- *Technical note*

Combined effects of gamma irradiation and silver nano particles packing on the chemical characteristics and sensory properties of saffron, using hurdle technology

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Background: Saffron, the dried stigmas of crocus sativus, is the world's most expensive spice which has been used as food additive and flavoring agent. The aim of the present study has been to investigate the combination effects of gamma irradiation and silver nano particles packing on the chemical properties of saffron during storage time. A combination of hurdles can ensure stability and microbial safety of foods. ***Materials and Methods:*** To investigate the combination effects, saffron samples were packed by Poly Ethylene films which were possed 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 Gy/sec) to 0, 1, 2 ,3 and 4 kGy at room temperature. UV-spectrophotometer was used to quantify the most important components crocin, picrocrocin and safranal which were respectively responsible for color, taste and odor. ***Results:*** Statistical analysis showed that irradiation and silver nano particles films packing could increase the flavor and aroma of saffron, and the best optimum dose of irradiation was 2 kGy. Also, sensory evaluation showed no significant difference between them (P<0.05). ***Conclusion:*** Hurdle technology has been more effective than irradiation or nano-silver particles packing methods. Therefore combined method can be used for microbial decontamination of saffron with no significant differences on chemical characteristics and sensory attributes. **Iran. J. Radiat. Res., 2012; 9(4): 265-270**

Keywords: *Saffron, gamma irradiation, silver nano particles, crosin, picrocrocin, safranal.*

INTRODUCTION

Saffron, the world's most expensive spice, is a plant from Iridaceae family with red-orange tripartite stigmas, which are used as food additive and flavoring agent [(1-4)].

Irradiation of food at a dose level of 10 kGy or below has been toxicologically safe and nutritionally adequate [(5-7)]. During last two decades, food irradiation with ionizing radiation was introduced as an easy and reliable technological process for reducing spoilage losses and improving hygienic quality with extended shelf life [(8-11)].

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 looking for new antibacterial agents. Adding nano-composites or nano particles in to packing materials to ensure better protection of foods have emerged up as novel antimicrobial agents with unique chemical and physical properties [(12, 13)] .The potential benefits for consumers and producers of these new products are widely emphasized [(14)] .The films with antimicrobial activity could help to control the growth of pathogenic and spoilage microorganisms [(15, 16)].

It is well known that silver ions and silver-based compounds are highly toxic to microorganisms and show severe bactericide

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effects on as many as 16 species of bacteria including *E. coli*. Thus, silver ions, as an antibacterial component, have been used in the formulation of dental resin composites and ion exchange fibers and in coatings of medical devices [17].

Gamma irradiation and silver nano particles have positive effects in preventing decay by sterilizing the microorganisms and by improving the safety without compromising the nutritional properties and sensory quality of the foods, and their use are gradually increasing worldwide [18-22]. A combination of hurdles can ensure stability and microbial safety of food [23].

In this study, we have quantified the most important components: crocin, picrocrocin and safranal which are respectively responsible for color, taste and odor.

MATERIALS AND METHODS

Sample preparation

Saffron samples were packed by Poly Ethylene films containing possed silver nano particles up to 300 ppm, as antimicrobial agents for the experimental group, and without silver nano particles for the control group (each sample was weighted 1g)

Gamma irradiation

The Saffron samples were irradiated in ^{60}cobalt irradiator (Gamma cell Model: PX30, dose rate 0.55 Gy/sec) to 0, 1, 2, 3 and 4 kGy at room temperature, and for each dose of gamma irradiation 3 samples were used. This procedure is commercially used for the irradiation of prepackaed spices.

Chemical tests

Fifty mg of saffron in 1000 ml distilled water was used for chemical analysis in UV-visible spectrophotometer whose results were inserted in the below formulation [24, 25]:

$$E_{1cm} = \frac{A \times 100}{m} \times \frac{100}{100 - H}$$

A= Optical Density in three wavelength, 257, 330 and 440 nm

H= humidity (10%), m= mass

Spectrophotometer in 257nm was used for quantifying picrocrocin which was related to saffron samples, flavor.

Spectrophotometer in 330nm was used for quantifying safranal which was related to saffron samples aroma.

Spectrophotometer in 440nm was used for quantifying crocin which was related to saffron samples color.

Sensory evaluation

Sensory acceptances of the irradiated and non-irradiated saffron samples, which were packed by nano silver films and poly ethylene films without nano silver particles, were done with 6 expert panelists who have already been trained on the irradiated foods. Panelists were instructed to evaluate each attribute using a four-point hedonic scale ranging from dislike to like extremely. About fifty ml of each saffron solution were given individually to the panelists and a three digit code was used for the samples. The sensory test was carried out as triplicate and the panelist were advised to carry out thrice.

