Decontamination of Saffron (*Crocus sativus* L.) by Electron Beam Irradiation

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Keywords: crocin, microorganisms, picrocrocin, safranal

Abstract

Dried stigmas of saffron were inoculated with three levels (10^3, 10^4, and 10^6 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 cultures used were, 0.82, 0.86, and 2.69 kGy, respectively. Bitterness, aroma and color indices were determined by measuring absorbance of saffron extracts at 257, 330 and 440 nm, respectively. Irradiation had little effect on picrocrocin (bitterness) and safranal (aroma) values; the color strength index (crocin) decreased at doses higher than 10 kGy.

INTRODUCTION

Saffron (*Crocus sativus*) is treasured for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods. According to the Codex Alimentarius definition saffron is considered a spice (Anon., 1991). It is the costliest spice because of its low yield as well as labor-intensive harvesting and processing practices (Winterhalter and Straunbinger, 2000). Saffron is believed to have originated in Iran (the current major producer), Greece and India. It was introduced later to Spain and is now grown in many other countries (Raina et al., 1996; Sampathu, 2000; Winterhalter and Straunbinger, 2000). Saffron, like other spices and vegetable seasonings, is of agricultural origin and is in contact with dust and natural fertilizers. Therefore, it may become contaminated with microorganisms (bacteria, molds and yeasts) and/or insects. The traditional method of handpicking is still the only method for harvesting the crop, which increases the risk of pathogenic bacteria being transferred to the product through workers' hands. Once the saffron is completely dried, the chance for bacterial growth is minimal.

The temperature and time required for drying the saffron are interrelated. Traditionally, the three stigmas are handpicked from each flower, spread on trays or cloths, and dried over charcoal fires or at ambient temperature. Solar drying, which can destroy the color quality, is avoided (Basker, 1993). Incomplete drying results in total loss of the product through decomposition and mold growth. Electrical or other powered dryers have not made significant inroads into the scattered rural areas of saffron fields.

The inherent sensitivity of aroma and flavor of saffron to heat processing has made it difficult to maintain the required aroma/flavor quality indices of saffron. Fumigation by ethylene oxide as an alternative practice for spices is now banned in many countries because of potential health hazards (Zareena et al., 2001).

The microbial quality of saffron has not received attention and most countries use the same microbial quality standards for saffron as for other spices.

Irradiation is widely accepted as a safe and effective tool for reduction of foodborne pathogens and decontamination of spices and other seasonings (Anon., 2000). Many countries now permit irradiation of spices for disinfection. An average dose of 10 kGy is prescribed by 24 countries. Six countries, including the United States, allow higher doses up to 30 kGy (Wilkinson and Gould, 1996). While the effects of gamma irradiation on aroma and color properties of saffron have been investigated (Zareena et al., 2001), the

effect of irradiation on the microbial quality of saffron has not been reported. The objective of this study is to evaluate the stability of contaminating microflora and chemical characteristics of saffron upon electron beam irradiation.

MATERIALS AND METHODS

Samples

Commercial whole saffron stigmas, produced in Spain, were obtained from a local supermarket in Ames, IA. The saffron was distributed aseptically in 1-gram portions into sterile disposable petri dishes and kept at ambient temperature in the dark.

Isolation and Enumeration of Normal Flora

To determine the level of microbial contamination, in saffron, four saffron samples of different brands were analyzed. Samples were diluted 1:20 with O.1% peptone and stomached at the normal setting for 1 min. The dilutions were surface-plated in duplicate onto Plate Count Agar (PCA, Difco) and Potato Dextrose Agar (PDA, Difco) for aerobic bacterial counts and yeast and mold counts, respectively. Plates were incubated aerobically at 32^{o}C for 48 h for bacterial counts or for 3 days for yeast and mold counts.

Inoculation

Aerobic bacterial counts on saffron samples ranged between 1.2×10^2 and 4.6×10^4 CFU g^{-1}; yeast and molds ranged between 5.0×10^2 and 9.0×10^2 CFU g^{-1}. These values are lower than those found on saffron harvested in some areas. For irradiation studies, higher levels of inoculation were used to mimic those anticipated high contamination levels.

