Comparing the effects of thermal and irradiation treatments on reducing the levels of acrylamide and furan and improving the antioxidant properties of semi-dried dates.

Serag A Farag[1], Noha M. Mohamed[2]*, Sayed Rashad[2]

[1]Department of food Irradiation, National Centre for Radiation Research &Technology, Nasr City, Egypt
[2]Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt

Abstract

Many thermally processed foods contain the potential carcinogens furan and acrylamide. They are acknowledged as hazardous and "*potentially carcinogenic to humans,*" according to the International Agency for Research on Cancer. The present study's objectives are to investigate the potential impact of low heat and low irradiation doses on reducing the levels of contaminants such as acrylamide and furan that are present in semi-dried dates, and examine the impact of thermal treatment and irradiation on the antioxidant activity, polyphenol content and butylated hydroxytoluene (BHT) concentration of semi-dried dates. The results of the study show that the thermal treatment of date fruits resulted in a significant increase in the levels of both acrylamide and furan in dates by 30% and 7%, respectively. On the other hand, the irradiation treatments resulted in a significant reduction of acrylamide and furan by 30 and 53%, respectively for the dose 3.0 KGy and by 68 and 81.2%, respectively for the dose 5KGy. These findings imply that low irradiation doses may have commercial applications, such as decontamination or disinfestations, and that they may even make it simple to reduce acrylamide and furan levels to safe and healthful levels at the same time.

Keywords: Electron beam accelerator , Irradiation, Date fruits, Thermal treatments , Acrylamide, Furan.

Introduction

Date (*Phoenix dactylifera L*) is an important fruit worldwide. Date fruits are amongst the most frequently consumed all the year in Arabic countries. Dates are healthy fruits owing to their high content of vitamins, sugars, antioxidants, polyphenols and flavonoids [1]. After harvest, dates could be processed in different ways. Dates processing comprises cleaning, grading, heating, drying, fumigating, irradiating, moistening, pitting, coating, packing, and pasteurization. The products made from date palms include unfinished and ready-to-use date products, items made from dates such date syrup and date juice, date spread and date sugar in liquid form [2]. During fruits processing, some toxicants such as heterocyclic aromatic amines, acrylamide, furan and acrolein could be produced. Acrylamide (AA) and Furan are two possible carcinogens commonly produce in processed food due to thermal processing [3].

Acrylamide ($CH_2{=}CHC(O)NH2$) is an unsaturated amide; it can be present in a variety of thermally processed foods. The efforts to reduce acrylamide content in foods have been in the forefront of the food safety authorities ever since Swedish scientists discovered an unfavorable acrylamide concentration in heat-treated meals in 2002. When heated at a high temperature (over 120°C), acrylamide is produced in food products with a high quantity of reducing carbohydrates like glucose and proteins, particularly those that are rich in the amino acid asparagines [4]. Furan (C_4H_4O) is cyclic dienyl ether, a low boiling point of 31.4°C, and poor water solubility. Its derivatives are naturally occurring substances produced in a variety of heat-processed foods and beverages; these substances have a low threshold for odour and greatly influence the sensory qualities of heated meals and beverages over 150°C [5]. Several international food bodies, including the US Food and Drug Administration (US FDA) and the European Food Safety Authority (EFSA) have recently given the existence of furan in foods a lot of attention. The initial findings on the presence of furans in a number of chosen foods, primarily heat-processed infant foods packaged in jars and cans [6]. Acrylamide and furan are classified as "*reasonably anticipated to be a human carcinogen*" by different international agency [7].

Thus, the food processing sector is currently faced with the task of reducing these toxicants without impairing the beneficial effects of heat processing. Recently, irradiation became safe durable alternatives for decontamination,

***Correspondence to:** Noha M. Mohamed, Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt, E-mail: noha_mahmoud31@yahoo.com

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disinfestations of stored food products including dates. Irradiation sources such as gamma or electron beam (EB) became applicable, economical use for food preservation on large scale [8]. In Egypt, irradiation has been commercially used since 1977 for sterilization of dried foodstuffs, herbs, and medical products [9]. Numerous gamma irradiation researches have concentrated on the radiolysis and radical scavenging properties of polyphenols. Recent research, however, have turned their attention away from radiolytic effects and onto the biological impact of gamma irradiation-induced changed structures. Because several studies found that the flavonoid structures were preserved after irradiation, it is assumed that the altered functional groups caused by irradiation lead to increased or novel biological effects [10].

The aims of the present study are (1) examining the possible role of low heat and low irradiation doses on suppressing the levels of acrylamide and furan present as contaminants in semi-dried dates; and (2) investigating the effect of thermal treatment and irradiation treatment on the antioxidant capacity, polyphenols and flavonoid content of semi-dried dates.

