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Study of Microbial Critical Points of Saffron from Farm to Factory in Iran

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***Abstract*—**In this research saffron samples were prepared from farms and sampling was done in four states contain : sampling from fresh saffron of petal with forceps , sampling from fresh saffron of petal by hands, sampling from dried sample by warm air in shadow, sampling from dried sample which dried by dryer. Samples collected and kept in sterile tubes and containers and carried to laboratory and maintained until experiment. Microbial experiments were performed to determine microbial load such as total count, Staphylococcus aureus, coli form, E.coli, mold and yeast. Results showed that in picking and drying stages the contamination amount increases in saffron samples. There was a significant difference between the microbial load of picked up saffron by forceps and by hands, and also between dried saffron by warm air in shadow and by dryer.

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***Keywords*—**saffron; contaminations; preparation method

I. Introduction

TODAY, saffron producing in Iran is a process which because the lack of techniques and hygiene in different parts of process such as harvesting gathering, handling, draying, packaging and storage, causes contamination increasing in saffron so the quality goes wrong. In addition this product`s quality is less than most countries standard and this causes many exporting problems of this valuable product each year. Consequently, Iranian saffron will not be able to compete 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. "Quality" is made up of many factors, beginning with guaranteed authenticity and secondly the assurance of non-adulteration. The process of preparation of saffron before packaging, contain:

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A. Picking

Picking crocus flowers requires intensive stoop-labor [1]: the flowers Are only a few centimeters above-ground and are frequently, but not always [2], surrounded by long thin leaves which must not be damaged—or else Daughter-corms will not form to replace the current generation. Flowering takes Place in autumn, lasts only two to three weeks [3] and picking is required Almost daily [4 ,1]: the flowers wilt rapidly And once this has happened they cannot readily be separated into their constituent Parts. Reports that whole flowers are "dried in the sun as such" and the stigmas later "picked by hand" should not be given much credence if the product is expected to be saffron in more than just name.

B. Separation

Cutting the style with its three attached stigmas is followed by physical separation from the petals and anthers. These stages are also traditionally labor-intensive but can be performed while seated in comfortable surroundings. One common Method of doing both simultaneously is to pluck the stigmas from the flower [1, 5], or with a fingernail. Mechanical cutting is possible theoretically but difficult in practice, to be followed by fan-separation [6, 7, 8]. A third reported separation method Consists of water-flotation [9, 10], almost guaranteed to result in a low-quality product because of leaching of water-soluble constituents, including the important color parameter.

C. *Drying*

Three market forms of saffron are known: "hay", "cake" and powder. The drying Process employed is always the most critical with regard to quality [11, 12]. The relationship between temperature and the time required To dry hay saffron—stigmas in the loose state [13]— To completion has been studied [3], but use of the resultant product to Estimate optimal-drying sensory conditions failed because, with hindsight, All volatiles had been lost. Proximate analysis of commercial saffron also shows that drying to zero moisture is not an appropriate model (see Table 1 in the chapter on saffron chemistry, this volume). Zarghami (1970) recommends "proper Roasting" at unspecified low temperatures [11]. Ameloti and Mannino (1977) write positively of a "fermentation process" [as opposed to a negative connotation for Fermentation during storage [1] but do not elucidate [14]. Charcoal Fires [1, 8, 15, and 16] are used for "Artificial heating" [5, 13] with few practical Details save a remark that "too much heat" destroys the aroma. Solar drying, in sun or in shade, has been used as an alternative to "artificial Heating" [7, 10, 17, and 18] even though it is almost

guaranteed to result in a photochemical decrease in color intensity (see the chapter on saffron chemistry, this volume). Drying by solar exposure may be “natural”, but as the resultant product shows, it is also crude; the constraint does not apply if sunlight is used only as the heat source, without exposure.The third drying method, apparently no longer in use, is for the production of Cake saffron [13, 19] For this method, a layer of stigmas approximately 6 cm thick was “kiln-dried” under the pressure of a board [20, 21, 22], first for 2 h at one unstated temperature and for a further 24 h at a lower one, also unstated. Our own trials were unsuccessful, possibly because insufficient raw material was available to build up a layer of adequate thickness. The statement that honey and safflower are added to the saffron cake [23] must be treated warily, use of honey may be justified technologically as a binder (doubtful), if permitted by local legislation and properly declared on the label; but the addition of safflower could constitute a *prima facie* case of adulteration. The final product has been described as a "compressed matted mass” [19].In Iran saffron pick and separate from the petals and anthers by hand. Saffron Drying is performed in two ways: drying by warm and dry air in shadow and drying by dryer. However primitive method (hot air) uses more in Iran. We select the best and biggest field of saffron in Torbat-e-heydarieh of khorasan that has best quality of color and flavor [24] and then sampling was done from picking to drying before packaging to determine contamination critical points. 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 physic 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].

II. Materials and Methods

A. Sample Preparation

2.1. *Sample Preparation*

The samples collected from the saffron field in Torbate-Heydarieh County of khorasan where the most amount of producing and the best quality saffron have in this region. Picking and separating performs by hand and drying carries out by 2 method (warm air & dryer) and then dried saffron transport to factory for packaging and carring to markets.

Samples collected and kept in sterile containers and carried to laboratory of Agricultural, medical and industrial research school- nuclear science and technology research institute (NSTRI), in Karaj for microbial tests.

