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Study of γ Irradiation and Storage Time on Microbial Load and Chemical Quality of Persian Saffron

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***Abstract*—**Irradiation is considered one of the most efficient technological processes for the reduction of microorganisms in food. It can be used to improve the safety of food products, and to extend their shelf lives. The aim of this study was to evaluate the effects of gamma irradiation for improvement of saffron shelf life. Samples were treated with 0 (none irradiated), 1.0, 2.0, 3.0 and 4.0 kGy of gamma irradiation and held for 2 months. The control and irradiated samples were underwent microbial analysis, chemical characteristics and sensory evaluation at 30 days intervals. Microbial analysis indicated that irradiation had a significant effect ($P < 0.05$) on the reduction of microbial loads. There was no significant difference in sensory quality and chemical characteristics during storage in saffron.

***Keywords*—**gamma irradiation; saffron; microbes; contamination

I. Introduction

APPLICATION of ionizing radiate treatment of food on an industrial scale was started at the beginning of the 1980s after the joint FAO/IAEA/WHO expert committee accepted The application of a 10 KGY overall average dose for food [1] in the past four Decades, a vast knowledge has been accumulated on the chemical and biological effects of ionizing irradiation, which has contributed to promote its utilization [2, 3, 4, 5 and 6] The recommended dose levels are: low level at 1 KGY to inhibit insect infestation and delay ripening; medium at 1 to 10 KGY to reduce Bacterial load (particularly of pathogens); and high at 10 to 50 KGY for Commercial sterilization and elimination of viruses [7]. Ionizing radiation is a method for preservation of foods that uses the high energy of gamma rays or accelerated electrons, thereby ionizing molecules [8]. Spice irradiation is the treatment with radiant energy to obtain some beneficial effects, which include disinfestations, improvement of the shelf life by the inactivation of spoilage organisms, and improvement of the safety of spices by inactivating food- borne pathogens. Ray irradiation is now internationally recognized as an effective method for maintaining the quality of spices for along time. The directive 1999/3/EC established a community list of food and food ingredients that may be treated with ionizing radiation and maximum overall average absorbed dose may be 10 KGY for dried aromatic herbs, Spices and vegetable seasonings the FDA limit for culinary herbs, seeds, spices, vegetable seasonings, and blends of these aromatic vegetable substances its up to exceed 30 KGY [9].Today, production saffron in Iran is such a way that in the course of different phases of harvesting, gathering. Handling, draying, packaging, and storage, due to non- observance of technical and hygienic principles, the product be comes contaminated and loses its original quality in addition to having hidden and evident effects on consumers, this causes a great part of the exported precious crop be will not be able to complete in the world markets with similar products from other countries, and naturally. This is not only will hinder saffron from being economically profitable for the country, but also damages the good reputation of Iranian product considering the above facts, it seems necessary to choose a suitable procedure for removal of contamination from saffron and increasing its shelf life. The aim of this study was to study effects of gamma irradiation and storage time as the process for microbial decontamination and improvement physico chemical characteristics of saffron were treated with 0,1,2,3 and 4 KGY of gamma irradiation and kept in room temperature for 2 months microbial and chemical analysis was done at zero, 30 and 60 days after irradiation in this study the optimum dose of gamma radiation in order to decrease the total count of mesosphiliic bacterial, coli form, E. Coli, mold and yeast was obtained at 2 KGY. Microbial analysis indicated that irradiation and storage at room temperature had a significant effect on the reduction of microbial loads. There was no significant difference in chemical characteristics during storage in saffron. Also, other researches in other countries showed same results [10 and 11].

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II. Materials and Methods

A. Sample Preparation

Saffron samples weighting 45 ± 0/001g were purchased at fields of Torbate Hyedariyeh County (Khorason- Iran) on the day of removal. Samples of saffron before packaged were pondered in sterile pounder. The samples were divided in to 45 equal parts and then were packed in commercial bags. (1 g per each package). Samples divided into 5 groups. One group of each sample wasn't exposed to gamma ray and kept as control and 4 groups were exposed to 1, 2,3 and 4 kilo- gray (KGY).

