

Chemical, Physical Properties and Aflatoxins Content of Palm Date Fruit Sprinkled with Some Spices and Herbs

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Abstract: This study was carried out on palm date fruit Rutab and Sukkary from Egypt and Saudi Arabia respectively. Dates were sprinkled with some spices as well as herbs (seed or powder) such as, anise, nigella, cinnamon, thyme and ginger. Moisture content, water activity, fungal contamination, the ability of isolated fungal flora to produce aflatoxins were determined. Total phenols and flavonoids content as well as Antioxidant activity were estimated. Finally, sensory evaluation was done. Results obtained revealed that, all samples under investigation were free from aflatoxins. Six toxigenic *Aspergillus flavus* were isolated from Rutab dates which produced of aflatoxins (B₁, B₂, G₁ and G₂), ranged between 0.528-2.671 ng/ml. Sukkarydates fruits were sprinkled with spices and herbs as (seed or powder) as, anise, nigella, cinnamon, thyme and ginger. Sprinkled Sukkary dates fruits with anise seed or cinnamon powder recorded the highest value of total phenolic and flavonoid content at concentration 970.67 and 930.83 ug/g and 4.19 and 4.05 mg/g and had higher of antioxidant activity. The dates sprinkled with cinnamon powder was excellent than all spices and herbs tested for sensory evaluation as well as, fungal flora during storage.

Key words: Palm date fruit • Spices and Herbs • Fungi • Aflatoxins • Phenols • Flavonoid • Antioxidant activity

INTRODUCTION

Dates, (*Phoenix dactylifera* L.) are one of the oldest cultivated plants dating back 7000 years. It is one of the most important fruit crops in tropical and subtropical region in the world. The palm dates was referred to as the (Tree of life). The economic importance of date is due largely to the nutritional value of the fruit, which contain 44-80% carbohydrates, 0.2-0.5% fat, 2.3-5.6% protein and 6-12% dietary fiber [1]. Spices and herbs such as garlic, black cumin (nigella) cloves, cinnamon, thyme, anise and ginger. Have been used for generation by humans as food and to treat ailments, or prevent diseases. Anise (*Pimpinella anisum* L. *Apiaceae*) is an annual herb widely cultivated in the Mediterranean rim and South Asia including India.

p-Anisaldehyde (4-methoxybenzaldehyde) extracted from anise seed oil and structurally related to vanillin is regarded as one of the world's principal flavoring compounds [2]. Nigella sativa had crude oil and

thymoquinone (TQ) extracted from its seeds and oil are effective against many diseases like cancer, cardiovascular complications, diabetes, asthma, kidney disease [3]. Cinnamon (*Cinnamomum verum*), has been used as a spice and as traditional herbal medicine for centuries. The available *In vitro* and animal *In vivo* evidence suggests that cinnamon has anti-inflammatory, antimicrobial, antioxidant, antitumor, cardiovascular and cholesterol-lowering effect [4]. Rhizomes of *Zingiber officinale Roscoe* (Zingiberaceae), commonly known as ginger, is one of the most widely used spice and condiment. Ginger shows an antioxidant activity, more than 50 antioxidants isolated from the rhizomes of ginger [5] and thyme (*thymus vulgaris*) is a small shrubby plant with a strong, spicy taste and odor. Thyme has carminative, diaphoretic, expectorant, sedative, antibacterial, antifungal properties and antispasmodic effects [6]. Mycotoxins are toxic compound, produced by the secondary metabolism of toxigenic fungi *Aspergillus*, *Alternaria*, *Calviceps*, *Fusarium* and *Penicillium* genera occurring in food commodities both pre- and post-harvest.

Although mycotoxin contamination of agricultural products still occurs in the developed world, the application of modern agricultural practices and the presence of a legislatively regulated food processing and marketing system have greatly reduced mycotoxin exposure in these population [7]. Aflatoxins (B₁, B₂, G₁ and G₂) are mycotoxins produced by fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, the most toxic and carcinogenic member of this family is aflatoxin B₁ (AFB₁) [8]. This study aimed to evaluate the effect of sprinkle palm dates fruits with some spices and herbs on the chemical and physical properties beside the ability of fungal flora to produce aflatoxins.

