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Raw milk from vending machines: Effects of boiling, microwave treatment, and refrigeration on microbiological quality

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ABSTRACT

In Italy, the sale of raw milk from vending machines has been allowed since 2004. Boiling treatment before its use is mandatory for the consumer, because the raw milk could be an important source of foodborne pathogens. This study fits into this context with the aim to evaluate the microbiological quality of 30 raw milk samples periodically collected (March 2013 to July 2013) from 3 vending machines located in Molise, a region of southern Italy. Milk samples were stored for 72 h at 4°C and then subjected to different treatments, such as boiling and microwaving, to simulate domestic handling. The results show that all the raw milk samples examined immediately after their collection were affected by high microbial loads, with values very close to or even greater than those acceptable by Italian law. The microbial populations increased during refrigeration, reaching after 72 h values of about 8.0 log cfu/mL for *Pseudomonas* spp., 6.5 log cfu/mL for yeasts, and up to 4.0 log cfu/mL for *Enterobacteriaceae*. Boiling treatment, applied after 72 h to refrigerated milk samples, caused complete decontamination, but negatively affected the nutritional quality of the milk, as demonstrated by a drastic reduction of whey proteins. The microwave treatment at 900 W for 75 s produced microbiological decontamination similar to that of boiling, preserving the content in whey proteins of milk. The microbiological characteristics of raw milk observed in this study fully justify the obligation to boil the raw milk from vending machines before consumption. However, this study also showed that domestic boiling causes a drastic reduction in the nutritional value of milk. Microwave treatment could represent a good alternative to boiling, on the condition that the process variables are standardized for safe domestic application.

Key words: unpasteurized raw milk, food safety, boiling, microwave treatment

INTRODUCTION

In Italy, as well as in different industrialized countries, raw milk consumption is becoming rather popular, thanks to the current trend toward "consuming natural" and "purchasing locally." Enhanced nutritional qualities, taste, and health benefits are advocated as reasons for the increased interest in raw milk consumption (Oliver et al., 2009). Raw milk supporters claim the suitability of unpasteurized milk for the treatment or for the prevention of some diseases, even if no scientific evidence supports this notion (Oliver et al., 2005). Only few epidemiological studies suggest that early-life exposure to unpasteurized milk could reduce the risk for developing asthma, allergic rhinitis, hay fever, pollen allergy, and atopic sensitization (Barnes et al., 2001; Waser et al., 2007; Loss et al., 2011).

It is a widespread belief that heating destroys the nutritional and health benefits of milk, but it is as important to underscore that raw milk can harbor a variety of microorganisms and it could be an important source of foodborne pathogens, such as *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, and *Listeria monocytogenes*, among others (Hill et al., 2012; Claeys et al., 2013; Serraino et al., 2013). The presence of pathogenic bacteria in raw milk is well documented: in Italy, several studies on raw milk collected from vending machines detected the presence of different pathogens, such as *Salmonella* spp., *E. coli* O157:H7, *Campylobacter* spp. and *L. monocytogenes* (Giacometti et al., 2012a; Bianchi et al., 2013; Serraino et al., 2013); in the United States and in Ireland, some cases of listeriosis due to the consumption of raw milk were recently described (Latorre et al., 2011; Hunt et al., 2012); in Canada, an outbreak due to the presence of *E. coli* in dairy products made from raw milk was reported (Gaulin et al., 2012).

In Italy, the sale of raw milk through vending machines has been allowed since 2004. Specific norms for both milk quality and characteristics of vending ma-

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chines have been imposed (EU, 2004; Italian Republic, 2007). Moreover, the Italian law imposed the restriction on users to store raw milk at 4°C for a maximum period of 72 h. After several episodes of hemolytic-uremic syndrome in children associated with the consumption of raw milk, the Italian Ministry of Health (2009) published the ordinance that obliges the posting on vending machines of the prominent notice "Milk must be boiled before consumption." Moreover, in the light of the latest scientific evidence (Scavia et al., 2009), in 2013, the Italian Ministry of Health reiterated the need to consume the raw milk after boiling.

