

Microbiological Quality, Aflatoxin Level, Phytochemical Compounds, Antioxidant Potential And Proximate Mineral Composition Of Date Palm Fruits (*Phoenix dactylifera L.*)

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ABSTRACT

Date palm fruits are edible fruits widely consumed for nutritional and health benefits. The microbiological quality, aflatoxin level, phytochemical compounds, antioxidant potential and proximate mineral composition of Refined Arabian Date Palm Fruit (RADPF), Nigeria Date Palm Fruit (NDPF) and Black Arabian Date Palm Fruit (BADPF) were investigated. Microbial counts, aflatoxin level, phytochemical components and proximate mineral composition were determined using standard methods while antioxidant potential was determined using DPPH free radical scavenging, Ferric Reducing Power and Total Phenolic Content assays. Total bacteria and fungi count ranged from 5.5 – 11.3 CFU/g and 0.5 – 13.0 CFU/g in which NDPF had the highest fungi and bacteria count. Aflatoxin level in RADPF, NDPF, and BADPF were 4.3ppb, 3.9ppb, and 2.00ppb respectively. Saponins, flavonoids, anthraquinones, terpenoids, and alkaloids were present in all the samples. All the date palm fruit samples have significant mineral contents. NDPF had highest DPPH scavenging activity (77.83%) while RADPF had highest FRAP (4.765 μ M/ μ g) and Total Phenolic Content (1.615 mgGAE/mL). The study revealed that the three date palm fruits sampled have some level of aflatoxin and also have substantial antioxidant activities.

Keywords: Date palm fruit, aflatoxin, antioxidant, microbial count, phytochemical

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1. INTRODUCTION

The date palm (*Phoenix dactylifera L.*) is one of the ancient and most abundant fruits in the world with different varieties having different texture, colour and flavour. It belongs to the family *Arecaceae* with about 200 genera and over 2,500 species (Hussain *et al.*, 2019). The fruits are sources of numerous nutrients which makes them advantageous to our health (Alahyane *et al.*, 2019). The components of dates are mainly protein, fat, carbohydrates, minerals, vitamins and amino acids (Ghnimi *et al.*, 2017).

Beside the nutrients present in date fruits, they also contain disease-preventing properties such as anti-cancer, anti-inflammatory, antioxidant, antimicrobial, hepato-protective and gastro-protective activities (Baliga *et al.*, 2011; Khalid *et al.*, 2017). Both the internal and external layers of date palm fruits are loaded with different kinds of microorganisms such as bacteria, yeast and fungi, with the latter causing the most damage (Mouloud *et al.*, 2017). Diseases such as boyoud disease (caused by *Fusarium oxysporum*), wilt diseases (caused by *Fusarium proliferatum*, *Fusarium solani* and other species of *Fusarium*), inflorescence rot disease (caused by *Mauginiellascaetiae* Cav.) and diplodia leaf-base disease (caused by *Diplodiaphoenicum*) amongst other are diseases that have been associated with date palm fruit (El-Hassni *et al.*, 2007; Abdullah *et al.*, 2010; Mouloud *et al.*, 2017).

The variations in both the internal and the external constituents of the fruits such as colour and chemical compositions have been attributed mainly to growth conditions and the region where the fruits are cultivated (Hamad *et al.*, 2015). It has been reported that the quality of dates from Egypt vary significantly in the phenolic contents, flavonols and sugars compared to those fruits from Bahrain, Oman and Algeria (Farag *et al.*, 2014). In this vista, this study was aimed at evaluating the microbiological quality, aflatoxin level, phytochemical compounds, antioxidant potential, and proximatemineral compositions of three most consumed date fruits in Nigeria. They were fruits of Refined Arabian Date Palm Fruit (RADPF), Nigeria Date Palm Fruit (NDPF) and Black Arabian Date Palm Fruit (BADPF).

2. MATERIALS AND METHODS

Plant Materials

Three varieties of date palm fruits were used in this study. They were Refined Arabian Date Palm Fruit (RADPF), Nigeria Date Palm Fruit (NDPF) and Black Arabian Date Palm Fruit (BADPF). RADPF and BADPF were obtained from Medinah, Saudi Arabia while NDPF was obtained from Kano, Nigeria.

Microbiological Assessment of the date palm fruit samples

The total heterotrophic bacterial count and total fungal count were determined using standard microbiological methods. The date palm fruits were surface-sterilized using 1% hypochloride and then ground using an electronic blender. Thereafter, the powdered samples were serially diluted in three folds, and then 1 mL inoculum was inoculated on sterile Nutrient Agar and Sabouraud Dextrose Agar. The plates were incubated at 37 °C for 24 hours and 28 °C for 48-72 hours for fungi and bacteria count respectively.

Determination of Total Aflatoxin of the date palm fruit samples

The total aflatoxin in the date palm fruit samples were evaluated using the modified method of Adebayo-Tayo *et al.* (2006). Ten gram of the samples were subjected to extraction using chloroform and then concentrated. The extracts were further subjected to thin layer chromatography (TLC) on silica gel DG254. Then 5, 10 and 15 µL of the extracts were spotted on three different points on a ruled base line of the TLC plates. Also 5, 10 and 15 µL of the aflatoxin standard were spotted on another three points close to the previous spotted points. The plates were first developed with diethyl ether and then with chloroform and acetone at 9:1 v:v.

The aflatoxins were identified on the basis of co-migration with aflatoxin standards (Fluka) and by their characteristic fluorescent colour under long ultra violet (UV) illumination at 360 nm. The fluorescent spots of aflatoxins were scraped off the TLC and eluted by chloroform and methanol at 9:1v:v). The solvent was evaporated under nitrogen to dryness and the residue was dissolved in methanol. The concentration of aflatoxins (B1 and G1) in solution was determined by measuring its absorbance at 360 nm then calculated.