MATERIALS AND METHODS

Dates: Unpackaged Rutab dates purchased from Ramses street in Cairo Governorate in Egypt. Four samples were purchased each one 2Kg. FiveKg of El-Duhayan Sukkary dates packed under vacuum were purchased from local market in Cairo, Egypt, which is exported from the Kingdom of Saudi Arabia (KSA).

Organic Spices and Herbs: Some spices and herbs were purchased from the organic market in Egypt such as anise or nigella seed. Cinnamon, nigella, thyme and ginger powder were also purchased from local market.

Dates Sprinkled with Spices and Herbs: Sukkary dates was sprinkled with 2 % of some spices or herbs (seeds and powder) in a plastic bag and shaken for 1 min.

Determination of Moisture Content and Water Activity: Moisture content (MC) and water activity (Wa) were determined in all samples under investigation. [9] Procedures.

Isolation and Identification of Fungi: The dilution plate count was used for fungi isolated in all samples under investigation, including sprinkled dates stored for four weeks. Sprinkled dates Samples were tacked at zero time, 1, 2, 3 and 4 weeks. Serial dilution was carried out. The fungi count was determined and results were expressed as CUF / g on potato dextrose agar [10]. Fungal isolates were identified according to Barnett and Hunter [11].

Detection of the Toxigenic *A. flavus* under Ultra Violet (UV) Lamp: After counting *A. flavus* and identification, detection of its ability for aflatoxin production was ensured through inoculation of six inoculates in coconut-milk agar. Slant were examined under long-wave

ultraviolet (UV; 365 nm) illumination to search for the presence of fluorescence in the growing colonies according to [12].

Aflatoxins (AFS) Production in Liquid Medium: Determination of aflatoxins (B₁, B₂, G₁ and G₂) production ability of *Aspergillus flavus* isolated from Rutab or Sukkary dates as well as organic spices and herbs in liquid media Yeast Extracted Sucrose (YES) for 21 days. The *A. flavus* was cultivated in Yeast Extract Agar (YEA) liquid medium, then the presence of aflatoxins was confirmed by extracting the medium with chloroform and examining the extracts by High Performance Liquid Chromatography (HPLC) with fluorescence detection.

Determination of Aflatoxins Produced by Isolated *A. flavus*: The aflatoxins content were determination by the HPLC. The system consisted of Waters Binary Pump Model 1525, a Model Waters 1500 Redone manual injector, a Waters 2475 Multi-Wavelength fluorescence detector and a data workstation with software Breeze 2. Aphenomenex C₁₈ (250, 4.6 mm i.d.), 5um from Waters corporation (USA).

Analysis of AFS from All Samples: Analysis of AFS from all samples was done by Enzyme-Linked Immunosorbent Assay (ELISA) method. Preparation of the samples and separation with immune affinity Enzyme Immunoassay for the quantitative analysis of aflatoxins Art. No: 1211 and Rid aflatoxin column Art. No: R5001/5002. R-Bio pharm AG, Darmstadt, Germany, the method described by Iqbal *et al.* [13].

Effect of Spices and Herbs on Sukkary Dates Through Storage: Sukkary palm dates fruit were sprinkled with some spices or herbs as mentioned before. The dilution plate count was used for determination of fungi isolated through storage of the samples for four weeks. Samples were taken at zero time (after sprinkled), 1, 2 and 4 weeks. Serial dilution was carried out. The fungi count was expressed as CFU / g [10]. Total fungal count were counted on Martin agar medium.

