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# Inactivation of *Mycobacterium paratuberculosis* and *Mycobacterium tuberculosis* in fresh soft cheese by gamma radiation

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ABSTRACT

The effectiveness of gamma irradiation on the inactivation of *Mycobacterium paratuberculosis, Mycobacterium bovis* and *Mycobacterium tuberculosis* in fresh soft cheese that prepared from artificially inoculated milk samples was studied. Irradiation at dose of 2 kGy was sufficient for the complete inactivation of these mycobacteria as they were not detected in the treated samples during storage at $4 \pm 1$ °C for 15 days. Moreover, irradiation of cheese samples, that were prepared from un-inoculated milk, at this effective dose had no significant effects on their gross composition and contents from riboflavin, niacin and pantothenic acid, while significant decreases in vitamin A and thiamin were observed. In addition, irradiation of cheese samples had no significant effects on their pH and nitrogen fractions contents, except for the contents of ammonia, which showed a slight, but significant, increases due to irradiation. The analysis of cheese fats indicated that irradiation treatment induced significant increase in their oxidation parameters and contents from free fatty acids; however, the observed increases were relatively low. On the other hand, irradiation of cheese samples induced no significant alterations on their sensory properties. Thus, irradiation dose of 2 kGy can be effectively applied to ensure the safety of soft cheese with regards to these harmful mycobacteria.



## 1. Introduction

The epidemiology of foodborne diseases has been changed as new pathogens have emerged. This led to increase in the awareness of risks involving microbiological contamination of food (Adams and Moss, 2008; Loaharanu, 2001). Mycobacterial diseases, caused by *Mycobacterium paratuberculosis*, *Mycobacterium bovis* and Mycobacterium tuberculosis, are a significantly reemerging infectious diseases of great health concern in many parts of the world (Käser et al., 2009). *M. paratuberculosis*, also known as *M. avium* subsp. *paratuberculosis*, represents a real threat to the agriculture and dairy food industries and believed to be a potential public health problem for susceptible humans (Shanahan, 2007). The organism causes paratuberculosis, commonly known as Johne's disease, in cattle, sheep, goats, and other ruminants (Cocito et al., 1994) and has been reported having a possible etiological role in Crohn's disease, a human gastrointestinal disorder characterized by severe abdominal pain, diarrhea, bleeding, bowel obstruction and a variety of systemic symptoms that can impede the ability to lead a normal life during chronic episodes that span from months to years (Nacy and Buckley, 2008).

Public health concerns about the presence of *M. paratuberculosis* in milk and dairy products are increasing due to increased accumulating of data that link this bacterium to Crohn's disease (Grant et al., 2002; Hruska, 2004; Hermon-Taylor and Bull, 2002). *M .paratuberculosis* has been isolated from milk samples obtained from cows with clinical Johne's disease as well as from clinically healthy animals and is likely to be present in most bulk milk (Ayele et al., 2005; Cocito et al., 1994; Donaghy et al., 2004; O'Reilly et al., 2004; Streeter et al., 1995; Sweeney et al., 1992).The most interesting is that the organism has been shown to survive the standard pasteurization of milk, including high-temperature short-time pasteurization in both artificially and normally infected cows' milk, in addition to the presence of viable *M. paratuberculosis* in commercially pasteurized cows' milk manufactured for retail sale (Ayele et al., 2005; Chiodini and Hermon-Tylor, 1993; Grant et al., 2002, 2005, 1998; Keswani and Frank, 1998; Millar et al., 1996; Sung and Collins, 1998). Furthermore, it was found to be present in retail cheeses (Ikonomopoulos et al., 2005) and will be able to survive the low temperature used in manufacturing of many cheeses as well as the ripening time up to consumption in both Swiss semi hard (Skovgaard, 2007; Spahr and Schafroth, 2001). The bacterium is more resistant to adverse conditions such as salt and low pH than most other pathogenic bacteria (Sung and Collins, 2000).

More importantly, tuberculosis remains the greatest cause of mortality and represents a major public health problem, especially in developing countries, now compounded by the rising

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incidence of multi-drug resistant tuberculosis and the human immunodeficiency virus pandemic (Affolabi et al., 2008; LaBombardi, 2009). One third of the world's population is infected with *M. tuberculosis* and about 9 million tuberculosis cases were diagnosed worldwide in 2006 (WHO, 2008). Human tuberculosis is caused mainly by *M. tuberculosis*. However, *M. bovis*, the etiological agent of bovine tuberculosis, is also an important zoonotic bacterium that affect humans and constitutes a public health problem in a large number of countries (Ayele et al., 2004; Biet et al., 2005; CDC, 2005; Harris et al., 2007; Humblet et al., 2009; Rodwell et al., 2008). The association of tuberculosis with young people makes this disease an important factor in the economy of many countries. The majority of tuberculosis patients are 15–45 years of age, the most economically productive sector of economy (Buu et al., 2009; Enarson, 2003; WHO, 1998).

