

STUDY THE EFFECT OF GAMMA RAYS ON CHEMICAL COMPONENTS, ANTIMICROBIAL ACTIVITY AND THE NUTRITIONAL VALUE OF DATE SEEDS

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Abstract. The objective of this study is to investigate the effect of treatment of three varieties of date (Bondoky, Sakkoty and Kaboshy) with gamma rays (0, 2.5, 5 and 10 kGy) on the chemical components and bioactive compounds of seeds and maximizing the benefit of these compounds, especially phenolic compounds, which have an important role as antioxidants as well as antimicrobial activity of seed extracts. Also, the fatty acids composition of seeds oil was assessed. The data indicated that most of the chemical components of the seeds were affected by irradiation in all varieties, as the content of protein and fat increased while the moisture content decreased by increasing the radiation dose compared with the control (untreated seeds). The content of sugars differed according to the variety, since Sakkoty variety has the highest content of sugars, followed by Kaboshy and Bondoky varieties. Moreover, the observed increase in antioxidant and antimicrobial activity was parallel to increase in phenolic compounds and flavonoids content due to irradiation treatment. There was an increase in saturated fatty acids and a decrease in polyunsaturated fatty acids with irradiation. Our experimental trial revealed that date seeds are rich source of bioactive compounds such as phenolic compounds and flavonoids and have antioxidant and antimicrobial activities which enhanced by irradiation treatment that may help develop antimicrobial drugs.

Keywords: Gamma rays; bioactive compounds; antioxidant and antimicrobial activity; date seeds.

INTRODUCTION

Date palm trees are very important trees in the Middle East countries because of their great benefits and high nutritional value, which protect the human from many diseases. Egypt is the world's first producer of dates in the world ahead of Iran and Saudi Arabia, according to the United Nations Food and Agriculture Organization (FAO). Annual production of dates is estimated at 1.5 million tons (17.7% of world production). The date fruit consists of a fleshy pericarp and seed, which represents about 10 to 15% of date fruit weight [21]. The date seeds are the waste produced during the processing of dates for use in many industries such as the confectionery and food industry waste. Currently, seeds are used mainly for animal feeds. Seed characteristics of date varieties vary depending on species and environmental conditions. Factors that affect the physical properties of date fruits, fertilization, irrigation, temperature and postharvest treatments. These factors can affect date seeds [18]. The date seeds contain a wide range of nutritional functional compounds such as protein (2.30-6.40%), fat (5-13.20%), moisture (3.10-7.10%), ash (0.9-1.80%) and dietary fiber (22.50-80.20%) [5]. This difference in dietary fiber is related to maturation stage and variety. Also, the seeds contain high levels of phenolic compounds (3.10-4.43%), antioxidants (580-929 μm trolox equivalents/g fresh weight). Thus, it is an important source of natural antioxidants [4]. The nutritional value of date seeds is also due to their high content of dietary fiber, which is of therapeutic importance in some cases such as diabetes and obesity. Date seeds have been used to make caffeine-free coffee [8] and also, listed in folk remedies for the management of diabetes, liver diseases and gastrointestinal disorders in traditional Egyptian medicine [14]. It has a protective effect against

hypertension, coronary heart disease (CHD), metabolic disorder, prostate cancer and intestinal disorders [36]. Also, dates seeds are rich in many minerals such as Na, K, Mg, Ca, P, Fe, Mn and Zn [1]. Due to the continuous increase in the number of pathogenic microorganisms that resist drugs, the composition of antibiotic and antifungal must be updated or addition of a new active agent has become a challenging research field [11]. Antioxidant activity is related to the presence of phenolic compounds and flavonoids in the plant, and there is a close relationship between them [24]. It is considered natural sources of antioxidants that protect the body from many diseases, instead of synthetic antioxidants such as butylated hydroxytoluene or butylated hydroxyanisole, which may cause cancer [2].