Materials and methods

Samples

Ten kilo grams of Saudi Arabian mature date variety (Sukkary, semi-dry) were obtained from an orchard near Al-Ahsa governorate in Saudi Arabia. The dates were directly collected after harvesting and completely sun dried and was not fumigated.

Heat treatment and irradiation process

Date samples were divided into four groups: (1) control (untreated samples), (2) thermally treated at 90°C by electrical oven for 2 hours, (3) irradiated at a dose of (3.0 kGy) and (4) irradiated at a dose of (5.0 kGy). The irradiation process was done at the laboratory of food irradiation department, National Center for Radiation Research and Technology (NCRRT), Nasr City, Cairo, Egypt using an electron beam Accelerator (EBA) as presented in (**Tables 1 and 2**). Each treatment was replicated five times.

Chemical analysis

The different date samples were subjected to different analysis in the laboratories of the Regional Center for Food and Feed at the Agricultural Research Center, Giza, Egypt.

Determination of acrylamide and furan in dates

The determination of acrylamide and furan in dates was done using gas chromatography-mass spectrometry GC/MS/MS (Agilent 7890N GC with Agilent 7000 MSD with Dynamic headspace), auto-sampler, GC column: HP-5, 15 m, 0.32 mm I.D., 20 µm film.

The procedure employed for acrylamide determination in dates was done following the method described by

[11].The extraction of acrylamide was done by mixing 25 g of each sample with 75 ml of water and the mixture was allowed to swell in a water bath for 30 minutes at 70°C. Then, 10 g of the homogenate were thoroughly mixed with 40 ml of 1-propanol. After centrifugation, 10 ml of the supernatant was mixed with 200 mg of vegetable oil and subjected to evaporation using a rotary evaporator. Finally the residue was extracted with acetonitrile and defatted with hexane and Butyramide solution was added as an internal standard solution.

For furan extraction, the approach suggested by [12] was employed with some modifications. The samples were processed into slurries by macerating for the ideal amount of time at room temperature, according to their weight, and then homogenizing with a homogenizer. Then 6 g part of the homogenized sample was added to chilled headspace vials holding an optimum concentration of 20 percent NaCl (W/V) solution, with a material to solvent ratio of 1:1 (solvent refers to water content and material refers to each sample concentration in that water). For further analysis, each vial was immediately sealed and submerged in an ice bath.

Determination of total antioxidant capacity

The total antioxidant activity of date samples was determined by the "*phosphomolebdenum assay*" using ascorbic acid as a standard antioxidant. The procedure described by [13] was followed and the total antioxidant activity was expressed as mg Ascorbic Acid Equivalent (AAE)/ 100 g.

Determination of total phenolic compounds

Total phenolic content (TPC) of the extract was determined according to the Folin Ciocaleau method [14]; Gallic acid (GA) was used as a standard and the results were expressed as mg gallic acid equivalent (mg GAE)/ 100 g extract.

Table 1. *Electron beam accelerator conditions.*

Conditions	Characters
Room Temperature	20^0C
Max.Voltage	3.0 MeV.
Max.Beam Current	30 Ma
Max.Conveyer Speed	16 m/min(50HZ)
Scan Width	8 cm X 90 cm
Min.Dosimeter	2 kGy
Deep Penetration	1 gm/1 cm^3

Table 2. *Dose conditions for samples.*

Dose	HV	Beam	Conveyer speed
3.0kGy	2.5 MeV	4mA	12.73 m/min-40 Hz
5.0kGy	2.5MeV	4mA	7.6 m/min-23 Hz

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Determination of Butylated hydroxytoluene (BHT)

BHT content was determined by a HPLC system Agilent (1260 series) with auto sampler, a UV detector, a quaternary pump and a thermostatted column compartment. The procedure for extraction and determination of BHT was done following the method described by [15] with some modifications.

Determination of DPPH radical scavenging activity

The free radical scavenging activity of date extract on the stable radical 1, 1diphenyl-2-picrylhydrazyl (DPPH) was also evaluated as earlier reported by [16]. Different extract concentrations were tested and the IC50 defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay was calculated as mg/ml.

Statistical analysis

Five replicates were performed for each analysis and the values were expressed as mean ± S.D. One-way analysis of variance (ANOVA) with significant differences between means determined at $p < 0.05$. A Linear regression analysis was applied using Excel program; a Microsoft computer software to get value of R2. All values were average of three replicates with standard deviation.

