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Microbiological safety and quality of Mozzarella cheese assessed by the microbiological survey method

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ABSTRACT

Dairy products are characterized by reduced shelf life because they are an excellent growth medium for a wide range of microorganisms. For this reason, it is important to monitor the microbiological quality of dairy products and, in particular, the total viable count and concentration of *Escherichia coli*, as they are indicators of the hygienic state of these products. In addition, in dairy products such as Mozzarella cheese, it is important to monitor the concentration of lactic acid bacteria (LAB), as they are the major components of starter cultures used in cheese production, contributing to the taste and texture of fermented products and inhibiting food spoilage bacteria by producing growth-inhibiting substances. For these reasons, to ensure the quality and safety of their products, cheese makers should monitor frequently, during fresh cheese production, the concentration of LAB and spoilage bacteria. However, usually, small- to medium-size dairy factories do not have an internal microbiological laboratory and external laboratories of analysis are often too expensive and require several days for the results. Compared with traditional methods, the microbiological survey (MBS) method developed by Roma Tre University (Rome, Italy) allows faster and less-expensive microbiological analyses to be conducted wherever they are necessary, without the need for a microbiological laboratory or any instrumentation other than MBS vials and a thermostat. In this paper, we report the primary validation of the MBS method to monitor LAB concentration in Mozzarella cheese and the analysis, using the MBS method, of total viable count, *E. coli*, and LAB concentrations in the production line of Mozzarella cheese as well as during the shelf life of the product stored at 20°C. The results obtained indicate that the MBS method may be

successfully used by small- to medium-size dairy factories that do not have an internal microbiological laboratory. Using the MBS method, these dairy factories can monitor autonomously the microbiological safety and quality of their products, saving both time and money. **Key words:** Mozzarella cheese, lactic acid bacteria, microbiological analysis

INTRODUCTION

Microbial contamination, causing approximately one-fourth of the world's food supply loss, has become an enormous economic and ethical problem worldwide (Huis in 't Veld, 1998). Dairy products are an excellent growth medium for a wide range of microorganisms and, thus, display a reduced shelf life (Ruegg, 2003). The microbiological quality of dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatments. Spoilage bacteria and various bacteria of public health concern can be found in these products and their concentrations should be kept as low as possible (Varga, 2007). In contrast, lactic acid bacteria (**LAB**), occurring in the indigenous microflora of raw milk and being the major components of starter cultures used in fermentation, contribute to the quality of fermented cheese products by improving the taste and texture and inhibiting food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Jana and Mandal, 2011). Thus, to be confident of fermented cheese quality, LAB concentration should be monitored during cheese production.

Traditional Mozzarella cheese made from water buffalo milk is one of the most highly valued unripened pasta filata cheeses in Italy, certificated with the European Protected Designation of Origin. Although mainly produced in Italy, it is widely exported and it is also industrially produced in other countries. The specific taste characteristics of Mozzarella cheese mainly arise from the raw milk used, the area of production, the environmental conditions, the traditional tools, and

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manufacturers (Kindstedt and Fox, 1993; Mauriello et al., 2003). Mozzarella cheese belongs to the category of stretched curd, or pasta filata cheeses. Pasta filata cheeses are distinguished by a unique plasticizing and kneading treatment of the fresh curd in hot water, which imparts to the finished product its characteristic fibrous structure and melting and stretching properties (Mijan et al., 2010). As for other typical cheeses, its manufacture and composition is regulated by law (DPR 54/97, DPR, 1997; Reg. EC 2527/98; EC, 1998). Traditionally, water buffalo Mozzarella cheese is manufactured from raw milk using natural whey cultures as starters. Starter cultures fulfill several important functions in cheese making: acid production from lactose, inhibition of spoilage and pathogenic microorganisms, improvement of cheese-keeping quality, and direct and indirect contributions to flavor and aroma (Coppola et al., 1990). In Mozzarella cheese, the main function of starter cultures is to ensure rapid acidification of the curd, which promotes the transformation of dicalcium paracasein into monocalcium paracasein during stretching in hot water. The amount and variety of microorganisms in the starter depend on the technological process and, in particular, by the use of natural whey starters (Suzzi et al., 2000).