B. Irradiation

Treatment the samples were irradiated by Co- 60 source (gamma cell px-30 dose rate= 0/749 Gy/sec and activity= 4600Ci) in Nuclear agriculture department of agricultural, medical and industrial research institute, atomic energy organization of Iran.

C. Microbial analysis

Microbial analysis of aerobic Bacteria, coli form, E. coli, mold and yeast was carried out a day after the arrival day on 30 and 60 days. For total count of aerobic mesophilic bacterial, 10 g powdered saffron sample was isolated aseptically and diluted with 90ml sterile peptone water (%0.1) and blended for 10 min to prepare 1:10 dilution subsequent dilutions were prepared 1:100 and 1:1000. for bacterial counts 1ml of each dilution was added into the sterile plate in duplicate, plate cont agar media (Merck) was added and incubated for 48h at 35^{oC} the number of viable bacteria colonies expressed as microbial counts (log CFU/g). for coli form counts 1ml of each dilution was spread in duplicate on plates containing violet red bile agar (Merck) and incubated for 48h at 37^{oC}, also the maximum possibility number (MPN) of coli farm was obtained by the 3 tubes in lactose broth media for 24 h at 37^{oC} and 1ml of each tube, which was positive for gas production, was added to another tube containing brilliant green broth (Merck). For clostridium per 1 ml of each dilution was spread in duplicated on plates containing sulfite polymixin sulfadiazine (Merck) and for mold and yeast 1 ml of each dilution was spread in duplicated on plates containing potato dextrose agar (Merck) and incubated for 7-10 day at 28^{Oc}

D. Chemical Analysis

E. Saffron samples milled to made equal powder for performance spectrophotometer experiment. 500mg of saffron powder mix to 900ml water slowly and shake with magnetic mixer for 1 hour. Then send out magnet of container and infusion therein water to reach 1000cc. then pickup 20ml of dilution with pipette and carried at to balloon and infusion there water amount 180cc. crystal dilution make by compressive syringe and membrane (0/45 micron). Crystal dilution injected to the spectrophotometer cell and reading at 200-700 nm was done. Quality parameters of the saffron such as picrocrocin, safranal, croc in were analyzed by spectrophotometer in 257, 330 and 442nm respectively. (Table II).

E. Statistical Analysis

Data obtained were analysis statistically (ANOVA) wherever possible and percent loss against control was computed differences among the results obtained by different treatment were analyzed statistically and means were separated by least significant difference (LSD) at %5 probability level.

III. Results And Discussion

Total count of aerobic bacterial decreased with increase of irradiation dose (Table 1). Mean of bacterial total count coli form count, mold and yeast and E. coli were 7×10^4, $6/9\times10^3$, 2×10^3 and 8×10^3 colony forming units (cfu) per gram at control Sample (0.0 KGY) respectively (table 1) according ANOVA analysis, the number of aerobic bacterial, coli form, E. coli and mold & yeast decrease with increase of irradiation, before irradiation significantly ($P<0.05$) reduce them (table 1). In this study optimum dose of gamma radiation to reduce the microbial contamination was obtained at 2KGy. (Achieve to under stander level) and microbial load reduce to zero at 4 KGY in 30 day for all of type of microbes.