Statistical analysis

Statistical analysis was done by Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test. The data were analyzed by SAS software (V.8.02, SAS Institute, Cary, NC, USA).

RESULTS

The effects of the gamma radiation treatment and silver nano particle packing on saffron components, the most important of which are, crocin, picrocrocin and safranal, are shown respectively in tables 1, 2 and 3 (P<0.05).Table 1 is for picrocrosin which relates to saffron flavor, table 2 is for safranal which relates to saffron aroma and table 3 for crosin which relates to saffron color.

As the data in table 1 show, when saffron samples have been packed by Poly

Ethylene film, without nano silver particles, the optimum gamma irradiation dose was 2 kGy. But, Saffron samples packed by Poly ethylene film with nano silver particles showed a little decrease in flavor which should be notified and statistical analysis show significant difference between them ($P<0.05$). The tables 1-3 show a little increase in aroma, color and flavor during storage after 30 days.

Table1. The concentration of picrocrocin (µmolar/mg) in irradiated and non-irradiated saffron samples (flavor).

Treatment	Poly ethylene film	First day	30days
Non-irradiated	WNSF	48.86±4.27	65.51±3.67
	NSF	58.83±9.12	66.62±7.38
Irradiated(1kGy)	WNSF	46.63±1.21	59.96±2.95
	NSF	57.74±15.78	67.68±4.21
Irradiated(2kGy)	WNSF	53.27±1.23	55.52±13.36
	NSF	53.31±7.92	63.26±11.72
Irradiated(3kGy)	WNSF	48.92±1.8	75.45±9.57
	NSF	59.96±7.26	62.18±10.73
Irradiated(4kGy)	WNSF	51.08±1.56	59.43±5.3
	NSF	56.58±7.6	65±7.22

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

Table 2. The concentration of safranal (µmolar/mg) in irradiated and non-irradiated saffron samples (aroma).

Treatment	Poly ethylene film	First day	30days
Non-irradiated	WNSF	26.65±1.22	34.43±6.29
	NSF	35.82±4.25	36.65±5.23
Irradiated(1kGy)	WNSF	24.43±2.45	32.14±1.51
	NSF	32.15±10.35	37.69±2.73
Irradiated(2kGy)	WNSF	29.26±2.01	37.7±7.27
	NSF	34.38±0.56	43.27±6.67
Irradiated(3kGy)	WNSF	26.67±3.06	29.94±5.95
	NSF	29.93±4.24	34.42±4.47
Irradiated(4kGy)	WNSF	24.39±2.45	28.59±4.21
	NSF	28.83±2.42	29.94±3.51

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

Table 3. The concentration of crocin (µmolar/mg) in irradiated and non-irradiated saffron samples (color).

Treatment	Poly ethylene film	First day	30days
Non-irradiated	WNSF	112.08±11.56	136.52±21.8
	NSF	115.4±12.72	140.92±3.85
Irradiated(1kGy)	WNSF	89±9.09	118.75±29.24
	NSF	102.14±40.64	133.21±16.93
Irradiated(2kGy)	WNSF	117.62±7.29	106.55±13.64
	NSF	133.18±3.03	163.66±4.27
Irradiated(3kGy)	WNSF	90.01±1.83	121.03±5.27
	NSF	93.21±16.99	129.9±11.56
Irradiated(4kGy)	WNSF	88.75±2.7	108.75±17.83
	NSF	108.74±6.98	122.03±7.29

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

The sensory evaluations of gamma irradiated saffron samples were shown in tables 4 and 5. The scoring was done as numerals, 4-excellent to 1- unacceptable. Table 4 is the judgment of 6 panelists on aroma of saffron samples, and table 5 is saffron samples for taste.

DISCUSSION

Food irradiation is performed to increase the hygienic quality of foods by reducing the number of pathogenicity and spoilage by microorganisms and extend the shelf life [10]. Many reports have been found on the influence of irradiation procedures on the chemical properties of foods [5- 9].

Lee *etal.* (2009) reported microbiological assay of fresh and stored ready-to-use tamarind juice showed better quality after gamma irradiation. Gamma irradiation of tamarind juice at 1, 3 and 5 kGy also resulted in a significant increase or maintenance of the antioxidants which is essential to preserve them for transportation. The color in both fresh and stored ready-to-use tamarind juice changed to bright color, which gave the possibility use in food industrial application. Irradiated ready-to-use tamarind juice was found to maintain its sensory properties until 5 kGy irradiation [27].

Vedadi *et al.* (2003) showed aroma differentiation by saffron experts indicated no change in irradiated saffron samples compared with the non- irradiated saffron samples [26].

