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EFFECT OF ELECTRON BEAM IRRADIATION ON SURVIVAL OF *BRUCELLA* SPP. IN TRADITIONAL ICE CREAM

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ABSTRACT

Irradiation is a new technology which can be used for foods especially for the ones which common methods such as thermal method cannot be applied. Traditional ice cream samples were purchased from a local market. After applying a 15 kGy dose for sterilization, *Brucella abortus* and *Brucella melitensis* were inoculated to samples and then treated with four doses of 1, 2, 3 and 5 kGy electron beam irradiation at −18C storage temperature. Microbial examinations were performed in 3, 7, 14 and 21 days after treatment with electron beam irradiation. Results showed that no bacteria were observed in the initial test after use of 5 kGy dose. Applying 2 and 3 kGy reduced significantly ($P < 0.05$) the microbial population but could not eliminate it completely. This study confirmed that electron beam irradiation is a reliable way to reduce microbial population of *Brucella* spp. in traditional ice cream and therefore improve food safety.

PRACTICAL APPLICATIONS

To maintain palatability of traditional ice cream, milk must not be too heated or else pasteurization is not achieved. So, electron beam irradiation could ensure the safety of ice cream, instead of heat treatment.

INTRODUCTION

Ice cream is a popular frozen dairy product which is identified as a high-risk food for consumers due to neutral pH, nutrient composition and long storage time (Lee *et al.* 2009). The risk of contamination with pathogens in industrial ice cream is lower than handmade ones because of some processes such as milk pasteurization, freezing and proper sanitary conditions at all stages from production to distribution. This becomes more important when the ice cream is manufactured in nonstandard, poor hygienic conditions with raw material in local shops and is sold as a traditional product (Kuplulu and Sarimehmetoglu 2004).

The most common techniques that are used to reduce the microbial population in food industry are thermal pasteurization and sterilization (Piyasena *et al.* 2003). But in the case of traditional ice cream, there is no possibility to apply the mentioned methods, especially in Iranian traditional ice cream where producers use raw or nonpasteurized milk; thus, it should be replaced by other reliable methods.

Irradiation is a physical treatment in which the food is exposed to a defined dose of ionizing radiation and is used on more than 60 food types in more than 55 countries worldwide. It can be used as novel method to ensure safety and quality of food (Badr 2011). This technique is used as an effective tool to eliminate pathogens present in foods (Aguirre *et al.* 2012), to reduce the spoilage, control microbial growth and delay or eliminate natural biological processes such as germination or sprouting and ripening in fresh food (Ioannis 2010a). Furthermore, irradiation can be used at the end of food processing, after packaging (Badr 2011; Roberts 2014). Irradiation can eliminate pathogens directly or indirectly with impact on DNA and production of free radicals (Kamat *et al.* 2000; Tahergorabi *et al.* 2012).

Three types of ionizing radiation are used in commercial radiation to process products, including Gamma rays, X-rays and accelerated electrons (Kim *et al.* 2010a). The two most widely used techniques of irradiating foods are gamma and electron beam radiation (Ioannis 2010b). Application of electron beam irradiation has been

emphasized as a successful technology in elimination of microbial contamination, disinfestation and improvement of the quality of foods and agricultural products (Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh 2011). Although there is a restriction in the use of electron beam irradiation due to its permeability limitation, it is preferred to gamma radiation because of its higher dose rate which requires less time to achieve pasteurization conditions (Farkas and Mohácsi-Farkas 2011; Li *et al.* 2015) with no need to use radioisotopes. Electron beam irradiation with high energy electrons using machine-generated electrons has significant impact on inactivation of some microorganism, with minimal thermal changes in products; thus, it is an efficient method to increase the shelf life of some different kinds of food (Ahn *et al.* 2013). Since the electron beam irradiation has no radioisotopes and minimal damaging effects on environment, it can be more accepted to use by people as an effective technology for destruction of some microorganisms (Li *et al.* 2015).

Brucellosis is one of the most common zoonotic diseases. In many countries, e.g., in Middle East and South America, the disease is known as an endemic disease (Seleem *et al.* 2010; Dasari *et al.* 2013). The disease in human and animals is caused by some species of *Brucella* including *B. abortus, B. melitensis, B. suis, B. canis, B. ovis* and *B. neotomae* (Godfroid *et al.* 2005). However, the two most prominent human pathogen species are *B. melitensis* and *B. abortus* (Franco *et al.* 2007), with the extreme virulence for humans caused by *B. melitensis* (Samaha 2008). There are several ways to transmit this pathogen to humans, such as direct contact with the infected animal, inhalation, ingestion of raw milk and unpasteurized dairy products, especially ice cream and fresh cheese (Kuplulu and Sarimehmetoglu 2004). The disease caused by these pathogens is called Malta fever or undulant fever (Sun and Zhang 2014).