One Gram-positive bacterium, one yeast, and one mold, selected at random from organisms enumerated in saffron samples, were inoculated separately into 20 ml of Trypticase Soy Broth (TSB, Difco) and incubated at 32^{o} C until visible turbidity was observed (48 hr). Equal volumes of these cultures were mixed and diluted to final population of about 10^3, 10^4 and 10^6 CFU ml^{-1}. A 4-ml aliquot of the mixture was added to 1 g saffron in a sterile Petri dish; dishes were kept at room temperature in a laminar flow hood with covers slightly ajar until the original moisture content (8%) of the saffron was regained.

Irradiation

Irradiation was conducted at the Iowa State University Linear Accelerator Facility (LAF). Samples were maintained at ambient temperature before, during, and after irradiation. Irradiation was achieved by a CIRCE III R Electron Beam (EB) irradiator (Thomson-CSF Linac, St, Aubin, France) with an energy level of 10 MeV and a dose rate of 96.3 kGy min^{-1}. Target doses were 2, 5, 10 and 15 kGy; actual absorbed doses, measured in alanine dosimeters by a 104 Electron Paramagnetic Resonance instrument (Bruker Instruments Inc., Billerica, MA), were 1.960, 5.208, 10.498, and 15.450 kGy. For simplicity, instead of actual absorbed doses, target doses are referred to throughout the paper.

Enumeration and *D*-value

The entire saffron sample was stomached for 1 min in 20 ml of 0.1% peptone; dilutions were plated on PCA and PDA as described previously. The *D*- values (dose in kilograys resulting in a 90% reduction of viable organisms) were calculated from the reciprocals of the slopes of the linear regressions of the log survivor values.

UV Spectral Analysis and Moisture Content

Quality attributes of bitterness (picrocrocin concentration) aroma (safranal concentration), and color (crocin concentration) were measured. Aqueous extract of powdered saffron was prepared according to ISO specifications (ISO, 1993), and

absorption values were determined at 257, 330, and 440 nm, respectively. Two measurements for each of two replicates were made by using a single beam spectrophotometer (Spectronic 601, Milton Roy Co., Rochester, NY). Comparison of means ($P<0.05$) was performed using Tokey' s multiple comparison test.

RESULTS AND DISCUSSION

Bacterial counts in inoculated saffron were little affected by irradiation doses up to 5 kGy, while doses of 10 and 15 kGy decreased survivors to below detectable limits (Figure 1). Given the initial bacterial counts and the current regulations regarding microbial quality of spices (Frank, 1988), irradiation of saffron at 10 kGy should be adequate to reduce the number of bacteria by at least three logs, to levels that meet standards.

Of the inoculated organisms, the mold was the most sensitive to irradiation (Figure 2), being reduced to undetectable levels by doses as low as 2 kGy. If high mold counts are expected, irradiation at 5 kGy could be sufficient to reduce counts to acceptable levels.

The yeast was the most radiation-resistant of the tested organisms (Figure 3); cells survived 15 kGy of irradiation. Irradiation at 10 kGy can decrease the number of yeasts by at least 2 logs and should reduce the yeast population to acceptable levels unless the starting population is very high. Since yeasts have large cells and contain large quantities of nuclear material compared with bacteria, they represent larger target for irradiation damage. However, the survival plots of yeasts vary in shape from sigmoidal or biphasic to simple linear and are dependent on the irradiation medium; a 4 kGy shoulder has already been reported in meat inoculated with *Trichosporon cutaneum* (McCarthy and Damoglou, 1996). Some yeasts are much more radiation-tolerant. For example a particularly radiation-resistant strain of *Saccharomuces cervisiae* var. *ellipsoideus*, with a *D*-value as high as 3 kGy has been reported (Stehlik and Kaindl, 1996)). Although the potential radiation resistance mechanism of the yeast was not studied here, it is important to remember that such resistance depends not only on the intrinsic properties of the organisms but also on the food substrate in which they are treated. In a dry product such as saffron, resistance can be high. Irradiation at a low dose could inactivate some potential spoilage microorganisms, thereby removing competitors of the more radiation-resistant contaminants. Irradiation at doses high enough to reduce all anticipated contaminants is preferred.

The *D*-value was calculated for each organism. It should be used when inactivation kinetics are exponential; however, in the current study we observed a shoulder in the curves for bacteria and yeast before exponential inactivation began. Therefore from the exponential portions of these curves, *D*-values of 2.69 kGy for the yeast, 0.86 kGy for the bacterium, and 0.82 kGy for the mold were calculated. *D*-values up to 3 kGy for yeasts have been reported; the values for bacterium and mold are in line with reported figures (Stehlik and Kaindl, 1996; Willkinson and Gould, 1996).

