Effects of Gamma Irradiation and Silver Nano Particles on Microbiological Characteristics of Saffron, Using Hurdle Technology

E. Hamid Sales · F. Motamedi Sedeh ·
S. Rajabifar

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Abstract Saffron, a plant from the Iridaceae family, is the world's most expensive spice. Gamma irradiation and silver nano particles whose uses are gradually increasing worldwide, have positive effects on preventing decay by sterilizing the microorganisms and by improving the safety without compromising the nutritional properties and sensory quality of the foods. In the present study combination effects of gamma irradiation and silver nano particles packaging on the microbial contamination of saffron were considered during storage. A combination of hurdles can ensure stability and microbial safety of foods. For this purpose, saffron samples were packaged by Poly Ethylene films that possess up to 300 ppm nano silver particles as antimicrobial agents and then irradiated in cobalt-60 irradiator (gamma cell Model: PX30, dose rate 0.55 Gry/Sec) to 0, 1, 2, 3 and 4 kGy at room temperature. The antimicrobial activities against *Total Aerobic Mesophilic Bacteria*, *Enterobacteriaceae*, *Escherichia Coli* and *Clostridium Perfringens* were higher in the irradiated samples, demonstrating the inhibition zone for their growth. Irradiation of the saffron samples packaged by Poly Ethylene films

with nano silver particles showed the best results for decreasing microbial contamination at 2 kGy and for Poly Ethylene films without silver nano particles; it was 4 kGy.

Keywords Saffron · Gamma irradiation · Silver nano particles · Microbiological characteristics

Introduction

Irradiation of food at a dose level of 10 kGy or below is toxicologically safe and nutritionally adequate [1–3]. In the recent two decades, food irradiation with ionizing radiation was introduced as an easy and reliable technological process for reducing spoilage losses and improving their hygienic quality and extent of shelf life [4–7].

On the other hand, due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, the pharmaceutical companies and the researchers are searching for new antibacterial agents. Adding nano-composites or nano particles into packaging materials to ensure better protection of foods have emerged up as novel antimicrobial agents and the unique chemical and physical properties [8, 9]. Potential benefits for consumers and producers of these new products are widely emphasized [10].

The films with antimicrobial activity could help to control the growth of pathogenic and spoilage microorganisms [11, 12].

It is well known that silver ions and silver-based compounds are highly toxic to microorganisms and show severe bactericidal effects on as many as 16 species of bacteria including *Escherichia coli*. Thus, silver ions, as an antibacterial component, have been used in the formulation

E. Hamid Sales
Department of Food Industry, Faculty of Agriculture, Varamin
Branch, Islamic Azad University, Varamin, Iran

F. Motamedi Sedeh (✉) · S. Rajabifar
Agricultural, Medical and Industrial Research School (AMIRS),
Karaj, Iran
e-mail: fmotamedi@nrcam.org

of dental resin composites and ion exchange fibers and in coatings of medical devices [13].

Gamma irradiation and silver nano particles whose uses are gradually increasing worldwide, have positive effects on preventing decay by sterilizing the microorganisms and by improving the safety without compromising the nutritional properties and sensory qualities of the foods [14–18].

Saffron, the world's most expensive spice, is a plant from the Iridaceae family, possessing red–orange tripartite stigmas [19, 20].

In this study, the effects of gamma irradiation and silver nano particles packaging on microbial contamination of saffron are compared, also the combined effects are studied for both of the antibacterial agents on saffron. Each hurdle implies putting microorganisms in a hostile environment, which inhibits their growth or causes their death [21].

Materials and Methods

Sample Preparation

Saffron samples were packaged by Poly Ethylene (PE) films that posses silver nano particles up to 300 ppm as antimicrobial agents for test group and without silver nano particles for control group (each sample weighed 1 g).

Gamma Irradiation

The Saffron samples were irradiated in 60 cobalt irradiator (gamma cell Model: PX30, dose rate 0.55 Gry/Sec) to 0, 1, 2, 3 and 4 kGy at room temperature, for each dose of gamma irradiation three samples were used. This procedure is commercially used for irradiation of prepackaged spices.

Microbial Contamination

Saffron samples from both irradiated and the non-irradiated samples were analyzed immediately after irradiation and subsequently at regular intervals during storage, the first day, the 30th and 60th day at ambient temperature. Cultures media such as; Eosin Methylen Blue (EMB), Sulphadiazin Poly Mixin Sulphit Agar (SPS), Plate Count Agar (PCA) and Lactose Broth (LB) were used for *E. coli*, *Clostridium Perfringenes*, *Total Aerobic Mesophilic Bacteria* and *Enterobacteriace* respectively. Briefly, maximum probability number method (MPN) was used to detect *Enterobacteriace* quantitatively. Anaerobic condition by Anaerocult A, Merk and SPS media used for detection of *C. Perfringenes*. Serial dilutions of the saffron samples in peptone water 0.9% were cultured on PCA media to count the *Total Aerobic Mesophilic Bacteria* and EMB media were used for detection of *E. coli* [22–25].

Statistical Analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. Significance was defined at $P < 0.05$.