Several studies confirm the effects of gamma radiation to destroy some pathogens in dairy products, particularly in ice cream (Kamat *et al.* 2000; Adeil Pietrana *et al.* 2003; Jo *et al.* 2007), but there are not enough evidence about the effectiveness of electron beam irradiation. The purpose of this study was to determine the effect of electron beam irradiation on the survival of *B. melitensis* and *B. abortus* in traditional ice cream.

MATERIALS AND METHODS

Sample Preparation

Traditional ice cream sample, which was made from raw milk, cream, sugar and some flavors such as vanilla and saffron, was purchased from an Iranian local market. During transportation to the laboratory, the samples were stored in a cool box containing dry ice and attempted to be maintained at −18C. Ten grams of ice cream samples were transferred to sterile polystyrene containers in a laboratory (cell culture container, $15 \times 8 \times 4$ cm^3) and stored in −18C, and at the same time irradiation and inoculation were being performed as well. For this experimental study, the samples were divided into two groups including control and treatment and then the treatment group was divided into four groups according to the irradiation dose.

Culture and Media

B. abortus and *B. melitensis* strains used for this study were prepared by the Faculty of Veterinary Medicine, Semnan University, Semnan, Iran. Strains of *Brucella* were cultured on sterile *Brucella* agar media (Merck, Darmstadt, Germany) containing 5% sheep blood and were incubated in a humid atmosphere under 10% CO_2 at 37C for 3 days. To ensure purity of strains, colonies of *Brucella* spp. were identified by using different biochemical tests such as H_2S and urease production, catalase activities and growth on media containing thionine and fuchsin dyes. Bacterial suspension equivalent to McFarland turbidity standard 0.5 was prepared in normal saline 0.9%, for inoculation of bacteria. The concentration of the bacterial suspension was approximately 1.5×10^8 cfu/mL.

Inoculation and Irradiation

The prepared sample (10 g) was exposed to 15 kGy of electron beam irradiation in order to inactivate the entire existing bacteria. By using sterile sampler, 1 mL bacterial suspension of each *Brucella* strains was inoculated into sterilized samples, and in order to unify the inoculation, samples were homogenized manually for 2 min. Then, the inoculated ice cream samples were irradiated with 1, 2, 3 and 5 kGy doses of electron beam irradiation at −18C using dry ice. Irradiation processes were performed at the Radiation Applications Research Center, Nuclear Science and Technology Research Institute, Yazd, Iran. In order to measure the absorbed dose of irradiated samples, the FWT dosimeter film (9F9-Far West Technology, Inc., Goleta, CA) and cellulose triacetate dosimeter film (4091611-L15, FujiFilm, Tokyo, Japan) with Fourier transform infrared spectroscopy spectrometer (8300 model, Shimadzu, Kyoto, Japan) were used. Irradiation was done by using electrons with energies from 10 MeV electron Rhodotron accelerator TT200 model (manufactured by IBA Industrial, Louvain-la-Neuve, Belgium). Finally, the samples were transferred to a laboratory for microbial analysis and the remaining samples stored at −18C immediately for subsequent tests.

TABLE 1. *BRUCELLA ABORTUS* COUNTS (CFU/G) IN TRADITIONAL ICE CREAM STORED AT –18C†

Irradiation dose (kGy)	1 day	3 days	7 days	14 days	21 days
0 (Control)	$2.8 \times 10^5 \pm 36.1$*,a	$2.4 \times 10^5 \pm 11.3^a$	$2.9 \times 10^5 \pm 25.2^a$	$2.2 \times 10^5 \pm 43.7^a$	$3.1 \times 10^5 \pm 60.1^a$
1	$5.5 \times 10^4 \pm 50^b$	$3.3 \times 10^4 \pm 57.3^b$	$2 \times 10^4 \pm 0^b$	$10^4 \pm 0^b$	$1.6 \times 10^3 \pm 5.3^b$
2	$3.8 \times 10^4 \pm 28.7^b$	$2.1 \times 10^4 \pm 28.8^b$	$1.7 \times 10^4 \pm 25.6^b$	NG^c	$3.3 \times 10^2 \pm 7.3^b$
3	$1 \times 10^4 \pm 11.5$	$10^4 \pm 0^b$	$6.6 \times 10^3 \pm 15.5^b$	NG^c	NG^c
5	NG‡^{c}	NG^c	NG^c	NG^c	NG^c

* Different letters on the same column indicate that there is a statistical significant difference between each other ($P < 0.05$).