Determination of Total Phenolic Content in Dates: Total Phenolic Content (TPC) of the all palm dates fruit sprinkled withspices or herbs as well as non-sprinkled dates were determined calorimetrically according to the method described by [14, 15] using Folin-Cio Calteureagent. The amount of total phenolic was calculated as garlic acid equivalent (GAE) from a calibration curve of garlic acid (0-50 ug/ml).

Determination of Total Flavonoids Content in Dates:

Flavonoids content were determined according to the method described by [16] using Shimadzu, spectrophotometer, the absorbance was measured at 430 nm. Catachine (0-50 ug/ml) was used as a standard for calibration curve.

Antioxidant Activity in Dates: Radical scavenging activity using 1, 1-diphenyl 1-2-picrylhydrazil (DPPH) reagent was used for determination of the antioxidant activity according to [17]. The absorbance was measured at 517nm and compared with a control. The inhibition of DPPH free radicals was calculated as follows:

$$\text{Inhibition \%} = \frac{A_{517\text{nm}}\text{Control} - A_{517\text{nm}}\text{sample}}{A_{517\text{nm}}\text{control}} \times 100$$

Sensory Evaluation: Organoleptic evaluation of different sprinkled dates or non- sprinkled was done on ten panelists to evaluate color, odor, taste, shape, texture and overall acceptability according to the method of [18].

Statistical Analysis: Data were subject to analysis of variance (ANOVA) using SPSS software [19] Differences between means were determined by the least significant difference test and significance was defined at $P < 0.05$. All measurements were carried out in triplicates.

RESULTS AND DISCUSSION

Moisture Content (MC) and Water Activity (Wa):

Temperature, oxygen and moisture content are the most important factors that influence the type of microbial growth and spoilage of foods. High sugar tolerant microorganisms, temperature of storage and water content are the major factors which affect the shelf life of date's fruit [20]. Spices and herbs were investigated for their MC and Wa and results were given in Table (1). It is obvious that cinnamon powder had the highest moisture content than the other spices and herbs. On the other hand thyme recorded the highest water activity (0.54). The moisture content and water activity of dates purchased from Cairo Governorate (Rutab) and dates of the KSA (Sukkary) were 20.50 -15.92% and 0.80-0.50, respectively. These results are close to that obtained by [21] who found that date fruits (Rutab) contain moisture content ranging from 10% to 22%. [22] found a water activity below 0.6.

Fungi Associated with Rutab and Sukkary Date Fruit:

Data in Table (2) indicated that, *Aspergillus flavus*,

A.niger and *Rhizopus* spp. were the common fungi found on Rutab and Sukkary date fruit. Fungal count on Sukkary fruit was less than that on Rutab fruits. These results are in agreement with those obtained by [23] who found that the dominant fungi were *Aspergillus niger* and *Penicillium* spp. on spoilage dates Rutab collected from markets in. Also, *Aspergillus niger* was the most predominant fungi on Sukkary palm dates variety in Saudi Arabia [24].

Data presented in Table (3) revealed that *Aspergillus niger* was the common fungi associating spices and herbs used in this study. High count of *Aspergillus niger* was observed on anise powder followed by moderate count on nigella seeds, meanwhile, least count of *Aspergillus niger* was recorded on nigella powder and Cinnamon powder [25] reported that the most predominant fungal genera associating with some spice in the Libya state market such as cumin, anise, cinnamon, sesame, fennel, black pill, nutmeg, coriander, black pepper, turmeric, ginger, galangal, caraway, cardamom were *Aspergillus* spp, *Penicillium* spp, *Alternaria* spp and *Fusarium* spp.

Detection of the Toxigenic *A. flavu* Sunder Ultra Violet (UV) Lamp:

This method describes a simple for screening the aflatoxin production by the *Aspergillus flavus* isolated from Rutab dates under UV light, it exhibited blue fluorescence (Figure 1). These results are agreement with those found with [26], who development a method for direct visual determination of aflatoxin production by colonies of *Aspergillus flavus*.