This work was conducted to study the effect of treatment of three varieties of date with different doses of gamma rays on the nutritional & chemical composition and antimicrobial activity of date seeds.

MATERIAL AND METHODS

Sample of dates

Three date (*Phoenix dactylifera* L.) varieties (Bondoky, Sakkoty and Kaboshy) were purchased from the local market in Cairo, Egypt. Mature fruits of uniform size, free of physical damage, insects injury and fungal infection were selected and used in the study.

Chemicals

The chemicals, solvents and media used for microbiological assay were of analytical grade and purchased from Merck (Darmstadt, Germany).

Irradiation treatments

Date varieties were irradiated with gamma rays Co^{60} at doses (0, 2.5, 5 and 10 kGy with a dose rate of: 1.9 kGy/h) in the National Center for Radiation

Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt.

Preparation of date seeds

After removing the date flesh, the seeds were washed to get rid of any adhering date flesh and air-dried. The seeds were oven dried for 2 days at 50 °C. Date pits of each variety were separately milled and subjected to the following analyses.

Proximate analysis

Dried seeds were used for determination of proximate analysis; moisture content, ash and fiber were determined by [7] methods. Crude protein was calculated as $N \times 6.25$ [22], while total fat was determined in accordance with [12]. The amount of total carbohydrates was obtained by method of [29].

Sugars content

For mono and di saccharides extraction, dried samples (0.1 g) were dissolved in 100 ml distilled water. All samples and the standard solution were filtered by using syringe filter (0.45 μm) and sugars determined using HPLC with refractive index (Agilent 1200 series) at 80 °C column temperature according to [6] method. Results were given by g/kg DW.

Methanolic extract of date seeds

Powder samples of date seeds were soaked in methanol 80% for 48 hours with shaking [34]. After filtration and solvent evaporation at 40°C using rotary evaporator, the extracts were re-dissolved in methanol and used to determine:

Phenolic compounds content

Phenolic compounds content was determined according to the method of [35] using the Folin-Denis reagent. The results were expressed as g of gallic (GAE) equivalent / 100g date seeds.

Flavonoids content

Flavonoids content was determined in the methanolic extract by the aluminum chloride colorimetric assay as described by [27]. The absorbance was measured against the blank at 510 nm and total flavonoids were expressed as g quercetin (QE) equivalent / 100g date seeds.

Hydrolysable tannins content

Hydrolysable tannins content was determined according to a modified vanillin assay [31]. The absorbance was read at 500 nm and total tannin content was expressed as g tannic acid equivalents / 100g date seeds.

Antioxidant activity by DPPH radical

The radical scavenging activity of the methanolic extracts against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical was determined as described by [17] at 517 nm.

The varieties that gave the highest content of phenolic compounds, flavonoids and antioxidant activity (Bondoky and Sakkoty) were selected to estimate the following analysis:

Evaluation of antimicrobial potentiality of the tested extracts

The used bacterial and fungal strains were supplied by the Department of Microbiology, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt. Antimicrobial activity of the tested extracts against the tested microbial strains (*Escherichia coli*, *Enterobacter cloacae* and *Bacillus cereus*, *Staphylococcus aureus* represent the Gram-negative and positive bacterial strains respectively, and *Candida albicans*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium sp.* represent fungal strains) was evaluated by disc diffusion assay. Whatman No. 1 filter paper discs, 6.0 mm in diameter, sterilized in dry heat at 80 °C in an oven for 1h were used to determine antimicrobial activity. Sabouraud dextrose agar and Muller Hinton agar media were prepared for fungal and bacterial strains, respectively. After sterilization, it was poured into sterilized petri plates and allowed to solidify. Using a sterile cotton swab, culture was swabbed on the surface of medium plates. The discs were loaded with 20 μl from each extract and aseptically placed over plates containing medium. Along with the tested extracts, discs of Nystatin (100 μg) as antifungal and Gentamycin (10 μg) as antibacterial taken as standards. Three replicates were maintained for each sample. The plates were incubated at 28 °C and 37 °C for fungal and bacterial strains respectively, and the zone of inhibition was observed after 4 days and 18h for fungal and bacterial strains, respectively. Control was maintained with filter paper discs dipped in methanol.

