lactic acid streptococci and lactobacilli, which generally suppress the spoilage and pathogenic organisms very effectively (Kosikowski, 1982). In the earlier days, fermentation was used to control the growth of harmful bacteria and some pathogens while making indigenous milk products.

Rayeb is a traditional curdled dairy product that has been known and highly appreciated by consumers for centuries, in Tunisia and some Arab countries. It is produced by spontaneous fermentation of cow's milk. It can be consumed as a fresh beverage or accompanied with some foods such as bread and couscous. Some studies reported by Benkerroum and Tamine (2004) and Samet-Bali and Attia (2012) have shown that Rayeb can be considered as a product with important nutritional value (total nitrogen: 32.81±0.45 $g.Kg^{-1}$, caseins: 26.11±0.61 $g.Kg^{-1}$, fat: 34.10±0.66 $g.Kg^{-1}$ and lactose: 30.60±0.38 $g.Kg^{-1}$). Recently, Rayeb is produced in Tunisia using industrial manufacturing practice in order to obtain safe fermented milk and to provide the product with standard characteristics. Nevertheless, consumers prefer the traditional product due to its organoleptic quality (fresh taste and characteristics aroma).

Rayeb is a product highly consumed in our region. Since it is traditionally made, the risk that it is contaminated with pathogenic microflora is very high. Therefore the objective of this study was to evaluate the hygienic quality of traditional Tunisian fermented milk « Rayeb ».

Materials and Methods

Fermented milk

"Rayeb" preparationRaw milk from cow (Holstein breed) was left spontaneously at 25 ± 2°C for coagulation, requiring up to 18 h. After gelation, the product was called "Rayeb". Rayeb microflora is composed of stable associations of LAB (*Leuconostoc*

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and *Lactococcus* genus) and yeasts, in particular due to metabolic interactions.

Sample analysis

Sixty samples of fermented cow's milk "Rayeb" were randomly collected from different dairy shops in different areas in Sfax governorate, Tunisia. Samples traditionally made, were collected under possible aseptic conditions in sterile airtight sampling jars and transferred to the laboratory in an insulated ice-box without delay to be immediately examined for determination of pH, total yeast and mold count, coliform count, *Staphylococcus aureus* count and detection of *Salmonella* according to Bradley *et al.* (1992).

Sensory analysis was conducted by 36 panelists, who were experienced in sensory evaluation of foods, but received no specific training relevant to the product. Panelists were asked to indicate how much they liked or disliked the product (fair, good, very good or excellent) according to Tamime and Robinson (1988).

Statistical analysis

The data were analyzed by using Graph pad prism computer program prepared by Graph pad software Inc. USA (Motulsky, 1999).

Results and Discussion

Results revealed that the pH was ranged between 3.8 and 4.7 with a mean of 3.94 ± 0.05. Although pH lower than 4.0 is considered inhibitory to the vegetative cells of pathogenic microorganisms, but also, it considered promising to growth of yeasts and molds. Nearly similar results were found in the literature (Adebesin *et al*., 2001; Abdel-Rahman *et al*., 2009). However, higher values were also recorded (Beukes *et al*., 2001). Fermented foods are normally considered to be safe against food-borne diseases because of their low pH (Gadaga *et al*., 2004). The pH in lactic acid fermented foods is usually reduced to less than 4 and this is usually sufficient to suppress the growth of most food-borne pathogens (Kingamkono *et al*., 1994).

The analytical grades of sensory evaluation of the examined samples revealed that 40 out of 60 (66.67 %) were good, 18 out of 60 (30%) were very good and only two samples (3.33%) was fair, while no sample was excellent. However, higher results were reported by Al-Otaibi (2009). Off flavor of fermented milks may be caused by contaminating microorganisms. The off-flavors may be characterized as yeasty, fruity, musty, cheesy or bitter and occasionally, soapy-rancid. A flavor threshold is generally reached at a count of about 10^4 yeasts and moulds / ml (Walstra *et al.*, 1999).