Aflatoxin Production by Isolated *A. flavus*: Although AFB₁ was not detected in all sample under investigation the aflatoxigenic *Aspergillus* spp were detected in (Rutab) dates in six isolated *A. flavus* which had the ability to produce AFS at high concentration (Table 4). The highest concentration was found from isolate No. 4 (.0528 ng/ml) and the lowest concentration of AFS from isolate No. 3 (2.671 ng/ml). This may be due to unhygienic handling of the date fruit from the local sellers and the nutrition content of the date fruit that may serve as good source of nutrition to fungi. Date fruits should be packed and processed under hygienic conditions for public health importance.

[27] detected aflatoxins in 12% from twenty five of dates and aflatoxigenic *Aspergillus* were detected in 40% of the three stage of dates maturation (Kimri) (Rutab) and dried (Rutab).

Table 1: Moisture content and water activity of some spices and herbs tested

Determination	Spices and Herbs						
	Seed		Powder				
	Anise	Nigella	Anise	Cinnamon	Ginger	Nigella	Thyme
Moisture (%)	8.28	6.39	9.06	11.82	7.49	5.21	7.85
Water activity	0.52	0.50	0.50	0.50	0.50	0.50	0.54

Table 2: Frequency of fungi associated with Rutab date fruit

Dates fruit	Fungal count (CFU×10 ³ /g)		
	<i>A. flavus</i>	<i>A. Niger</i>	<i>Rhizopus spp.</i>
Rutab	2.05	2.0	0.1
Sukkary	1.0	1.0	0.0

Table 3: Frequency of fungi associated with spices and herbs

Spices and Herbs	Isolated Fungi			
	Fungal count (CFU×10 ³ /g)			
	<i>A. flavus</i>	<i>A. niger</i>	<i>Alternaria sp.</i>	Unknown
Seed				
Anise	0.0	0	1.0	0.0
Nigella	0.0	1.5	0.0	1.00
Powder				
Anise	0.0	5.0	0.0	0.00
Cinnamon	0.0	0.5	0.0	3.00
Ginger	0.0	1.0	0.0	1.50
Nigella	0.0	0.5	0.0	2.00
Thyme	0.0	0.0	0.0	4.05

Table 4: Aflatoxins production by *A. flavus* isolated from Rutab dates fruit

Isolated number	Concentrations of aflatoxins (ng/ml)				
	AFB ₁	AFB ₂	AFG ₁	AFG ₂	Total AFs (ng/ml)
One	0.106	0.020	0.525	ND	0.651
Two	0.124	0.013	0.915	0.356	1.408
Three	0.198	0.030	ND	0.300	0.528
Four	1.520	0.725	0.345	0.081	2.671
Five	0.125	0.250	0.225	0.099	0.699
Six	0.238	0.787	0.365	0.600	1.990

ND: Not Detected



Fig. 1: Blue fluorescence of toxicogenic *A. flavus* under UV lamp

Analysis of AFBS: AFS was not detected by ELISA in any of the palm dates fruits (Rutab and Sukkary) or in organic spices and herbs. All the samples were free from

any contamination with AFS. These results are not in agreement with that of [12] who found mycotoxins in spices such as aflatoxins which was found in red peppers (paprika, chili and cayenne) nutmeg, mustard, ginger, black and white peppers, coriander.

Effect of Sprinkling Palm Date Sukkary Fruit by Spices and Herbs on Fungal Association During Storage

Periods: Data in Table (5) indicated the presence of common fungal genera on Sukkary palm dates fruits *i.e.*, *Aspergillus niger*, *Aspergillus flavus*, *Penicillium spp.* And *Rhizopus spp.* *Aspergillus niger* more frequency followed by *Aspergillus flavus*. Meanwhile, *Penicillium spp* and *Rhizopus spp.* were the least fungal frequency. In general, all fungi were less frequently found on Sukkary dates fruit treated by seed of anise or powder of cinnamon compared with untreated fruits. During storage intervals of 0, 1, 2 and 4 weeks after sprinkling fruits by seed of anise or powder of cinnamon, frequency of fungal count were reduced. Meanwhile, untreated dates fruits recorded high fungal count. Sprinkling of dates fruit with powder of cinnamon reduced fungal count more than seeds of anise at different storage periods [28] reported that cinnamon completely inhibited the development of *A. niger* and inhibited production of B₁AF and B₂AF at all concentrations of cinnamon and anise.