Irradiation of saffron up to 15 kGy had little effect on bitterness and aroma indices (Table 1). However, the color of saffron (crocin) was affected at 10 and 15 kGy. Some carotenoids have been shown to be influenced by irradiation, particularly when ample moisture was available in the product (Bhushan and Thomas, 1990). We did not see the same decrease in safranal and pigments at 5 kGy of irradiation as were reported by Zareena et al. (2001). One possible reason could be the different methods used in these two studies; a simple spectroscopic measurement in this study *vs* GC/mass spectrometry method used by Zareena et al. (2001). Alonso et al. (1996) have argued the suitability of spectroscopic methods for evaluation of safranal. Although, safranal was long considered to be the primary compound responsible for saffron aroma, recent sensory studies have revealed additional trace constituents in the volatile fraction that are equally important for the typical aroma of the spice (Winterhalter and Straunbinger, 2000). Therefore, more elaborated methods such as HPLC may be essential if a more complete spectrum is required.

We suggest that radiating saffron at a dose of 10 kGy should be enough to reduce

microbial contaminants to an acceptable level, without significantly affecting important chemical characteristics. Radiation-tolerant yeasts, if present in high enough numbers initially, may survive such treatment and become the major, though harmless, flora. We selected examples of common contaminants for this study; for completeness, other less frequently found contaminants could also be tested . We examined only the immediate effects of irradiation. Some reports suggest that microorganisms surviving irradiation of spices are damaged and incapable of reproduction in the medium in which they were irradiated, even under conditions favorable to growth (Muhamed et al., 1986). Chemical and microbial stability of irradiated saffron upon storage should be investigated.

ACKNOWLEDGEMENTS

The authors are grateful to Mr Michael J. Holtzbauer for his assistance in irradiating the samples. Partial sponsorship was provided through I-HELP program of Iowa State and Purdue universities.

Literature Cited

Alonso, G.L., Salinas M.R., Esteban-Infantes, F.J. and Sánchez-Fernández, M.A. 1996. Determination of safranal from saffron (*Crocus sativus* L.) by thermal desorption-gas chromatography. J Agric. Food Chem. 44:185-188.

Anon., 2000. Food irradiation: available researches indicate that benefits outweigh risks. US General Accounting Office (GAO). RCED-00-217. [Internet, www], ADDRESS: http://www.frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=gao&docid=f:rc 00217.pdf

Anon, 1991. Code of good irradiation practice for the control of pathogens and other microflora in spices, herbs and other vegetable seasoning. International Consultative Group on Food Irradiation (ICGFI), Document No 5. Vienna.

Basker, D. 1993. Saffron, the costliest spice: drying and quality, supply and price. Acta Hort. 344: 86-97.

Bhushan, B. and Thomas, P. 1990. Effect of gamma irradiation and storage temperature on lipoxygenase activity and carotenoid disappearance in potato tubers (*Solanum tuberosum* L.). J Agric Food Chem. 38: 1586-1590.

Frank J, 1993. Irradiation of Dry Food Ingredients. CRC Press. Boca Raton. FL. p.17.

ISO, 1993. Saffron: Specification and Test Methods. International Organization for Standardization. ISO 3632-1&2.

McCarthy, J.A. and Damoglou, A.P. 1996. The effect of substrate on the radiation resistance of yeasts isolated from sausage meat. Lett. App. Microbiol. 22: 80-84.

Muhamed, L.J., Ito, H., Watanabe, H. and Tamura, N. 1986. Distribution of microorganisms in spices and their decontamination by gamma-irradiation. Agric Biol. Chem. 50: 347-352.

Raina, B.L., Agarwal, S.G., Bhatia, A.K. and Guar, G.S. 1996. Changes in pigments and volatiles of saffron during processing and storage. J. Sci. Food Agric. 71: 27-32.

Sampathu, S.R, Shivashankar, S. and Lewis, Y.S. 2000. Saffron (*Crocus sativus* Linn.) - cultivation, processing, chemistry and standardization. CRC Crit. Rev. Food Sci. Nut. 20: 123-157.

Stehlik, G. and Kaindl, K. 1966. Microbiological studies on the influence of combined processes of heat and irradiation on the survival of *Saccharomyces cervisiae* var. *ellipsoideus*. In: Food Irradiation. Proceeding of a Joint Symposium by IAEA & FAO. International Atomic Energy Agency. Vienna. pp. 299-305.