Table 1. The incidence of different microorganisms in the traditional fermented milk "Rayeb" samples

	Yeast & mold	Coliform	Total staphylococci	*S. aureus*
No. of positive	60	40	24	8
%	100	66.66	40	13.33

Table 2. Microbial counts (CFU/mL) in the traditional fermented milk "Rayeb" samples

	Yeast & mold	Coliform	Total staphylococci	*S. aureus*
Minimum	1.8×10^3	0.2	0	0
Maximum	10^8	1.1×10^5	2.7×10^4	4.6×10^2
Mean	1.34×10^7	3.707×10^3	5.10×10^3	68.4
± SEM	2262054	1510	1257	13

Results presented in Table 1 revealed that yeast and mold were recovered from all of the examined samples. However, lower results were reported by Al-Otaibi (2009). Coliforms were present in 66.66% of the examined samples; despite, lower results were recorded by Marthara *et al.* (2004) and Al-Otaibi (2009). Staphylococci were present in 40% of the examined samples, while *S. aureus* was recovered from 13.33%. Higher results were reported by Bendahou *et al*. (2008).

Results presented in Table 2 revealed that the total yeast and mold count ranged from 1.8×10^3 to 10^8 CFU/mL; with mean count of 1.34×10^7 ± 2262054 CFU/mL. Nearly similar results were previously reported (Adebesin *et al*., 2001; Olasupo *et al.*, 2002; Uzeh *et al*., 2006). However, lower results were recorded by Lore *et al*. (2005), Abdel-Rahman *et al.* (2009) and Al-Otaibi (2009). In the current study, the mean coliforms count was 3.707×10^3 ± 1510 CFU/mL, with minimum of 0.2 and maximum of 1.1×10^5 CFU/mL. Similar results were found by Adebesin *et al*. (2001). However, lower results were recorded by Lore *et al*. (2005) and Al-Otaibi (2009) and higher results were recorded by Uzeh *et al.* (2006).

The mean count of total staphylococci was 5.10×10^3 ± 1257 CFU/mL, with a minimum of 0 and a maximum of 2.7×10^4 CFU/mL. Nearly similar results were found by Olasupo *et al.* (2002). The present mean S. aureus count was 68.4 ± 13 CFU/mL, with a minimum of 0 and a maximum of 4.6×10^2 CFU/mL.

Similar results were reported by Abdel-Rahman *et al*. (2009). *Salmonella* organisms were not detected in all the examined samples of fermented skimmed milk. Similar results were recorded by Beukes et al. (2001) and Abdel-Rahman *et al.* (2009).

The presence of staphylococci in high count is a potential health hazard as this high count furniture the presence of enterotoxigenic strains, lastly the presence of enterotoxigenic strains in food does not always necessarily mean that the toxin will be produced, but it demonstrates the need for proper examination by public health inspectors of the production, storage and commercialization of milk and other products made with unpasteurized milk.

The results obtained from analysis of fermented milk samples showed that they are contaminated with microorganisms of public health concern. The high coliforms count may be a consequence of the low level of hygiene maintained during the processing and sale of this product. This includes the handlers and the utensils. Exposure of the product while it is displayed for sale in bowls can serve as source of contamination. The presence of pathogenic microorganisms in milk and dairy products during different processing procedures indicated the lower standards of hygiene in the selected dairy process. The presence of coliforms bacteria, furthermore, the presence of *Escherichia coli* and *Staphylococcus aureus* in some of the products emphasized the importance of production hygiene during manufacturing of dairy products in small-scale operations (Beukes, 1999). Preparation procedures for most products are still traditional arts and the fermentation is uncontrolled starter cultures are not normally used therefore variations in the quality and stability of the products is often observed. The technological and hygienic problems of traditional fermented foods need to be addressed in order to reduce losses due to wasteful and insufficient fermentation pathways, poor quality and unstable shelf-life of products (Odunfa and Oyewelo, 1998).

Initial levels of contamination with pathogens in traditional fermented milk are critical for the safety of fermented foods. Minimizing contamination of the raw materials is therefore another way of controlling pathogen levels in the final product. Measures should be taken to interrupt the transmission of pathogens to fermented foods at both the household and commercial levels. At the commercial level, improvement of product quality and safety could be achieved by applying Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP) and the Hazard Analysis and Critical Control Point (HACCP) system, attempts to provide HACCP guidelines for some traditional fermented foods. However, educating food handlers, particularly mothers and food vendors, the food hygiene is a strategy that can be used in efforts aimed at to preventing food-borne diseases.

In order to obtain safe fermented milk "rayeb", it is therefore critically important to ensure high quality raw milk can be produced from healthy animals under good hygienic conditions. The equipment, tools and conditions under which milk is produced must fulfill certain minimum requirements. In fact, the milking equipment should be cleaned as soon as possible after milking, the cattle housing should be clean and airy. Furthermore, rayeb storage conditions should be designed to provide an environment which minimizes the deterioration and contamination of rayeb (e.g. by temperature and humidity control).

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