Oil extraction from date seeds

The oil extraction of date seeds was carried out in a Soxhlet apparatus using hexane. The fatty acids composition of date seeds oil was identified by GLC analysis.

Statistical analysis

All the statistical analyses were performed using an ANOVA, and we applied Duncan's multiple range tests [15] to compare the results of the experiments ($P \leq 0.05$) and the data were presented as the means \pm SD.

RESULTS

Proximate analysis

The proximate analysis of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation was shown in Table 1. The data indicated that the content of protein and fat increased by irradiation compared to the control (0 kGy) and the doses of 2.5 and 5 kGy gave the highest value of protein and fat, respectively, but crude fiber decreased and then increased by radiation. The highest content of crude fiber was obtained at a dose of 10 kGy compared with the control in all varieties of date seeds. On the other hand, moisture content decreased by increasing the radiation dose, where the dose of 10 kGy gave the lowest value of moisture content. Concerning of

Table 1. Proximate analysis of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation

Variety	Dose (kGy)	Crude protein %	Ash %	Crude fat %	Moisture %	Crude fiber %	Carbohydrate %
Bondoky	0	5.15±0.05 ^f	1.21±0.05 ^{abcd}	6.34±0.23 ^{ef}	7.71±0.26 ^{bc}	24.96±1.51 ^b	54.63±0.84 ^{cd}
	2.5	5.50±0.20 ^{cd}	1.24±0.09 ^{ab}	6.42±0.24 ^{cd}	7.60±0.22 ^{cd}	22.97±0.81 ^{ef}	56.27±1.83 ^b
	5	5.38±0.46 ^c	1.26±0.04 ^a	6.47±0.20 ^c	7.50±0.31 ^d	23.66±1.23 ^d	55.73±1.79 ^b
	10	5.21±0.16 ^f	1.27±0.03 ^a	6.36±0.27 ^{def}	7.50±0.30 ^d	26.45±0.73 ^f	53.21±1.84 ^c
Sakkoty	0	5.55±0.17 ^{cd}	1.16±0.05 ^{cd}	6.21±0.26 ^h	7.82±0.19 ^{ab}	23.46±1.71 ^{de}	55.80±1.03 ^b
	2.5	5.88±0.10 ^a	1.23±0.07 ^{abc}	6.32±0.19 ^{fg}	7.70±0.22 ^{bc}	20.94±0.89 ^h	57.93±1.13 ^a
	5	5.73±0.16 ^{ab}	1.22±0.08 ^{abcd}	6.41±0.21 ^{cde}	7.60±0.25 ^{cd}	22.92±0.92 ^f	56.12±0.79 ^b
	10	5.61±0.17 ^{bc}	1.23±0.06 ^{abc}	6.26±0.23 ^{gh}	7.60±0.29 ^{cd}	25.00±1.05 ^b	54.30±0.90 ^{cd}
Kaboshy	0	5.43±0.22 ^{de}	1.15±0.09 ^d	7.29±0.15 ^b	7.90±0.21 ^a	24.23±1.64 ^e	54.00±1.29 ^d
	2.5	5.75±0.14 ^{ab}	1.16±0.04 ^{cd}	7.37±0.28 ^a	7.80±0.34 ^{ab}	21.94±0.88 ^g	55.98±0.95 ^b
	5	5.63±0.27 ^{bc}	1.17±0.07 ^{bcd}	7.43±0.14 ^a	7.70±0.18 ^{bc}	23.20±0.97 ^{def}	54.87±1.42 ^c
	10	5.50±0.10 ^{cd}	1.18±0.08 ^{bcd}	7.28±0.22 ^b	7.70±0.26 ^{bc}	26.30±1.75 ^a	52.04±0.80 ^f

Values are mean of three replications ± standard deviation and different letters indicate statistically significant differences at P ≤ 0.05.

carbohydrate content, it was increased at dose of 2.5 followed by 5 kGy and then decreased by high dose (10 kGy) compared to control in all varieties of date seeds. Also, there was a slight increase in ash content by radiation doses. These results are consistent with those obtained by [23].

