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EFFECT OF GAMMA IRRADIATION ON THE TOTAL PHENOLIC CONTENT AND FREE RADICAL -SCAVENGING ACTIVITY OF IRANIAN DATE PALM *MAZAFATI* (*PHOENIX DACTYLIFERA* L.)

Fereshteh Ezzati Ghadi[1]*, Abdollah Ramzani Ghara[2], Taghi Ghanbari[3]

[1]Department of Plant Biology, Faculty of Science, University of Jiroft, Kerman, Iran; f.ezzati@ujiroft.ac.ir

[2] Department of Plant Biology, Faculty of Science, University of Jiroft, Kerman, Iran; a.ramzani@ujiroft.ac.ir

[3]Genetics & Agricultural Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Sari, Iran; tg_ghanbari3@yahoo.com

* Author to whom correspondence should be addressed; E-Mail: f.ezzati@ujiroft.ac.ir

Abstract- ***The present work was carried out to investigate the effects gamma rays on phenolic content and antioxidant activity of common date variety in Kerman Province, Iran, namely, Mazafati .In the present study, the radiation processing of dates samples was carried out at dose levels of 0, 0.5, 1 and 2.5 kGy. The phenolic content was measured by the Folin–Ciocalteau method and the antioxidant activity by reducing-power assay, total antioxidant capacity and DPPH methods. The results of the effect of different doses of gamma radiation (0.5 and 1kGy) on the dates shows that no significant effect on antioxidant and the amount of phenolic content was observed. Gamma irradiation of dates at 2.5 kGy increased antioxidant activity and total phenolic content. In summary, gamma irradiation(2.5 kGy) increased the total phenolic content, as well as enhancing the free radical-scavenging activity.***

Keywords - Date-palm fruit, Gamma ray, Phenolic content, Antioxidant activity

I. INTRODUCTION

Date palm is one of the oldest trees cultivated by man. Date fruits have a great importance from both a nutritional and therapeutic point of view [1,2]. They are rich sources of sugars, vitamins, minerals and fibers. In some date varieties, the sugar content of the fruits reaches up to 88%, and such fruits are considered a high-energy food source [3]. Moreover, date fruits possess antioxidant and antimutagenic properties [4,5], attributable to their high levels of polyphenolic compounds and vitamins [5,6].

The potential health benefits of fruits and vegetables have been partially attributed to their polyphenols contents, in particular flavonoids that have received much attention in the literature over the past decade for its biological effects [7,8]. Recent studies have reported that palm date has a potent ability to suppress free radicals using different methods [9,10] even within studies that found little contents of total phenols [11,5]. Date fruits are considered an excellent source of readily available energy (80-85% carbohydrate), supplying between 200 to 300 K cal./100 g, depending on the moisture content [12]. Dates also contain a moderate amount of thiamine, riboflavin and folic acid. They are a good source of the nutritionally important minerals such as iron, potassium, copper, sulphur and manganese. On the other hand, dates are relatively low in protein (2-3%) and fat (about 2-3%) [12].

The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases [13]. Studies have indicated that phenolic compounds are a major source of natural antioxidants in foods of plant origin [14] and exhibit a wide spectrum of biochemical activities such as antimicrobial, antimutagenic, anticarcinogenic as well as the ability to modify the gene expression [15,16]. Numerous epidemiological studies confirm significant relationship between the high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risks [17].

Reactive oxygen species (ROS) such as singlet oxygen (O_2), superoxide anion ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$) radical and hydrogen peroxide (H_2O_2) are often generated as by products of biological reactions or from exogenous factors [18,19]. The compounds that protect cells against the damaging effects of ROS are known as antioxidants. They result in a decrease of its toxic action and stop the radical-chain processes [20]. Antioxidants play a major role in protecting biological systems against many incurable diseases. Antioxidants have been widely used in different fields of industry and medicine as substances which interrupt radical-chain oxidation processes, improve general health, help cell rejuvenation, and prevent cancer [21].

Gamma irradiation as a phytosanitary treatment of food and herbal materials is increasingly recognized throughout the world. It improves the hygienic quality of various foods and herbal materials and reduces the losses due to microbial contamination and insect damage [22,23]. It reduces the reliance on chemical fumigants and preservatives currently used by the food and pharmaceutical industries. The chances

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of recontamination are also reduced, as it can be done after packaging. There is a growing scientific interest in the influence of irradiation processes on antioxidant activity and the compounds responsible for such activity. Several studies on plant materials showed that gamma irradiation does maintain or enhance antioxidant properties [23,25]. However, some studies have shown that gamma irradiation decreased the antioxidant properties [26,27] in plant materials.