In a previous study, Sorrentino et al. (2012) highlighted that refrigeration applied to raw milk during storage in vending machines is not a guarantor of microbiological safety, as some contaminating species are able to multiply at low temperatures. Furthermore, improper handling of milk by consumers may further increase the risk of contamination; in fact, Giacometti et al. (2012b) reported that 82% of consumers do not use insulated bags to transport raw milk home and the transport time often exceeds 30 min.

Boiling, as imposed by the Italian Ministry of Health (2009, 2013), remains the sole method to ensure the safety of milk. However, the responsibility of hygienic sanitation of raw milk is actually given to the consumer, who often underestimates or completely ignores the risk associated with raw milk consumption (D'Ascenzi et al., 2010; Giacometti et al., 2012b).

This study fit into this context with different aims: to evaluate the microbiological quality and safety of raw milk collected from different vending machines located in the Molise region, to study the quantitative variations in microbial populations of raw milk during storage at 4°C for 72 h, and to assess the effect of a domestic boiling process and of different microwave treatments on the safety and the quality of milk.

MATERIALS AND METHODS

Experimental Design and Milk Treatments

Raw milk samples were aseptically collected from March 2013 to July 2013 in 3 different vending machines located in the Molise Region (Southern Italy). Each month, 2 different samples were collected from each vending machine, and a total of 30 raw milk samples were obtained. At the time of sampling, the temperature of the milk was measured. All the samples were placed in a cool box, stored at 4.0 ± 0.5°C until delivery to the laboratory (Dipartimento di Agricoltura, Ambiente e Alimenti, University of Molise, Campobasso, Italy), and analyzed within 2 h after sampling. The samples from each vending machine, collected in different periods, were divided into 10 batches:

- C_0: untreated raw milk subjected to analysis immediately after its delivery to the laboratory;
- C_{24}: untreated raw milk subjected to analysis after 24 h of storage at 4.0 ± 0.5°C;
- C_{48}: untreated raw milk subjected to analysis after 48 h of storage at 4.0 ± 0.5°C;
- C_{72}: untreated raw milk subjected to analysis after 72 h of storage at 4.0 ± 0.5°C;
- B_0: raw milk subjected to a simulated domestic boiling treatment immediately after its delivery to the laboratory;
- B_{24}: raw milk stored at 4.0 ± 0.5°C for 24 h and then subjected to a simulated domestic boiling treatment;
- B_{48}: raw milk stored at 4.0 ± 0.5°C for 48 h and then subjected to a simulated domestic boiling treatment;
- B_{72}: raw milk stored at 4.0 ± 0.5°C for 72 h and then subjected to a simulated domestic boiling treatment;
- M_{750}: raw milk subjected to a simulated domestic microwave treatment at 750 W for 75 s immediately after its delivery to the laboratory;
- M_{900}: raw milk subjected to a simulated domestic microwave treatment at 900 W for 75 s immediately after its delivery to the laboratory.

To simulate a domestic boiling treatment, 200 mL (approximately a capful) of raw milk from the B batches was poured into a small, previously sterilized pan and heated until the appearance of foam. The treatment was immediately stopped, and analyses were carried out within 15 min. During boiling, the milk temperature was monitored using a digital thermometer (Delta Ohm S.r.L., Padua, Italy).

To simulate domestic microwave treatment, 200 mL (approximately a capful) of raw milk from the M batches was poured into a sterilized 300-mL Pyrex beaker and the treatment was performed by using a domestic microwave oven with 950-W nominal output at 2,450 MHz (Whirlpool FT380; Whirlpool Europe Srl, Comerio, Italy). Two different programs were used: 750 W for 75 s and 900 W for 75 s. Immediately after treatments, the milk temperature was monitored as described above.

Microbiological Analyses

For microbial counts, raw and treated milk samples from each batch were serially diluted using 0.1% peptone water. The different microbial groups were detected on specific media and incubation temperatures. In detail, total mesophilic count (**TMC**) was performed on plate count agar (Oxoid Ltd., Basingstoke, UK) incubated