† All the counts are the average of three independent experiments ± standard deviation.

‡ Variables with no growth (NG) at a detection limit $<10^2$ cfu/g.

Microbial Analysis

For microbial analysis of the treatment and control groups, the samples were defrosted, mixed and homogenized manually for 2 min with 90 mL of sterile 0.1% peptone water (Merck). The amount of 0.01 mL samples was inoculated on *Brucella* agar media containing blood immediately after treatment. The plates were incubated in humid atmosphere at 37C for 7 days. Experiments for each strain of *Brucella* were performed three times in different period of times (1, 3, 7, 14 and 21 days). Because the pH plays an important role in survival of *Brucella*, this parameter was measured during analysis to maintain about 6.5 to 7 pH (Bakhtar Biochemistry, No. b32, Tehran, Iran).

Statistical Analysis

The total experiment was repeated three times. Data were analyzed by analysis of variance using SPSS 16.0 (Chicago, IL) software and Tukey's multiple comparisons. Significance was defined at $P < 0.05$.

RESULTS

The survival of *B. abortus* and *B. melitensis* strains in control and irradiated samples at different doses are presented in Tables 1 and 2, respectively. There is a significant difference between irradiated and control groups ($P < 0.05$). Also, it was obvious that increasing the irradiation dose resulted in increasing inactivation of bacterial strains ($P < 0.05$) so that no bacteria was detected in 5 kGy irradiated samples at initial count.

As shown in the Tables 1 and 2, *B. abortus* was more sensitive to irritation process than *B. melitensis*. The results showed significant differences between different days and number of the bacteria ($P < 0.05$); the only exception was shown between days 14 and 21 after irradiation about *B. abortus*, and days 1, 3 and 7 about *B. melitensis*.

DISCUSSION

In the present study, reduction of *Brucella* strains counts was observed at all irradiation doses. However, both strain of *Brucella* were observed over 21 days after treatment with 1 kGy irradiation. Our results indicate that 2 kGy dose of electron beam irradiation was sufficient to eliminate *B. abortus* after 14 days. Little regrowth on day 21 could be due to the ability of bacteria to rebuild or recover itself partially. This fact was also observed on *B. melitensis* treated with 5 kGy dose after 3 days. All *B. abortus* strains treated with 3 kGy were eliminated completely after 14 days, thus, this seems to be the effective dose and time to destroy the bacteria. So, according to our findings, in order to have complete elimination of *B. melitensis*, the amount of 5 kGy is required.

According to an experimental study, it has been seen that *B. abortus* was viable up to 1 month in ice cream (Kuplulu and Sarimehmetoglu 2004) that is in accordance with the

TABLE 2. *BRUCELLA MELITENSIS* COUNTS (CFU/G) IN TRADITIONAL ICE CREAM STORED AT –18C†

Irradiation dose (kGy)	1 day	3 days	7 days	14 days	21 days
0 (Control)	$3.7 \times 10^5 \pm 48.1$a,*	$3.1 \times 10^5 \pm 76.4^a$	$3.3 \times 10^5 \pm 53.3^a$	$2.9 \times 10^5 \pm 16.8^a$	$3.8 \times 10^5 \pm 19.5^a$
1	$6.5 \times 10^4 \pm 21.9^b$	$4.5 \times 10^4 \pm 0^b$	$6 \times 10^4 \pm 0^b$	$3.6 \times 10^4 \pm 57.5^b$	$1.3 \times 10^3 \pm 23.4^b$
2	$4.5 \times 10^4 \pm 15^b$	$6.6 \times 10^4 \pm 57.5^b$	$1.7 \times 10^4 \pm 25.6^b$	$2 \times 10^4 \pm 10^b$	NG^c
3	$2.5 \times 10^4 \pm 86.2^b$	$1.5 \times 10^4 \pm 50^b$	$6.6 \times 10^3 \pm 15.5^b$	$3.3 \times 10^2 \pm 57.3^b$	$6.6 \times 10^2 \pm 15.7^b$
5	NG^c,‡	$6.6 \times 10^2 \pm 14.7^c$	NG^c	NG^c	NG^c

* Different letters on the same column indicate that there is a statistical significant difference between each other ($P < 0.05$).

† All the counts are the average of three independent experiments ± standard deviation.