2-2 *Enumeration of microbes*

Microbial analysis of staphylococcus aurous, total bacteria count coliform, E. coli, mold and yeast was carried out a day after the arrival day on 30 and 60 days. For total count of aerobic mesospheric 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 count agar media (merck) was added and incubated for 48h at 37^{oC} the number of viable bacteria colonies expressed as microbial counts (log CFU/g) (table 1). for st.aureus count 1 ml of each dilution was added into the sterile plate in duplicated Baird Parker Agar media (merk)

was added and incubated 48 h at 37 ^{oC} then colonies counted , 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 form 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). (Fig 1) 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}. (Fig 2)

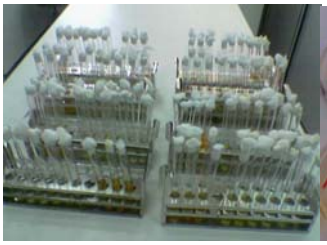

Fig. 1 Coli form growth in Lactose Broth (LB) Media

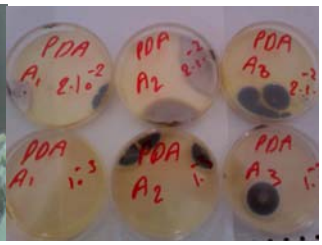

Fig. 2. Mold & yeast growth in Potato Dextrose Agar (PDA) media

TABLE I
MICROBIAL MEAN FOR SAFFRON SAMPLES IN TWO METHODS OF PICKING & SEPARATION (WORKER HAND & FORCEPS)

Type of Mic	Microbial loads (Mean ± SE)			
Kind of picking	Total count	St. aureus	E.coli	Coli form
By forceps	$2\times10 \pm 1.1 \times 10^2$	0	0	$3\times10^2 \pm 1.8\times10^2$
By worker hand	$4.66\times10^2 \pm 5\times10^3$	$1.1\times10^2 \pm 3.2\times10$	0	$3\times10^2 \pm 1.3\times10^2$

TABLE II
MICROBIAL MEAN FOR SAFFRON SAMPLES IN TWO METHODS OF DRYING (WARM AIR IN SHADOW & BY DRIER)

Type of Mic	Microbial loads (Mean ± SE)			
Kind of picking	Total count	St. aureus	E.coli	Coli form
By forceps	$1.05\times10 \pm 1.1\times10^2$	$1.5\times10^2 \pm 1.1\times10^2$	$2.3\times1 \pm 1\times10$	$2.5\times10^3 \pm 1.8\times10^3$
By worker hand	$7\times10^4 \pm 4\times10^4$	$8\times10^3 \pm 2.6\times10^3$	$7.5\times1 \pm 1\times10$	$8\times10^3 \pm 2\times10^3$

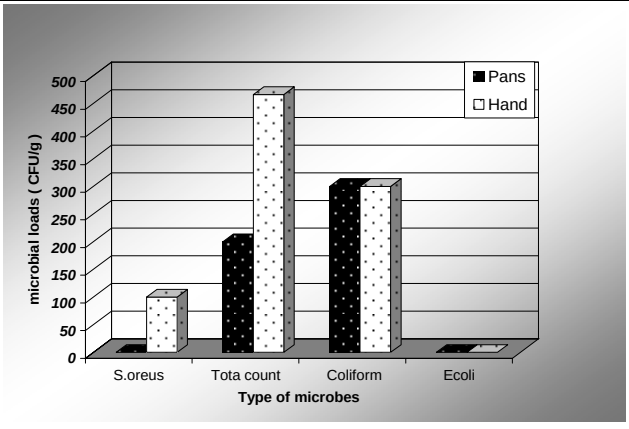

Fig. 3 Effect of picking on microbial load of saffron

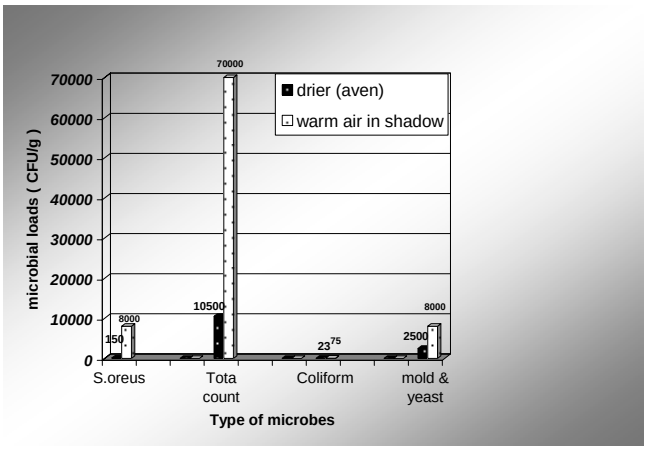

Fig .4 Effect of two types of drying on microbial load of saffron

Data dandy results showed that picking ad separation by hand (worker hand) increase microbial loads (total count, st.aureus) significantly. Also ancient method for drying (warm & dry place in shadow) increases microbial load (total bacteria count, st.aureus, coli form and mold & yeast) more than drying by drier.

2.4. *Statistical Analysis*

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

III. CONCLUSION

The effect of picking , separating and drying on microbial loads (total bacteria count , coli form , E.coli , staphylococcus aureus , mold & yeast) showed in (Fig 3 , 4) in conclusion , contamination level increases in saffron in two states (picking & drying), the results obtained from this study showed that two cp (critical point) exist before packaging: Contamination of hands, air, soil for picking and separation environment and material for drying and packaging.

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