The biological effect of gamma radiation is mainly due to the formation of free radicals by the hydrolysis of water, which may result in the modulation of an antioxidative system, accumulation of phenolic compounds and chlorophyll pigments [28-30]. Use of biotechnological approaches with low-dose irradiation treatment for enhancing the production of bioactive plant metabolites, such as, phenolic compounds, salicylic acids, coumaric acids, caffeic acids, flavonoids, and anthocyanins has been documented in medicinal plants [31]. The aims of this study were to evaluate the effect of gamma irradiation on phenolic contents and antioxidant activity of Iranian date fruit namely *Mazafati.*

II. EXPERIMENTAL SECTION

Fresh dates were collected from cultivars grown in the Jiroft of Iran. 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), gallic acid and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co., (St. Louis, MO). All the solvents and other chemicals used were of analytical grade from Sigma and Merck.

Sample Collection

The Iranian date palm fruit *Mazafati* were collected from agriculture organization of Jiroft, Iran. The fruit were segmented and their seeds were carefully removed and stored in sterile plastic bags in refrigerator.

Gamma irradiation

The samples of dates fruit were subjected to different doses (0, 0.5, 1 and 2.5 kGy) of gamma radiation using a ^{60}Co gamma source (Gammacell 220). The irradiations were carried out at room temperature.

Extraction of the Phenolics

Forty grams of date fruit were pitted, crushed and cut to small pieces with a sharp knife and blended for 3 min. Each sample was macerated in 120 ml methanol:water (80:20, v/v) for 10 h at room temperature and filtered with filter paper Whatman No. 1. After filtration, the alcohol is removed under vacuum at 40°C and then evaporated to dryness using a rotary evaporator.

Biological Activity

Antioxidant activity by reducing-power assay

The ability of extracts to reduce iron (III) was assessed by the method of Yildirim *et al*. [32]. The absorbance was measured at 700 nm. High absorbance indicates high reducing power.

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Shimada [33]. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

DPPH scavenging activity (%) = [Absorbance of control - Absorbance of sample]/ Absorbance of control

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH [34]. The absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample.

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin-Ciocalteu reagent method [34] and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve:

$Y= 0.001X+ 0.022 \qquad R^2 = 0.9982$

where Y is the absorbance and X is concentration as Gallic acid equivalents (µg/ml).

Statistical analysis

The data were analyzed by ANOVA. Each test was conducted with 3 replications. Comparison of the mean points was done by LSD test ($P<0.05$) in SPSS. The diagrams were drawn in EXCEL.

III. RESULTS AND DISCUSSION

Biological Activity

Total phenolic content

The total phenolic content of irradiated and non-irradiated samples of dates was determined using the Folin–Ciocalteu's phenol reagent. The results are expressed as mg equivalents of gallic acid/g dry weight of extract and given in Figure 1. For radiation-processed samples, the data (Fig. 1) showed significant ($p < 0.01$) increases in the total phenolic contents of irradiated as compared to control at dose level of 2.5 kGy. Moreover, no significant changes in phenolic contents were observed in the following 0.5and 1 kGy gamma irradiation as compared to control.

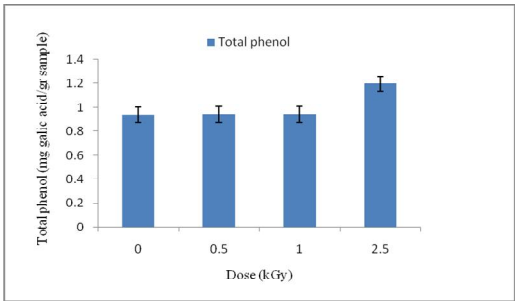

Fig.1 Effect of irradiation treatment on total phenolic content of *Mazafati* date fruit.

Antioxidant activity by reducing-power assay

Figure 2 illustrates a comparison of mean absorption capacity of different concentrations extract of date fruit at different gamma doses (0, 0.5, 1 and 2.5 kGy) at wavelength 700nm. Result shows that no significant changes were observed at dose levels of 0.5 and 1 kGy as compared to control sample. Whereas, results reveal that significant increase in antioxidant activity of date palm fruit after irradiation at dose level of 2.5 kGy.