Sugars content

The results in Fig. 1 showed the effect of irradiation on sugars content (glucose, fructose and sucrose) of different seeds. The results were varying partly depending on the seed varieties of date. The content of glucose and fructose increased by using radiation doses (2.5 and 5 kGy) then decreased with high dose (10 kGy) and the highest content of glucose and fructose obtained by 2.5 kGy dose compared with control. On the other hand, the content of sucrose decreased by increasing radiation dose and the dose of 10 kGy gave the lowest content of sucrose compared with control.

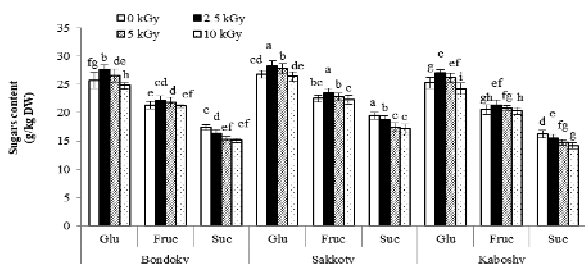


Figure 1. Sugars content (glucose, fructose and sucrose g/kg DW) of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at P ≤ 0.05.

Phenolic compounds, flavonoids and hydrolysable tannins content

Date seeds have a higher content of phenolic compounds than fruits of the same varieties [28]. The phenolic compounds content was increased by radiation doses in all date seed varieties (Fig. 2) and the highest value obtained by 5 kGy which gave 4.08, 4.20 and 2.16% in Bondoky, Sakkoty and Kaboshy respectively. Kaboshy seeds contain the lowest content of phenolic compounds, but Sakkoty seeds contain the highest content followed by Bondoky.

The same trend was found in flavonoid and hydrolysable tannin contents (Fig. 2) as their content increased by radiation. The highest content obtained from the dose of 5 kGy in all seed varieties which gave 2.40, 2.52 and 1.69% of flavonoids content and 3.32, 3.46 and 2.39% of tannins content in Bondoky, Sakkoty and Kaboshy respectively.

Antioxidant activity

Phenolic compounds and flavonoids are closely related to antioxidant activity. Increasing of phenolic compounds and flavonoids content resulted in enhancement of antioxidant activity. Data in Fig. 3 shown the scavenging activity% of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation. It is clear that the scavenging activity increased by irradiation and the dose of 5 kGy gave the highest content of scavenging activity (76.95%, 82.80% and 73.39%) in Bondoky, Sakkoty and Kaboshy respectively.

Antimicrobial activity

Table 2 showed antimicrobial activities of date seed extracts affected by gamma irradiation. The results indicated that the zone of inhibition around the discs varied according to date seed extracts and the sensitivity of bacteria. This may refer to differences in resistance of bacteria to anti-tested materials due to change in membrane permeability of cells, thereby hindering the entry or excretion of enzymes by the change in the chemical composition of the chemical constituents or by changing the nature of some of their components [33]. A dose of 5 kGy was more effective in inhibition of bacterial growth than antibiotic. On the other hand the fungal strains showed full resistance to all date seed extracts except *Candida albicans* and *Aspergillus parasiticus*.