Zareena *etal.* (2001) reported changes in aroma and coloring properties of saffron (*Crocus sativus*) after γ-irradiation at doses

Table 4. The sensory values of gamma irradiated saffron about aroma.

Treatment	Poly ethylene film	First day	30days
Non-irradiated	WNSF	2.5 ±0.54	2.5±0.83
	NSF	2.66±0.81	3±0.63
Irradiated(1kGy)	WNSF	2±0.89	2.5±0.83
	NSF	2.33±0.81	3±0
Irradiated(2kGy)	WNSF	2.66±0.81	2.83±0.51
	NSF	2.83±0.98	3±0.63
Irradiated(3kGy)	WNSF	1.83±0.98	2.16±0.4
	NSF	2±0.89	2.83±0.63
Irradiated(4kGy)	WNSF	2.5±1.04	2.33±0.81
	NSF	1.66±0.51	2±0.89

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

Table 5. The sensory values of gamma irradiated saffron about taste.

Treatment	Poly ethylene film	First day	30days
Non-irradiated	WNSF	2.66±0.81	2.5±0.83
	NSF	2.5±1.04	3.16±0.75
Irradiated(1kGy)	WNSF	2.33±0.81	3±0.63
	NSF	2.33±1.21	3±0.63
Irradiated(2kGy)	WNSF	2.66±0.81	2.83±0.75
	NSF	3.16±0.75	2.66±0.81
Irradiated(3kGy)	WNSF	2.33±1.21	2.16±0.75
	NSF	2.66±1.22	2.5±0.83
Irradiated(4kGy)	WNSF	1.83±0.98	1.83±0.75
	NSF	2±0.89	2±0.63

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

of 2.5 and 5 kGy (necessary for microbial decontamination). The volatile essential oil constituents responsible for aroma of the spice were isolated by steam distillation and then subsequently analyzed by gas chromatography/mass spectrometry (GC/MS). No significant qualitative changes were observed in the aroma upon irradiation although a trained sensory panel could detect slight quality deterioration at a dose of 5 kGy. Carotene glucosides which impart color to the spice were isolated by solvent extraction and then subjected to thin-layer chromatography and high-performance liquid chromatography (HPLC). Fractionation of the above pigments into aglycon and glucosides was achieved by using ethyl acetate and *n*-butanol, respectively. Analysis of these fractions by HPLC revealed a decrease in glucosides, and an increase in aglycon content in the irradiated samples. The possibility of degradation of pigments during gamma irradiation is discussed [28].

Cho *et al.* (2005) studied, the antimicrobial activity of silver nanoparticles (Ag-NPs) and platinum nanoparticles (Pt-NPs) aqueous solution which were prepared using different stabilizer, such as sodium dodecylsulfate (SDS) and poly-(*N*-vinyl-2-pyrrolidone) (PVP), for *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E.coli*) by measuring the minimum inhibitory concentration (MIC). Antimicrobial effect of Ag-NPs for *S. aureus* and *E. coli* was investigated using cup diffusion method. The growth of Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria were inhibited by Ag-NPs. The MIC of Ag-NPs for *S. aureus* and *E. coli* were 5 and 10 ppm, respectively. But the Au-NPs stabilized with SDS did not show antimicrobial activity. Also, the Pt-NPs stabilized with PVP (or SDS) did not show antimicrobial activity for the test organisms [29].

In the present study, the combination of gamma irradiation and silver nano-particle was investigated. There were significant difference between irradiated and non-irradiated saffron samples, which were packed by poly ethylene films, without nano silver particles, and the optimum gamma irradiation dose was obtained as 2 kGy ($P<0.05$). But, a little decrease in flavor was shown for the saffron samples which were packed by poly ethylene films with nano silver particles, and there were significant difference between them ($P<0.05$). The concentration of aroma, color and flavor during storage after 30 days was increased in comparison to the first day. Also, the irradiation of saffron samples packed by poly ethylene films with nano silver particles as shown in tables 1, 2 and 3 was 2 kGy, which has been appropriate for the use without any adverse change in sensory qualities.

However, tables 4 and 5 show no significant difference in the irradiated saffron samples and non-irradiated ones , and they were no significant difference between saffron samples packed by poly ethylene films without nano silver particles and those packaged by poly ethylene films with nano silver particles($P<0.05$). In sensory evaluation of irradiated samples, many investigations have been done [5, 8, 9] and sometimes significant differences were observed among them.

Finally, it can be concluded the combined method is more effective than their discrete application, and all data showed confirming results for the use of irradiation and nano silver films packing for decontamination of saffron. So these hurdles are proved to be useful in consideration of chemical saffron samples characteristics.

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