Total Phenolic, Flavonoid Content and Antioxidant Activity in Dates:

The total phenolic and flavonoid content as well as the antioxidant activity of dates either sprinkled with some spices and herbs or not is shown in Table (6). It can be seen from results that, the dates sprinkled with both anise seed and cinnamon powder recorded the highest value of total phenolic and flavonoid content at concentration of 970.67-930.83 ug/g and 4.19 - 4.05 mg/g, respectively.

In is respect [29] reported that, anise seed had various properties such as antimicrobial, antifungal, antiviral, antioxidant, muscle relaxant, analgesic and anticonvulsant activity as well as different effects on gastrointestinal system. So, antioxidant properties of dates sprinkled with some spices or herbs extracts are

Table 5: Effect of spices and herbs on fungal count of Sukkary date during storage

Treatment	Storage (week)	Fungal count (CFU×10 ³ /g)				
		Total fungi	<i>Aspergillus</i>			
Sukkary Date's			<i>Niger</i>	<i>Flavus</i>	<i>Penicillium spp.</i>	<i>Rhizopus spp.</i>
Dates Non-Sprinkled	0	2.8	1.0	0.3	1.5	0.0
	1	8.5	5.0	2.5	0.0	1.0
	2	8.3	5.0	0.0	3.0	0.0
	4	6.0	4.0	1.5	0.5	0.0
Dates Sprinkled with Anise Seed	0	2.4	1.7	0.7	0.0	0.0
	1	3.0	1.5	1.0	0.0	0.5
	2	1.5	0.5	1.0	0.0	1.0
	4	0.0	0.0	0.0	0.0	0.0
Dates Sprinkled with Cinnamon powder	0	1.3	1.0	0.3	0.0	0.0
	1	2.5	2.0	0.5	0.0	0.0
	2	0.5	0.5	0.0	0.0	0.0
	4	0.0	0.0	0.0	0.0	0.0

Table 6: Total phenolic and flavonoid content as well as antioxidant activity of dates sprinkled with some spices and herbs and non-dates sprinkled

Dates sprinkled with	Phenolic content (ug/g)	Flavonoid content (mg/g)	Antioxidant activity (%)
	Seed		
Anise	970.67±1.42 ^a	4.19±0.75 ^a	27.62±0.18 ^d
Nigella	831.50±1.34 ^b	2.64±0.6 ^b	49.66±0.35 ^c
	Powder		
Anise	702.67±3.43 ^b	2.58±0.46 ^b	55.03±0.29 ^{bc}
Cinnamon	930.83±2.37 ^a	4.05±0.97 ^a	33.84±0.32 ^d
Ginger	627.67±0.15 ^c	2.34±0.15 ^b	67.33±0.44 ^{ab}
Nigella	770.50±1.22 ^c	2.63±0.62 ^b	51.21±0.56 ^c
Thyme	737.83±2.33 ^{cd}	2.61±0.83 ^b	53.16±0.48 ^c
Non-date sprinkled	617.33±0.15 ^c	2.22±0.15 ^b	69.87±0.59 ^a

Values followed by the same letter in a column are not significantly different at P <=0.05