Fatty acids composition of date seeds oil

The results of fatty acid composition of date seeds oil are presented in Table 3. The data indicated that oleic acid was the major unsaturated fatty acid in two varieties (Bondoky and Sakkoty), while the main saturated fatty acid was lauric acid in Bondoky variety and palmitic acid in Sakkoty variety. Capric, myristic, stearic, linoleic, linolenic, arachidonic and behenic acids were also found in two varieties. The data showed an increase in saturated fatty acids and a

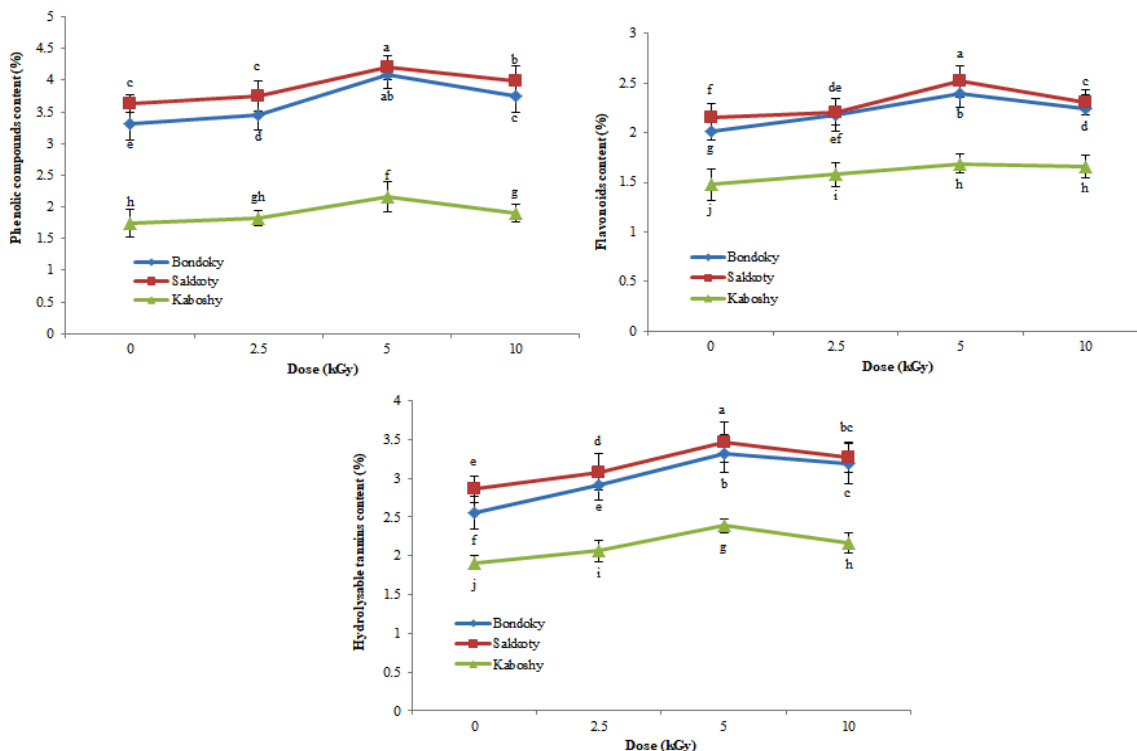


Figure 2. Phenolic compounds, flavonoids and hydrolysable tannins content (%) in extracts of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at $P \leq 0.05$.

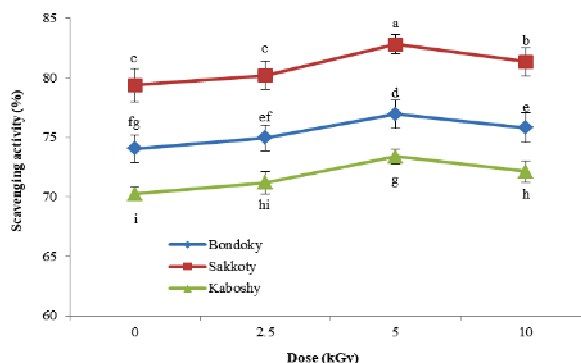


Figure 3. Scavenging activity (%) in extracts of date seed varieties (Bondoky, Sakkoty and Kaboshy) affected by gamma irradiation. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at $P \leq 0.05$.