Results

The effects of the gamma radiation treatment and silver nanoparticle packaging on the *Total Aerobic Mesophilic Bacteria*, *Enterobacteriace*, *E. Coli* and *C. Perfringenes* in saffron are shown in Figs. 1, 2, 3 and 4 respectively. Despite of Saffron antimicrobial properties, there were the bacterial contamination in the saffron samples, and decreasing of the contamination was considered by gamma irradiation and silver nanoparticles packaging. According to the results, however, the saffron has antimicrobial properties, but the *Total Aerobic Mesophilic Bacteria*, *Enterobacteriace*, *E. Coli* and *C. Perfringenes* in the non irradiated and without silver nanoparticles packaging saffron samples were $2 \times 10^5 \pm 50332.23$, $1.1 \times 10^3 \pm 0$, $1 \times 10^3 \pm 100$ and $6 \times 10^3 \pm 305.5$ at the first day, respectively. After gamma irradiation using 2 kGy the above bacterial contamination decreased $6 \times 10^3 \pm 305.5$, $4.3 \times 10^1 \pm 0$, $2 \times 10^2 \pm 23.09$ and 0 respectively. Also silver nanoparticles packaging of the saffron samples made to decrease the above bacterial contamination till $1 \times 10^3 \pm 115.47$, $4.6 \times 10^2 \pm 0$, $4 \times 10^2 \pm 115.47$ and $3 \times 10^3 \pm 200$, respectively.

Fig. 1 Decreasing of total count of *Aerobic Mesophilic Bacteria* $\pm$ SE in duration of storage using increasing of gamma irradiation. WNSF without nano silver films, NSF nano silver films, SE standard error

WNSF-without nano silver films, NSF-nano silver films, SE-Standard Error

Fig. 2 Decreasing of *Enterobacteriaceae* $\pm$ SE in duration of storage using increasing of gamma irradiation. WNSF without nano silver films, NSF nano silver films, SE standard Error

WNSF-without nano silver films, NSF-nano silver films, SE-Standard Error

Fig. 4 Decreasing of *C. Perfringenes* $\pm$ SE in duration of storage using increasing of gamma irradiation. WNSF without nano silver films, NSF nano silver films, SE standard Error

WNSF-without nano silver films, NSF-nano silver films, SE-Standard Error

Fig. 3 Decreasing of *E. Coli* $\pm$ SE in duration of storage using increasing of gamma irradiation. WNSF without nano silver films, NSF nano silver films, SE standard Error

According to the Figs. 1, 2, 3 and 4 increasing of gamma irradiation dose till 4 kGy causes 100, 99.7, 100 and 100% decreasing of *Total Aerobic Mesophilic Bacteria*, *Enterobacteriaceae*, *E. Coli* and *C. Perfringenes*, respectively at the first, the 30th and the 60th day. Also packaging by PE film with nano silver particles against packaging without nano silver particles shows positive effects on decreasing the above bacterial contamination till 99.5, 58.18, 60 and 50% respectively at the first day, the percent of decreasing at the 30th day were 97.14, 80.90, 56.66 and 50%, finally after 60 days 95, 82.5, 50 and 60% respectively. However combination of gamma irradiation and

packaging with nano silver particles has more severe positive effects on microbial safety.

According to ANOVA-one way method there are significant differences in different doses of gamma irradiation and packaging with nano particles or without nano particles ($P < 0.05$).

Discussion

Chaudhry et al. [26] in March 2008 reviewed nanotechnology applications for food packaging. They reported that nanotechnology derived food packaging materials are the largest category of current nanotechnology applications for the food sector, the main applications for food contact materials include (FCMs): (1) FCMs incorporating nano-materials to improve packaging properties such as flexibility, gas barrier properties, temperature/moisture stability. (2) “Active” FCMs that incorporate nanoparticles with antimicrobial or oxygen scavenging properties. (3) “Intelligent” food packaging incorporating nano-sensors to monitor and report the condition of the food. (4) Biodegradable polymer–nano-material composites [26]. H. B. Ghoddusi, B. Glatz in the International Symposium on Saffron Biology and Biotechnology in 2004 were studied on decontamination of saffron by electron beam. The dried stigmas of saffron were made by them, inoculated with three levels (103, 104, and 106 CFU g⁻¹) of a mixed culture (bacteria, yeast, and mold) isolated from the natural contaminant flora of saffron, and were irradiated at four dose levels (2, 5, 10, and 15 kGy) in an electron beam irradiator. Yeasts were most resistant to irradiation;

some survivors were found even at doses as high as 15 kGy. Molds and bacteria were less resistant and were eliminated at 5 and 10 kGy, respectively. The calculated D-value for the mold, bacterium and yeast used, were 0.82, 0.86, and 2.69 kGy, respectively [27].

In the present study, we have used a combination of gamma irradiation and silver nanoparticle packaging to decrease the microbial load in saffron as an important spice. As the data in Fig. 1 shows, the optimum gamma irradiation dose to decrease total aerobic mesophilic bacteria to zero was 4 kGy for PE film without nano silver particles. But it was 3 kGy for saffron samples packaged by PE film with nano silver particles. Also irradiation of the saffron samples packaged by PE films with nano silver particles showed the best results for decreasing microbial contamination at 2 kGy and for PE films without silver nano particles, it was 4 kGy. Finally, it can be concluded that the combined method is more effective than single method and all data show decrease in each microbial load.

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