Although respiratory route is considered the most important for transmission of tuberculosis bacilli; the alimentary route of infection plays an important role in spreading this disease through the consumption of contaminated milk or dairy products (Griffin and Dolan, 1995; Pardo et al., 2001; Thoen et al., 2006). Raw milk is inherently dangerous and may contain a whole host of pathogens including *M. paratuberculosis*, *M. bovis* and *M. tuberculosis*. Historically, tuberculosis was one of the most serious human diseases disseminated by the consumption of contaminated raw milk (Adams and Moss 2008; Boor and Murphy, 2002). Several studies supported the participation of milk and dairy products in the transmission of mycotuberculosis. Human and bovine mycobacteria were observed in milk collected from dairy herds (Appuswamy et al., 1980; Batish et al.,1989) and isolated from the milk of clinically normal cows (Batish et al.,1989) and from bulk milk tanks (Guindi et al., 1980).The epidemiological investigations indicated that the consumption of non-pasteurized dairy products, including fresh cheese, may have accounted for an elevated incidence of human tuberculosis due to *M. bovis* (CDC, 2005) and recent outbreaks of human tuberculosis by *M. bovis* have implicated cheese as a source of the infections (Harris et al., 2007).

The recovery of these mycobacteria from fresh cheese suggests that human infection through the consumption of dairy products is possible and support the epidemiological conclusions from recent outbreaks that milk products may serve as a reservoir for mycobacteria transmission to human populations. Whether mycobacteria are not effectively inactivated by pasteurization or recontaminate the final dairy products, there is a possibility that these bacteria may exist in a viable form in cheese especially when manufactured from raw milk. Thus a precautionary approach is very important and treatment aimed at elimination of mycobacteria from cheese products is a very high priority for dairy industry and should be encouraged.

Irradiation with ionizing energy is very effective in killing many of the common microbial pathogens that are significant contributors to foodborne illness. A major advantage of irradiation for this purpose is that the food can be processed after it has been sealed in its final packaging, thereby reducing or entirely eliminating the possibility of recontamination following irradiation (Borsa, 2006). Food irradiation has emerged as a safe and viable technology for ensuring the safety and quality of food. It is approved for use in over 55 countries worldwide for various applications and purposes in a wide variety of foodstuffs including cheese (Loaharanu, 2001; Chauhan et al., 2008; IAEA, 2009).Therefore, the present work aims to investigate the effectiveness of low gamma irradiation doses in the inactivation of *M. paratuberculosis*, *M. bovis and M. tuberculosis* in fresh soft cheese samples that are prepared from milk inoculated with these mycobacteria.

## 2. Materials and methods

### 2.1. Safety

All works with mycobacteria under investigation in this study were carried out with safety precautions normally used in microbiological laboratories (CDC, 2007; WHO, 1998).

### 2.2. Mycobacterial cultures

#### 2.2.1. M. paratuberculosis

*M. paratuberculosis* was isolated from one of a total of 146 cows' fecal samples (collected from several private farms in El-Sharkia Governorate, Egypt) by sedimentation processing (Whipple et al., 1991). The sediment was used to inoculate Herrold's egg yolk medium (HEYM) slants with and without mycobactin J to asses the mycobactin J dependence commonly associated with *M. paratuberculosis*. The slants were monitored weekly for 16 weeks of incubation at 37 °C. The positive culture showed colonies in the HEYM slants with mycobactin J, while the slant without mycobactin J had no growth (as will be illustrated later), was examined microscopically by using Ziehl–Neelsen stain and used in this study.

#### 2.2.2. M. tuberculosis

A confirmed culture of acid-fast *M.tuberculosis* was obtained from a clinical microbiology laboratory, which was isolated from an acid-fast positive clinical respiratory sputum collected from a patient and cultured on Löewnstein–Jensen slants with glycerol.

#### 2.2.3. M. bovis

*M. bovis* was isolated from milk samples of bovine tuberculosis infected dairy cattle. Milk samples of 50 ml were centrifuged at 2500*g* for 15 min, and the pellet was resuspended in 10 ml of 0.75 (w/v) hexadecylpyridinium chloride for decontamination and centrifuged as above following incubation at room temperature for 5 h. The decontaminated sediment was used for the inoculation of Löewnstein–Jensen slants containing pyruvate and others containing glycerol for confirmation, then the slants were incubated at 37 °C for 8 weeks.

### 2.3. Preparation of mycobacterial inoculum

The microbiological experiments with each of mycobacteria under investigation were carried out separately. The preparation of mycobacterium inoculum was carried out according to Pfyffer et al. (2003). Cells of mycobacterium were washed from slopes of the cultured medium with 10 ml of phosphate-buffered saline (PBS), centrifuged at 2500*g* for 20 min and resuspended in PBS. Standard suspensions containing approximately $10^9$ mycobacterium cells/ml (determined spectrophotometrically) were observed.

### 2.4. Inoculation of milk and cheese making

Four cheese making separate experiments (three different replicate trials within each experiment) were carried out in this investigation to be used in the mycobacterial studies (three experiments) and chemical and sensory studies (one experiment). For mycobacterial studies, cows' raw milk was heat treated at 75 °C for 5 min and cooled to 35 °C. Appropriate aliquots of the obtained mycobacterium suspension were added to milk with good mixing to yield inoculated milk containing $\sim 10^7$ cells of mycobacteria/ml . The salt was then added at rate of 3% (w/w) to milk followed by renneting using commercial animal rennet. The curd was cut into small pieces and carefully transferred to a