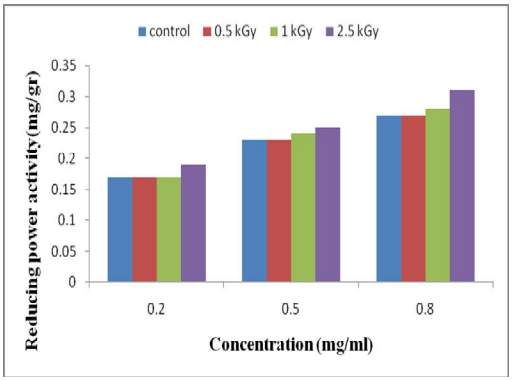

Fig. 2 Effect of irradiation treatments on antioxidant activity by reducing-power assay of *Mazafati* date fruit.

DPPH radical scavenging activity

Results in figure 3 show that regarding capacity to control free radicals, no significant changes were observed between control and irradiated sample at dose levels of 0.5 and 1 kGy. Moreover, gamma irradiation at 2.5 kGy significantly increases the radical scavenging activity of *Mazafati* dates. Furthermore, this characteristic increased along with increase in concentration of the extracts.

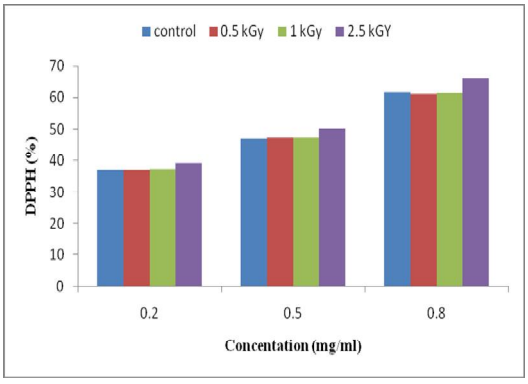

Fig. 3 Effect of irradiation treatments on DPPH of *Mazafati* date fruit.

Total antioxidant activity

Mean absorption capacity of date fruit are compared in figure 4. Results showed that there is significant increase ($p<0.05$) among different concentration of date fruit at dose level of 2.5 kGy regarding the absorption capacity. As results shown in Figure 4 the total antioxidant activity reveal no significant changes between control and irradiated samples at dose level of 0.5 and 1 kGy.

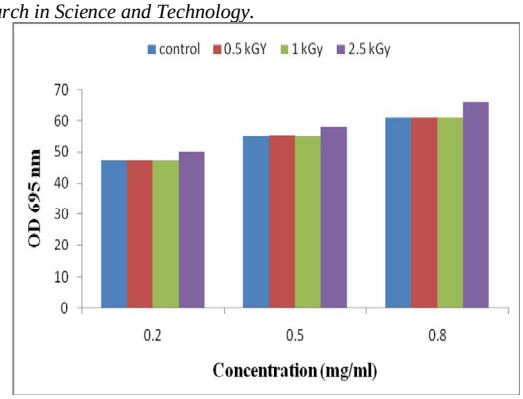

Fig. **4** Effect of irradiation treatments on total antioxidant activity of *Mazafati* date fruit.

Discussions

Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids and contribute to overall antioxidant activities in the plants. However, for plant materials, diverse effects of radiation on the phenolic content have been reported. Variyar, Bandyopadhyay, and Thomas [35] found increased amounts of phenolic acids in irradiated cloves and nutmeg. Harrison and Were [36] also reported increases in total phenolic content of gamma-irradiated almond skin extract, as compared to the control samples. Similarly, Huang and Mau [37] reported a higher content of tocopherols in irradiated than in non-irradiated lyophilised mushrooms. These increases in phenolic contents were associated with the degradation of tannins [38] and changes in the conformation of the molecules [39], as a result of the irradiation treatment. In contrast, Koseki *et al*. [40] reported a decrease in the amount of total phenolic compounds in dehydrated rosemary after irradiation doses of between 10 and 30 kGy, with respect to controls. The difference in the effect of radiation on total phenolic content may be due to plant type, geographical and environmental conditions, state of the sample (solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, dose of gamma irradiation, etc. Gamma irradiation is known to increase the activity of phenylalanine ammonia‐lyase which is responsible for the synthesis of polyphenolic acids [41]. Increase in the production of phenolics was observed in *Pterocarpus santalinus* when subjected to different doses of gamma radiation [42]. The *T. arjuna* seedlings exposed to increasing radiation doses up to 150 Gy showed a higher quantity of phenolic compounds, which are a major component of the plant secondary metabolites, with medicinal importance. The Increase in total phenols and total flavonoids contents in irradiated plants had been reported by Lee *et al*. [43]. Such increase in total phenols and total flavonoids is due to the release of phenolic compounds from glycosidic components and the degradation of larger phenolic compounds into smaller ones by gamma irradiation as suggested by Harrison and Were [36]. Irradiation exerts its effects as direct and indirect mechanisms. In case of indirect mechanism, radiolysis of water results in the production of free radicals such as hydroxyl radicals, hydroperoxide radicals and hydrated electrons. These radicals may break the glycosidic bonds of procyanidin trimer, tetramer and hexamer that are present in plants, leading to the formation of