Table 7: Organoleptic evaluation of dates sprinkled with some spices and herbs

Dates sprinkled with	Organoleptic Evaluation					
	Color	Odor	Taste	Shape	Texture	General appearance
	Seed					
Anise	7.8±1.11 ^{ab}	7.8±1.68 ^{ab}	8.10±1.59 ^a	7.3±1.63 ^{ab}	7.4±1.50 ^b	7.6 ±1.57 ^{ab}
Nigella	7.0±1.77 ^{bc}	6.7±2.11 ^{ab}	7.0 ±2.58 ^{bc}	6.6±1.83 ^b	6.9±2.42 ^{cb}	7.0±2.05 ^b
	Powder					
Anise	6.0±1.25 ^c	6.5±2.32 ^{bc}	6.70±2.16 ^{bc}	6.6±2.27 ^b	6.6±2.54 ^c	6.3 ±2.16 ^{bc}
Cinnamon	8.5±1.17 ^a	8.4±1.57 ^a	8.60±1.26 ^a	7.9±1.59 ^a	7.8±1.93 ^{ab}	8.4±1.71 ^a
Ginger	6.4±2.00 ^{bc}	6.3±1.56 ^{bc}	6.6±1.17 ^{bc}	7.0±1.41 ^b	5.9±1.10 ^c	7.4±1.26 ^b
Nigella	6.8±1.15 ^{bc}	6.6±2.01 ^{bc}	7.2±2.44 ^b	5.9±2.18 ^{bc}	6.8±2.20 ^{bc}	7.2±2.29 ^b
Thyme	5.6±2.40 ^c	5.7±2.26 ^c	6.0±1.82 ^c	5.5±2.12 ^c	5.9±2.37 ^c	6.0±2.49 ^c
Non-dates sprinkled	7.8±1.22 ^{ab}	7.6±1.07 ^{ab}	7.4 ±1.42 ^{ab}	7.2±1.13 ^a	7.0±1.57 ^a	8.6±0.91 ^a
LSD at 0.05	1.403	1.707	1.660	1.565	1.452	1.522

Values followed by the same letter in a column are not significantly different at P <=0.05

commonly associated with the presence of the phenolic compounds possessing the ability to donate hydrogen to the free radical. It is well know that phenolic compounds act as potent antioxidants [30]. Results in Table (6) recorded that, dates sprinkled with anise seed and cinnamon powder had higher of

antioxidant activity compared with all other dates sprinkled or not. The results were recorded 27.62 and 33.84%, respectively. These results are in agreement with [31] who found that, the DPPH radical scavenging activity positively correlated with the total phenolic content.



Fig. 2: Sukkary date sprinkled with some spices and herbs (1-Anise powder 2-Cinnamon powder 3- Anise seed 4- Ginger powder 5-Non-dates sprinkled 6- Nigella seed 7- Thyme 8- Nigella powder)

Sensory Evaluation of Dates Sprinkled with Some Spices or Herbs: Organoleptic evaluation is generally the final guide of the quality from the consumer's point of view. Results of the sensory evaluation of date sprinkled with some spices and herbs (seed or powder) are shown in Figure (2) and Table (7). The obtained results showed that, there were significant differences in color, odor, taste, shape and texture scores between non-dates sprinkled and date sprinkled with spices or herbs expect general appearance of non-date sprinkled which had the highest score. Taste and palatability of dates were improved when sprinkled with spices and herbs compared with control. This might be attributed to the highest content of volatile aromatic or essential oils in these spices and herbs. Results showed that the sensory characteristics of the dates sprinkled with cinnamon powder are excellent compared with other dates sprinkled with different spices and herbs or control, followed by dates sprinkled with anise seed, then dates sprinkled with nigella seed or powder, followed by dates sprinkled with thyme then dates with ginger powder. No significant differences were noted between the dates sprinkled with nigella seed or powder. Generally, it could be observed that the addition of medicinal herbs (essential oils and phenolic compounds) to date exhibited the highest of sensory properties [32].

CONCLUSION

Analysis of palm dates fruit (Rutab) indicated high fungi contamination. This may be the result of the fruits handling such as washing, sorting and exposure to

environmental contaminants. Thus, palm dates fruits need more hygienic processing to be suitable for human consumption. IT is recommended to besprinkled with some spices and herbs specially anise seed or cinnamon powder to prevent contamination.

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