Table 2. Inhibition Zone (mm) in extracts of date seed varieties (Bondoky and Sakkoty) affected by gamma irradiation

Microorganism	Bondoky				Sakkoty				CN	NS
	0 kGy	2.5 kGy	5 kGy	10 kGy	0 kGy	2.5 kGy	5 kGy	10 kGy		
Bacterial strains										
Gram-negative										
<i>Escherichia coli</i>	12±0.56	12.5±0.75	14.5±0.82	13±0.30	13±0.00	13.5±0.46	15.5±0.00	14±0.85	13±0.00	NT
<i>Enterobacter cloacae</i>	12.5±0.95	13±0.00	15±0.95	13±0.44	13±0.17	14±1.01	15.5±0.44	15±0.40	15±0.00	NT
Gram-positive										
<i>Bacillus cereus</i>	11±0.36	12.5±1.05	14±1.15	13±0.92	12±0.10	13±0.62	15±0.26	14±0.56	14±0.00	NT
<i>Staphylococcus aureus</i>	12±0.00	13.5±0.00	15±0.82	14±1.11	15±0.20	15±0.26	17±1.21	16±0.98	15±0.00	NT
Fungal strains										
<i>Candida albicans</i>	12±0.00	13±0.00	15±0.17	14±0.00	12±0.36	14±0.70	17±1.57	15±0.50	NT	10±0.00
<i>Aspergillus parasiticus</i>	9±0.00	11±0.00	12±0.92	11±0.00	11±0.66	12±0.30	14±0.00	12±0.00	NT	R
<i>Aspergillus flavus</i>	R	R	R	R	R	R	R	R	NT	R
<i>Aspergillus niger</i>	R	R	R	R	R	R	R	R	NT	10±0.00
<i>Penicillium sp.</i>	R	R	R	R	R	R	R	R	NT	12±0.00

CN (Gentamycin), NS (Nystatin), NT (Not tested), R (Resistant). Values are mean of three replications ± SD.

Table 3. Fatty acids composition of date seeds oil (%)

Compound name	Area %							
	Bondoky				Sakkoty			
	Cont	2.5 kGy	5 kGy	10 kGy	Cont	2.5 kGy	5 kGy	10 kGy
Caprylic (C8:0)	4.53	5.25	5.88	6.23	-	-	-	-
Capric (C10:0)	10.04	10.52	10.52	10.48	10.18	10.73	10.67	10.71
Lauric (C12:0)	12.78	12.53	12.29	12.33	10.62	10.42	10.28	10.32
Myristic (C14:0)	6.13	6.57	6.75	7.25	8.43	8.87	9.5	10.47
Palmitic (C16:0)	11.55	11.92	11.71	11.61	14.21	14.67	14.53	14.33
Stearic (C18:0)	4.89	5.43	4.59	4.41	5.02	5.4	4.9	4.79
Oleic (C18:1 n9)	41.28	42.89	43.23	42.91	41.56	42.28	43.85	43.69
Linoleic (C18:2 n6)	4.58	4.89	5.03	4.78	5.11	5.32	5.78	5.68
Linolenic (C18:3n3)	1.92	-	-	-	2.14	0.98	0.49	-
Arachidonic (C20:4n6)	1.64	-	-	-	1.71	0.69	-	-
Behenic (C22:0)	0.65	-	-	-	1.02	0.64	-	-
SFA ^a	50.57	52.22	51.74	52.31	49.6	50.88	49.89	50.62
USFA ^b	49.42	47.78	48.26	47.69	50.52	49.27	50.12	49.37
MUSFA ^c	41.28	42.89	43.23	42.91	41.56	42.28	43.85	43.69
PUSFA ^d	8.14	4.89	5.03	4.78	8.96	6.99	6.27	5.68
USFA/SFA	0.977	0.915	0.933	0.912	1.02	0.968	1.00	0.975

a: Saturated fatty acids

b: Unsaturated fatty acids

c: Monounsaturated fatty acids

d: Polyunsaturated fatty acids

decrease in polyunsaturated fatty acids with irradiation. Also, Linolenic acid is the most fatty acid that has been affected by irradiation. This result is harmony with [3], who reported that there were changes in unsaturated and saturated fatty acids by irradiation and these changes were significant in sesame and sunflower oils, but insignificant in peanut oil. On the other hand, [9] reported that radiation dose (3 kGy) did not stimulate any differences in monounsaturated and polyunsaturated fatty acids.