TABLE I
MICROBIAL MEAN FOR CONTROL (0.0KGY) AND IRRADIATED (1, 2, 3 AND 4 KGY) SAFFRON SAMPLE

Time (day)	DOSE (kGy)	TC[a] (Mean ± SE)	CC[b] (Mean ± SE)	EC[c] (Mean ± SE)	M & y[d] (Mean ± SE)
0 DAYS	0	$7\times10^4 \pm 10^2$	$6.9\times10^3 \pm 1.2\times10^2$	$2\times10^3 \pm 10$	$8\times10^3 \pm 4\times10^3$
	1	$3.2\times10^4 \pm 3\times10^3$	$4.6\times10^3 \pm 2\times10^2$	0	$8.6\times10^2 \pm 5\times10^2$
	2	$1.5\times10^3 \pm 2\times10^2$	$9.2\times10^2 \pm 2\times10^2$	0	0
	3	$1.1\times10^2 \pm 3\times10$	$8.3\times10^2 \pm 1.9\times10^2$	0	0
	4	7 ± 3	$4\times10^2 \pm 10^2$	0	0
30 DAYS	0	$4.9\times10^4 \pm 5\times10^3$	$4.1\times10^5 \pm 8\times10^2$	$10^3 \pm 2\times10^2$	$6.8\times10^3 \pm 3\times10^2$
	1	$1.5\times10^3 \pm 3\times10^2$	$2.4\times10^3 \pm 6\times10^2$	0	$1.3\times10^2 \pm 1.8\times10^1$
	2	$1.3\times10^2 \pm 2\times10$	$4.3\times10^2 \pm 3\times10^2$	0	0
	3	0	0	0	0
	4	0	0	0	0
60 DAYS	0	$1.2\times10^4_3 \pm 10$	$2.6\times10^3 \pm 3\times10^2$	$8\times10^2 \pm 1.2\times10^2$	$6\times8\times10^3 \pm 2.5\times10^3$
	1	$4.4\times10^2 \pm 2\times10^2$	$1.3\times10^2 \pm 3$	0	$4\times10^2 \pm 10^2$
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0

a= total count of aerobic bacteria **b= coli form count**
c= Ecoli count **d= mold and yeast count**
SE= standard error **T=time (day)**
ID= irradiation dose (kGy)

Results show that microbial load decrease with increase of irradiation dose for all microorganisms. Also with lapse (storage time 0-60 day), microbial load degraded for total

count, coli form, E.coli (Fig 1, 2, & 3), but increase for mold & yeast. (Fig4).

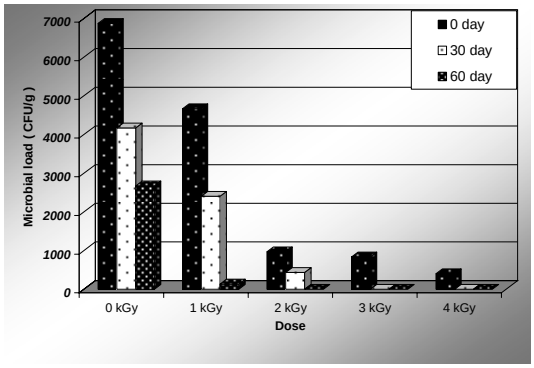

Fig. 1 Effect of irradiation treatment on coli form Count at zero, 30 and 60 days after irradiation

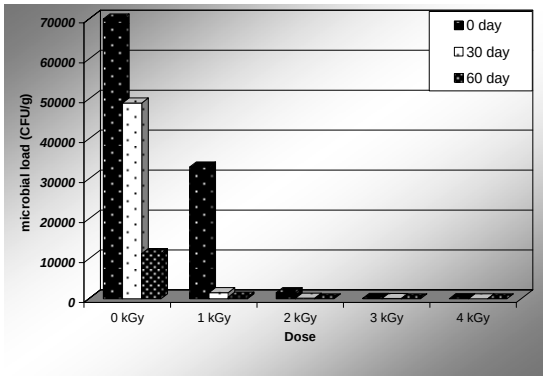

Fig. 2 Effect of irradiation treatment on Total count at zero, 30 and 60 days after irradiation

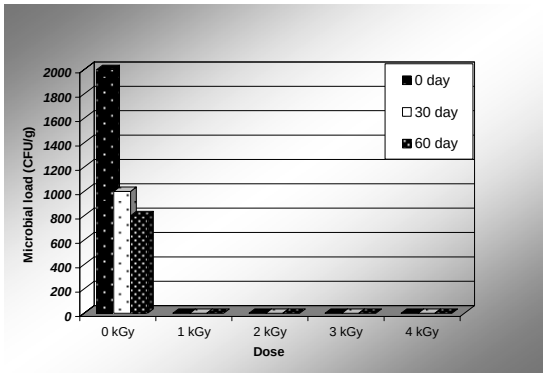

Fig. 3 Effect of irradiation treatment on Mold & yeast Count at zero, 30 and 60 days after irradiation