‡ Variables with no growth (NG) at a detection limit $<10^2$ cfu/g.

results of the present research, which after 21 days *Brucella* bacteria were observed in the control samples in traditional ice cream. *Brucella* spp. can survive 87, 60, 4 and 2 days in ultra-high temperature milk, water, yogurt with 3.5% fat and yogurt with 10% fat, respectively (Falenski *et al.* 2011). Estrada *et al.* (2005) reported that *B. abortus* can be viable in fermented milk after 10 days even in low pH below 4 due to its ability to recover itself (Estrada *et al.* 2005). The survival of *Brucella* spp. in milk and some dairy products were analyzed; one was in ordinary yoghurt stored at two conditions, room temperature and 4C in 5 and 8 days, respectively (Samaha 2008). Also, Kuplulu and Sarimehmetoglu (2004) showed that *B. abortus* was detected at the level of 5.4×10^2 MPN/g in vanilla ice cream samples, but there were no *Brucella* spp. in the chocolate and fruit flavor ice cream samples (Kuplulu and Sarimehmetoglu 2004). Similar research revealed that in irradiated samples at 5 kGy of gamma irradiation, no bacteria was observed, whereas coliform bacteria, *Listeria* spp., *Escherichia coli* and *Salmonella* spp. were found in nonirradiated ice cream samples as well (Lee *et al.* 2009). Another investigation revealed that the 3 kGy dose of gamma irradiation was enough to achieve a three-magnitude order reduction in total bacterial counts, a two-magnitude order reduction in moulds and yeasts and totally inactivation of coliform and *Staphylococcus* spp. (Adeil Pietrana *et al.* 2003). Applying 1 kGy of gamma radiation can improve the microbial quality of the ice cream and have good efficiency to eliminate some pathogens, such as *E. coli* O157:H19, *Listeria monocytogenes* and *Yersinia enterocolitica* (Kamat *et al.* 2000). Also, it has been shown that 3 kGy was an effective dose to inactivate the total aerobic bacteria in vanilla ice cream, whereas for the purpose of elimination in chocolate or strawberry ice cream, 5 kGy is needed (Jo *et al.* 2007). Another study indicated that 2 kGy dose of gamma irradiation was enough for complete inactivation of Enterobacteriaceae family and 3 kGy dose of gamma irradiation was sufficient for completely inactivation of the inoculated *Staphylococcus aureus* that is similar with our results (Badr 2012). Fallah *et al.* (2010) study results showed that 3 kGy dose of gamma radiation was enough to elimination of *E. coli* O157:H7 in ready-to-cook barbecue, but this dose could not completely eliminate *L. monocytogenes* and *Salmonella* Typhimurium (Fallah *et al.* 2010). For complete sterilization of beef jerky, high dose such as 25–40 kGy is required, and total aerobic bacterial count is significantly decreased with increasing dose of electron beam irradiation (Kim *et al.* 2010b). Elimination of fecal coliforms and reduction of *Staphylococcus* spp. count in ready-to-cook meat products were observed by gamma irradiation treatment, and showed that 2.5 kGy dose of gamma irradiation in combination with chilled storage can improve the safety and extends the shelf life of ready-to-cook meat products (Kanatt *et al.* 2010). Irradiation of 1 kGy for sliced cheese and 3 kGy for pizza cheese were enough to lower the total aerobic bacteria to undetectable levels, and results showed that a low dose of less than 3 kGy of irradiation (gamma and electron beam) can improve the microbial quality of sliced and pizza cheeses (Kim *et al.* 2010a). Velasko *et al.* (2011) study results showed 96% reduction of the *Clostridium tyrobutyricum* spores in cheese which was treated with 3 kGy dose of electron beam irradiation (Velasko *et al.* 2011). Another study that was conducted in 2012 showed that electron beam irradiation is an effective type of ionizing radiation to inactivate *Salmonella* in food products (Tahergorabi *et al.* 2012).

Although 3 kGy irradiation dose could reduce the microbial level notably according to the type of pathogen virulence and pathogenicity, it cannot be considered as a reliable dose because it is not clear how many *Brucella* bacteria is needed to cause brucellosis. Some reports have shown that presence of at least 10 to 100 bacteria is needed to be inhaled for creating brucellosis infections. So even the presence of 1 cfu/g in food should be considered as a risk factor (Kuplulu and Sarimehmetoglu 2004).

CONCLUSIONS

It can be concluded that electron beam irradiation technology is an acceptable method to reduce microbial contamination of *Brucella* spp. in traditional ice cream for which there are limitations to use common methods such as thermal processing.

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