DISCUSSION

Date seeds are also called pits, pips, stones and kernels. They are rich in oil, proteins, minerals and fiber, so they can serve as valuable materials for feeding animals [20]. From the above results Sakkoty seeds contain the highest content of protein and carbohydrate while Kaboshy seeds contain the highest content of fat. There were differences in the chemical composition of date seed varieties (Bondoky, Sakkoty and Kaboshy), and these differences may be due to variability of varieties and climatic conditions [32]. The rise of crude protein by radiation may be due to the dissociation of complex protein molecules into simpler forms. However, the moisture may also have contributed to the observed increase in crude protein concentration, where the probability of a decrease in moisture can be associated with a corresponding increase in the relative quantity of the main components in the sample [10]. Carbohydrates and fats are more important than proteins in date seeds because the content of protein in date seeds is low and may not be easy to digest [19]. Also, Sakkoty variety had the highest content of sugars, followed by Bondoky and Kaboshy varieties, respectively. Also, the increase in sugars due to the irradiation can be attributed to the breakdown of complex sugars (polysaccharides) into simple extractable forms [10]. Date pits of Khalti varieties in Tunisia contain 6.88% moisture, 8.12% total sugars, 6.63% reducing sugar, 1.49% sucrose,

5.31% crude protein and 8.33% fat [32]. The results showed that Sakkoty and Bondoky varieties contain the highest content of phenolic compounds, flavonoids and tannins while Kaboshy variety contains the lowest content. These results are in agreement with [25] who reported that γ -rays doses up to 12.5 kGy increased the phytochemicals (phenolic compounds, flavonoids, tannins and saponins) in *Ziziphus mauritiana* leaves and this increase is due to the release of active components from their complex structures that have degraded by radiation. On the other hand, the irradiation doses (from 10 to 30 kGy) decreased the phenolic compounds content in dried rosemary [26]. This difference can be attributed to several factors, including plant type, environmental conditions, sample case, method of extraction, extraction solvent and radiation dose.

The powerful antioxidant activity in date seed extracts can be attributed to the high content of phenolic and flavonoid compounds. These compounds are an important group of natural antioxidants and have beneficial effects on human health [5]. This result was in harmony with that obtained by [25], who stated that radiation doses led to a significant increase in scavenging activity in dried rosemary. Date seeds extract showed antibacterial activity and the bacterial inhibition of date seed extract may be attributed to its bioactive components such as phenolic and flavonoid compounds [33]. Natural products are a particularly rich source of anti-infection agents. Phenolic and flavonoid compounds have antimicrobial activity as they play a role in inhibition of DNA gyrase [13]. Also, some large molecules may degrade into small molecules that have antibacterial activity due to gamma irradiation [25].

The relative amounts of UFA and SFA in oil are important for health and nutrition. The ratio of UFA/SFA is important for illustrating the harmful effects of dietary fats [30] and this ratio was used to predict the seeds shelf life as the low ratio refers to the

longer shelf life of the product [16]. In the current study, ratio of UFA/SFA decreased by irradiation.

It is clear that date seeds are an important source of protein, crude fiber and fatty acids especially oleic acid which is very important for health to protect against cardiovascular diseases and can be a useful source of edible oils. Date seeds are suitable for preparation of fiber based foods because of their high fiber content. They are also a rich source of bioactive compounds such as phenolic compounds and flavonoids, so they have antioxidant and antimicrobial activities that may help develop antimicrobial drugs. Moreover, irradiation has positive effects on the chemical composition of seeds. It also enhances antioxidant and antimicrobial properties of seed extracts.

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