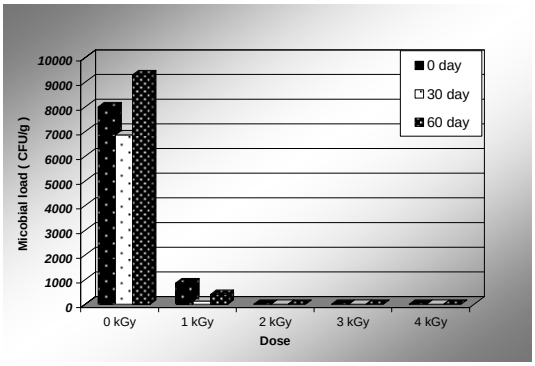

Fig. 4 Effect of irradiation treatment on E.coli Count at zero, 30 and 60 days after irradiation

A.Chemical analysis of Quality Parameters

For the spectral measurements of flavor, aroma and color in 251, 330 and 442 nm for picrocrocin, saffranal and crocin respectively. No significant qualitative changes were observed in these constituents upon irradiation, although, changes of flavor color and aroma at 3, 4 KGY in compare with 1, 2 KGY decrease significantly but there was no significant change with control sample (0.0 KGY).

TABLE II
SENSORY ATTRIBUTES OF CONTROL AND IRRADIATED SAFFRON SAMPLE DURING STORAGE (60 DAYS

Time (day)	DOSE (kGy)	Flavor [a](Mean ± SE)	Aroma [b](Mean ± SE)	Color [c](Mean ± SE)
0 DAYS	0	1.646 ± 0.007	0.856 ± 0.003	0.612 ± 0.008
	1	1.674 ± 0.002	1.346 ± 0.005	0.654 ± 0.006
	2	1.862 ± 0.011	1.376 ± 0.004	1.176 ± 0.002
	3	2.016 ± 0.003	1.684 ± 0.003	1.470 ± 0.005
	4	1.824 ± 0.002	1.282 ± 0.004	1.200 ± 0.002
30 DAYS	0	0.290 ± 0.002	0.402 ± 0.004	0.002 ± 0.003
	1	0.002 ± 0.003	0.340 ± 0.005	0.002 ± 0.003
	2	0.324 ± 0.002	0.374 ± 0.003	0.150 ± 0.002
	3	0.348 ± 0.003	0.340 ± 0.002	0.300 ± 0.007
	4	0.406 ± 0.005	0.444 ± 0.001	0.360 ± 0.003
60 DAYS	0	1.032 ± 0.006	1.263 ± 0.004	0.003 ± 0.007
	1	1.248 ± 0.003	1.116 ± 0.005	0.006 ± 0.002
	2	1.179 ± 0.006	1.233 ± 0.002	0.045 ± 0.002
	3	1.260 ± 0.004	1.446 ± 0.002	0.030 ± 0.003
	4	1.440 ± 0.005	1.905 ± 0.002	0.846 ± 0.004

[a] For flavor and aroma intensity and 0 (tender) to 3 (tough)
[b] For flavor and aroma intensity and 0 (tender) to 3 (tough)
[c] Scale ranged from 0 (none color (red)) to 3 (strong color)

IV. CONCLUSION

The results clearly indicated that quality deterioration occurred even in well packed untreated control samples. Various doses of γ irradiation resulted in complete absence of microbial growth and contained the quality deterioration during storage saffron. In conclusion, irradiation dose of 3 kGy can be effective to control microbial growth in saffron and in extending their shelf life without any significant deterioration of quality constituents. This technology will enable food processors to deliver larger amounts of high quality saffron with extended shelf life.

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