Results and Discussion

Effect of different treatments on Acrylamide and furan content of semi dry dates

Acrylamide and furan are well-known as thermal process contaminants in foods as a Maillard reaction (MRPs) products [17]. In the present work, the results of acrylamide and furan concentrations in different date samples are shown in **(Table 3).** The data revealed that the semi-dry dates (control) contain acrylamide and furan at concentrations of 10.0 ± 0.3 and 11.6 ± 0.6 ppb, respectively. This could be one of the first reports to detect the presence of acrylamide and furan in low concentration in date fruits (control). The presence of these two compounds could be due to the high temperature and low moisture conditions in desert areas where dates are grown. Some workers showed same levels of acrylamide in processed dates in the range (20-110 ppb) (FDA 2015).

High temperatures are required for the synthesis of acrylamide and furan, with the Maillard reaction serving as the primary mechanism. But these compounds could exhibit various characteristics. In fact, acrylamide is a harmful consequence of the Maillard reaction that may be found in a range of low-moistureheatedmeals. Acrylamideiscreated whenthecarbonyl group of sugars reacts with asparagine. Distinct reactions that take several different paths and use various precursors and intermediates produce furan which is usually present in almost all heated foods in rather significant concentrations [18]. The results of the present study show that the thermal treatment of date fruits resulted in a significant increase in the levels of both acrylamide and furan in dates comparing to control by 30% and 7%, respectively. According to [19] furan is a member of a group of substances that develop the tastes of much food. The thermal breakdown of carbohydrates including glucose, lactose, and fructose is thought to be the main source of furan and its compounds in diet. Some thermally degraded carbohydrate products, including glucose, contain furan, the most basic type of furans.

On the other hand, the irradiation treatments resulted in a significant reduction of acrylamide and furan by 30 and 53%, respectively for the dose 3.0 KGy and by 68 and 81.2%, respectively for the dose 5KGy. It has been confirmed that the non-thermal approach of ionizing radiation is commonly used to ensure food safety and disinfection. Irradiation is also used in the food processing industry to ensure food safety while preserving the nutritional, textural, and organoleptic qualities of food to some extent [20]. The decrease of acrylamide and furan concentrations upon irradiation treatments was in consistence with previously reported results [21,22].

The results also showed a linear strong correlation ($R2 > 0.9$) between the irradiation dose and the decrease in both acrylamide and furan concentrations in dates as presented in **(Figure 1)**. These results are consistent with the findings of [23] who stated that the radiation dosage will have an impact on how furan levels are affected by radiation. According to the same authors, radiation can have an impact either directly or indirectly. Ionized particles or rays directly target food constituents like proteins and DNAs in direct action. Through the radiolysis of water, which produces radicals such hydrated electrons, hydroxyl radicals, and hydrogen atoms, irradiation exerts its effects through the indirect process. These radicals then target food-related molecules. Given how well irradiation reduced furan and acrylamide, it appears that the two chemicals are destroyed by the radiation through an indirect process.

Effect of different treatments on the antioxidant capacity, phenolic content, BHT and DPPH scavenging activity of dates

The effect of tested treatments on the antioxidant capacity, phenolic content, BHT concentration and DPPH scavenging activity of dates is shown in **(Table 4)**. The results show that the different treatments applied to dates resulted in significant enhancement in the dates' antioxidant capacity, polyphenols and BHT content and DPPH scavenging activity. The data in **(Figure 2)** shows linear relationships between the irradiation dose and tested parameters. The highest correlation was obtained for DPPH scavenging activity (R2=0.75), then total antioxidant activity (R2=0.73) followed by total phenols (R2=0.63) and the weakest correlation was for BHT (R2=0.53).

Table 3. *Acrylamide and Furan contents (ppb) in date samples.*

Treatments	Acrylamide	Furan
Control	$10.0^{b} \pm 0.3$	$11.6^{b} \pm 0.6$
3.0 kGy	$7.0^{c} \pm 0.4$	$5.48^{c} \pm 0.4$
5.0 kGy	$3.2^{d} \pm 0.5$	$2.18^{d} \pm 0.3$
Thermal 90^{0}C/2hrs	$13^{a} \pm 0.4$	$12.4^{a} \pm 0.3$

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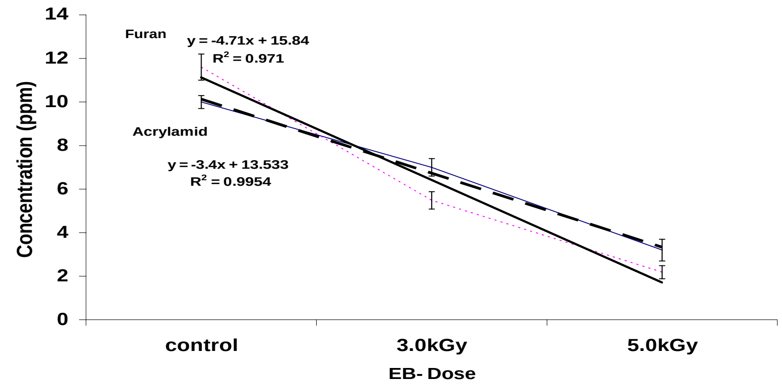