Lactic acid bacteria are the major component of the starters used in fermentation, and some of them are also natural components of the gastrointestinal microflora (Coeuret et al., 2003). The term LAB is conventionally reserved for genera in the order *Lactobacillales*, which includes the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*, in addition to *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Mack et al., 1999). Lactic acid bacteria are gram-positive, nonspore-forming cocci, coccobacilli, or rods. They generally are anaerobes, lack catalase, and ferment glucose primarily to lactic acid, or to CO₂ and ethanol (Coeuret et al., 2003). Lactic acid bacteria play an important role in the production and conservation of fermented foods, especially in the dairy industry (Loones, 1994; Moreira et al., 2000; Luquet and Corrieu, 2005). As starter cultures, LAB contribute to the development of the physical properties of cheese, particularly body and texture. The use of starter cultures that display different proteolytic characteristics can modify the stretch, melt, and color of Mozzarella cheese (Oberg et al., 1991). In addition, LAB produce several metabolic products, such as organic acids, FA, hydrogen peroxide, and bacteriocins, which have antimicrobial activity, resulting in the growth inhibition of spoilage and pathogenic bacteria and in the improvement of quality and shelf life of the product (Osman Mohamed Abdalla and Nouredin Mohammed Ibrahim, 2010).

The evaluation of the microbiological quality and safety of Mozzarella can be carried out through the evaluation of 3 parameters: total viable count (TVC), the number of *Escherichia coli*, and the number of LAB. The TVC gives a quantitative idea of the presence of mesophilic aerobic microorganisms of animal origin. It serves as an important criterion to evaluate the microbial quality of various foods and also the degree of freshness of food (Nanu et al., 2007). *Escherichia coli* are commensal organisms that reside within the host gut, but some pathogenic strains are recognized as a cause of gastroenteritis (Callaway et al., 2003). Contamination from human and animal waste is traditionally indicated by the presence of commensal *E. coli*. Although these organisms are essentially nonpathogenic, their presence warns of the possible concurrent existence of pathogenic microbes (Sherfi et al., 2006). Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs requires *E. coli* quantification as an indicator of the level of hygiene. In cheeses made from milk or whey that have undergone heat treatment, *E. coli* content is unsatisfactory at >1,000 cfu/g. In addition, Regulation (EC) No. 2527/98 of 25 November 1998, concerning the characteristics of Mozzarella, as per Regulation (EC) No. 2515/94, requires for Mozzarella cheese, the presence of “typical microflora resistant to curd stretching, in an amount of not less than 10⁷ cfu/g in samples analyzed within three days after the date of production” (EC, 1998).

The microbiological quality of Mozzarella cheese is assessed by quarterly controls performed by external laboratories on samples chosen randomly in the production lines in Mozzarella cheese manufacture. The analyses concern the presence of pathogenic and contaminating bacteria, but are still insufficient to guarantee the constant microbiological control needed for a particular product such as Mozzarella. In this case in fact, it would be important to assess the microbiological quality before the product is sold and consumed to allow rapid intervention. Analysis should, therefore, be rapid, reliable, economical, and executable by the personnel without relying on external laboratories.

In this context, MBS S.r.l. (a spin-off of Roma Tre University, Rome, Italy) has developed an alternative method, called the microbiological survey (MBS) method. It is a fast colorimetric system for the detection and the selective count of bacteria present in agro-food, water, and environmental samples. This method consists of an analytical kit, using disposable, ready-to-use reaction vials for fast microbiological analyses. The analysis is based on the color change of the vial content, which is induced by the presence of bacteria. The analyses can be carried out by untrained personnel and wherever analyses are necessary, without the need for

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