Visvanatas, S., Ravishankar, G. and Venkatarman, L. 1990. Induction of crocin, crocetin, picrocrocin, and safranal synthesis in callus cultures of saffron-*Crocus sativus* L, Biotechnol. Appl. Biochem. 12: 336-340.

Willkinson, V.M. and Gould, G.W. 1996. Food Irradiation-a Reference Guide. Butterworth Heinemann, Oxford.

Winterhalter, P. and Straunbinger, M. 2000. Saffron-renewed interest in an ancient spice. Food Rev. Int. 16: 39-59.

Zareena, A.V., Variyar, P.S., Gholap, A.S. and Bongirwar, D.R. 2001. Chemical investigation of gamma-irradiated saffron (*Crocus sativus* L.). J. Agric. Food Chem. 49: 687-691.

Tables

Table 1. Effect of irradiation on chemical compounds of saffron[1]

Irradiation doses (kGy)	Picrocrocin[2] (257 nm)	Safranal[2] (330 nm)	Crocin[2] (440 nm)
0	79.12[b]	46.08[a]	128.47[a]
2	82.17[a]	45.86[a]	128.69[a]
5	81.73[a]	46.30[a]	128.91[a]
10	79.99[ab]	45.86[a]	124.99[b]
15	81.95[a]	45.86[a]	123.47[c]

[1] Values are means of two replicates and duplicate measurements. Means separation was carried out by Tukey test

[2] Means within a column followed by a different letter are significantly different ($P<0.05$)

Figures

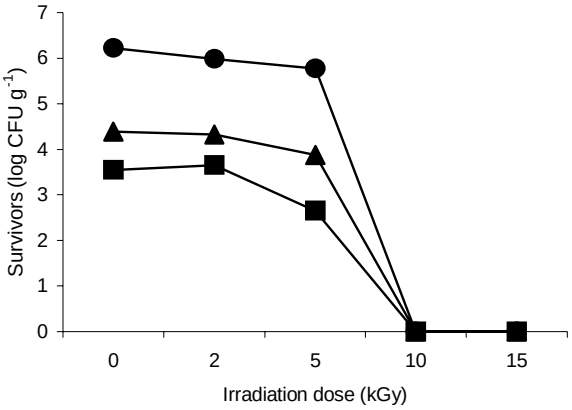

Fig. 1. Survival of bacteria at three inoculation levels (10^3, 10^4 and 10^6) in saffron exposed to electron beam irradiation

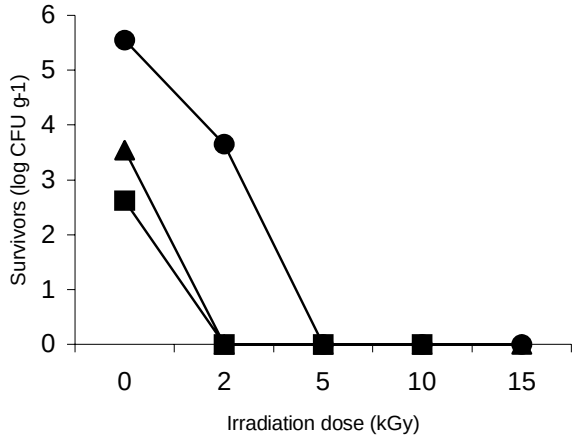

Fig. 2. Survival of molds at three inoculation levels (10^3, 10^4 and 10^6) in saffron exposed to electron beam irradiation

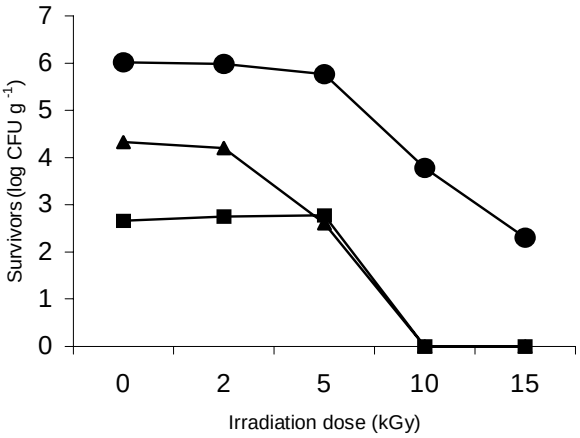

Fig. 3. Survival of yeasts at three inoculation levels (10^3, 10^4 and 10^6) in saffron exposed to electron beam irradiation