Figure 1. *Correlation between irradiation dose and the levels of acrylamide and furan.*

Table 4. *Effect of different treatments on some date constituents.*

Treatments	TAC* (mgAAE/100g)	TP** (mgGAE /100g)	DPPH (%)	BHT (mg/kg)
Control	2627.5^c ± 10.30	767.9^c ± 7.69	48.18^b ± 0.06	1320.0^c ± 6.08
Thermal 90^{0}C/2hrs	3448.1^b ± 23.05	786.9^b ± 5.6	50.40^a ± 0.07	1530.0^a ±10.14
3.0 kGy	3478.5^b ± 13.34	739.7^d ± 3.1	50.88^a ± 0.02	1430.0^b ± 9.50
5.0 kGy	3762.4^a ± 20.20	947.8^a ± 2.6	50.60^a ± 0.001	1480.0^b ±10.58

*TAC =Total antioxidant capacity (as ascorbic acid equivalent), **TP =Total phenols (as gallic acid equivalent).

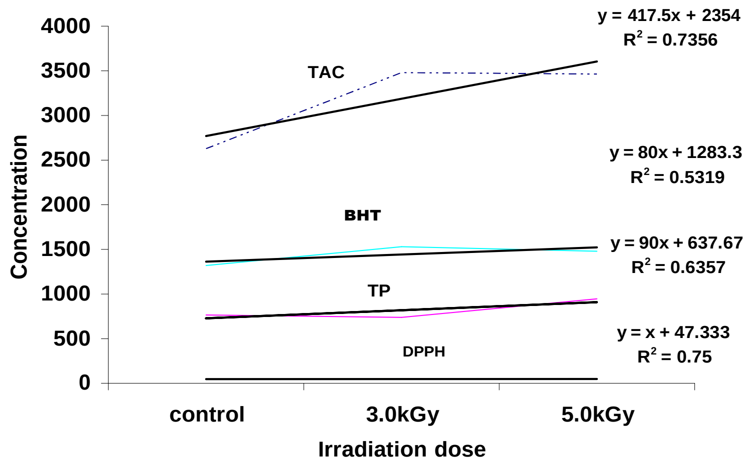

Figure 2. *Correlation between TAA, TP, BHT, DPPH and irradiation doses.*

The reduction of AA and furan was clearly seen to occur simultaneously with an increase in phenol activity rather than an increase in radiation exposures. Workers attained the same outcomes [24]. In the study by [25] on the impact of gamma irradiation on the antioxidant activity of peanut skin, it was found that the antiradical activity as measured by DPPH and ABTS had either slightly decreased or stabilized, and that the change in this activity was largely dependent on the doses of gamma rays.

The enhancement of phenol activity by irradiation in fruits was observed by different investigators [26,27]. Therefore, it is clear from obtained data that AA and furan molecules can degenerate by using γ- rays cause through conjugated many phenol compound with free sugars or asparagines to prevent Maillard pathway to complete produced any carcinogenic compound, especially through enhancement phenols by irradiation. In the same time, the main products of the degradable complicated tannins to soluble tannins were activated by direct effect of γ- rays or indirect by degradation of tannins then the re-arrangement consequently increased phenols or flavonoids concentrations .The mechanism of degeneration may be depend on indirect role of free radicals of phenols as(-OH,---H,--OOH) or others from available water of semi-dry fruits through irradiation process which activate the re-arrangements of the flavonoids and phenols to increase the potentiality of degradation of the carcinogenic by irradiation. Also, the free radicals (-OH,--OOH) can strongly react with the amide group of intermediates in MR and block AA formation. The suggested degradation mechanisms of AA, furan in presence of phenols after irradiation process could be due to a direct effects of irradiation for degradation AA or Furan or indirectly through enhancement of phenolic compounds to interact with the sequence of MR to stop the production of AA or Furan by blocking the start of the carbonyl moieties, or the intermediates in the MR then blocking the toxic compounds formation.

Conclusion

We can conclude that both the levels of acrylamide and furan in dates significantly increased as a result of the thermal treatment of date fruits. On the other hand, acrylamide and furan levels were significantly reduced as a result of the irradiation treatments. These results suggest that low doses of EB may have industrial uses, such as decontamination or disinfestations, and that they may even make it simple to reduce AA and furan levels to safe and healthy levels simultaneously. When compared to other treatments, irradiation has increased in effectiveness and cost-effectiveness for use on a broad scale in a variety of products. Lean data technologies PVT LTD.

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