procyanidin mono- mers, which increase the total phenolic and total flavonoids content in irradiated plants Lee *et al*. [43].

In plant tissues many phenolic compounds are potential antioxidants: flavonoids, tannins and lignin precursors may act as ROS scavenging compounds. Observed increase in total phenolic and tannin contents was beneficial for antioxidant proper ties of irradiated soybean seeds due to polymerization of phenolic constituents and also cross-linking and fragmentation, which were the key reactions controlling the properties of macromolecules such as proteins [44,45]. These results are in harmony with that reported by Mahmoud [46], Nassar *et al*. [47] and Moussa [48] who reported an increase in carbohydrates and soluble sugars in response to plant irradiation. Fan [49] studied the effects of ionizing radiation (0, 0.5, 1, and 2 kGy; followed by storing at 8 °C for 8 days) on antioxidant capacity, phenolics content, and tissue browning of three vegetables (Romaine and Iceberg lettuce, and endive). Their results revealed enhancement in the phenolic content and antioxidant capacity of both tissue types (midrib and non-midrib leaf tissues) of all vegetables at days 4 and 8 after irradiation. This increase in phenolic content and antioxidant capacity was attributed to increased phenolic synthesis contributing to the total antioxidant capacity.

Thus, it seems that 2.5 kGy of irradiation might induce some chemical reactions in components of dates, which possibly degrade or decompose large molecules into small phenolic molecules, which are readily soluble in methanol and may also be beneficial for the antioxidant properties of the plants.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability assay is widely used to evaluate the free radical scavenging capacity of antioxidants [50]. The decrease in the level of free radicals with the increase in the concentration of the palm date extracts indicates their role extract as antioxidants. The DPPH scavenging ability of date palm extract could be explained by higher availability of different antioxidants. Several studies indicated that palm dates contained flavonoids, such as luteolin, quercetin, and apigenin, as well as phenolics, such as *p*-coumaric, ferulic, and sinapic acids, and cinnamic acid derivatives. Accumulation of free radicals can damage cells at the level of nucleic acids, membrane lipids, and proteins, leading to generation of cancer and aging related diseases [51, 52]. A strong correlation between the antioxidant activity and the total phenolic and total flavonoids of palm dates was also recorded [53]. A research study conducted by Jo *et al*., [25] indicated that the scavenging ability on DPPH radicals was increased in green tea extracts following irradiation at 10 and 20 kGy. Variyar *et al*., [35] also found that the soybean scavenging ability on DPPH radicals increased with gamma irradiation doses from 0.5 to 5 kGy. On the other hand, Lampart-Szczapa *et al*., [27] reported that increased doses of irradiation decreased the antioxidant effects of lupin seed extracts. Ahn *et al*. [26] found that, immediately after irradiation at 2 kGy, the scavenging ability of Chinese cabbage was reduced. According to a Huang and Mau [37] report, methanol extracts of irradiated freeze-dried mushrooms did not show significant modifications in their scavenging activity as a result of irradiation doses between 2.5 and 20 kGy. Byun *et al*.,[24] observed no significant changes in the scavenging abilities of non-irradiated and 5, 10 and 20 kGy-irradiated Chungkookjang and Doenjang.

IV. CONCLUSIONS

The aim of this study is to investigate the effects of irradiation treatments on the quality properties of the phenolic content and antioxidant activity of the *Mazafati* date fruit. Irradiation treatment was found to be superior for improving the quality of date fruit.

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AUTHOR CONTRIBUTIONS

Fereshteh ezzati ghadi, Abdollah ramzani ghara designed the research; Fereshteh ezzati ghadi, abdollah ramzani ghara and Taghi ghanbari performed the experimental work; Fereshteh ezzati ghadi wrote the manuscript. All authors discussed, edited and approved the final version.

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