Proximate Analysis of the Date palm Fruit Samples

Standard analytical procedures for food analysis were used to determine the moisture content, crude protein, crude fibre, crude fat, ash and carbohydrate.

Determination of moisture content

Two gram of the date palm fruit samples were put into the crucibles, dried in an oven at 105 °C overnight. The dried sample was cooled in a desiccator for 30 min and weighed to a constant weight. The percentage loss in weight was expressed as percentage moisture content on dry weight basis (AOAC, 2006). This was repeated three times to obtain triplicate values.

Determination of ash content

From the dried and ground sample, 2.0 g was taken in triplicates and placed in pre-weighed crucibles and ashed in a muffle furnace at 600 °C for 3 h. The hot crucibles were cooled in a desiccator and weighed. The percentage residual weight was expressed as ash content (AOAC, 2006).

Crude fat content determination

From the pulverized sample, 2.00 g was used for determining the crude lipid by extracting the lipid from it for 5 h with (60 to 80 °C) petroleum ether in a soxhlet extractor (AOAC, 2006). Triplicate samples were extracted to obtain triplicate values that were later averaged.

Crude Protein determination

Crude protein was determined by the Kjeldahl method. Half gram of the sample was weighed in triplicate into a filter paper and put into a Kjeldahl flask, 8 to 10 cm³ of concentrated H₂SO₄ were added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with about 10 cm³ of 40% NaOH solution. The condenser tip was dipped into a conical flask containing 5 cm³ of 4% boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01 M HCl until the solution turned red (AOAC, 2006).

Determination of crude fibre

From the pounded sample, 2.0 g were used in triplicates for estimating the crude fibre by acid and alkaline digestion methods using 20% H₂SO₄ and 20% NaOH solutions (AOAC, 2006).

Carbohydrate determination

The carbohydrate content was calculated using the formula described by (Mathew *et al.*, 2014).

$$\text{Available carbohydrate (\%)} = 100 - [\text{Protein(\%)} + \text{Moisture(\%)} + \text{Ash(\%)} + \text{Fibre(\%)} + \text{Crude fat(\%)}] \quad (1)$$

Determination of mineral contents

The mineral elements were determined using the methods of Mathew *et al.* (2014). Potassium was determined using Gallenkamp Flame analyzer, while calcium and iron were determined using Buch Model 205 Atomic Absorption Spectrophotometer. Phosphorus level was determined using phosphovanadomolybdate colorimetric techniques on JENWAY 6100 Spectrophotometer.

Determination of Antioxidant Activities of the date palm fruit samples

The antioxidant activities of the date palm fruit samples were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, Ferric reducing power, and total phenolic content assay.

DPPH Free Radical Scavenging Assay

DPPH free radical scavenging activity was determined using the method of Shahdadi *et al.* (2013). Two milliliter of 1mM ethanolic solution of DPPH was added to 3 mL of the date palm fruit extracts, the mixture was shaken vigorously and allowed to stand in the dark at 28 °C for 30 min. The absorbance was measured at 517 nm. Ethanol was used as blank, while DPPH solution in ethanol serves as the control. The free radical scavenging activity of the samples was expressed as:

$$\% \text{ DPPH Activity} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100 \quad (2)$$

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing power was determined according to the method of Odeh *et al.* (2014). Three milliliter of freshly prepared FRAP reagent previously warmed at 37°C for 10 minutes was mixed with 40 µL of the date palm fruit extracts and the mixtures were incubated at 37 °C. After incubation, the absorbance of the solution was measured at 570 nm using reagent blank containing distilled water which was also incubated at 37 °C as reference. Aqueous solutions of known Fe²⁺ concentrations in the range of 2-5 mM were used for calibration

Total Phenolic Content Assay

Total phenolic content was determined using the Folin-Ciocalteu's method as described by Odeh *et al.* (2014). Forty microliter of the date palm fruit extracts were mixed with 1.8 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and allowed to stand at room temperature (28 °C) for 5 minutes. Then 1.2 mL of Na₂CO₃ (7.5%, w/v) was added to the mixture and allowed to stand for 60 min at room temperature (28 °C). Then the absorbance was measured at 750 nm using aqueous solutions of known gallic acid concentrations in the range of 10 - 500 mgL⁻¹ for calibration. The total phenolic content was determined from extrapolation of the calibration curve. Results were expressed as milligrams of Gallic Acid Equivalent (GAE)/100g sample.

3. DATA ANALYSIS, RESULTS AND DISCUSSIONS

3.1 Data Analysis

The data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 20.

3.2 Results and Discussion

Table 1 shows the total bacterial and total fungal counts from the date palm fruit samples. NDPF had the highest total bacterial and total fungal counts of 11.3×10^2 CFU/g and 13.0×10^1 CFU/g respectively while RADPF had the least counts of 1.5×10^2 CFU/g and 0.5×10^1 CFU/g respectively. The high bacterial count found in NDPF corroborates with Umar *et al.* (2014) which reported higher bacterial counts ($1.4 - 9.6 \times 10^4$ CFU/g) in date palm fruits sold in Katsina, Nigeria. The high bacterial count could be attributed to the unprocessed nature of NDPF and contamination through handling by the sellers. The high sugar content of the date palm fruits might also contribute to the microbial load.

Table 1: Microbial Load of the date palm fruits

Sample	Total Bacterial Count ($\times 10^2$ CFU/g)	Total Fungal Count ($\times 10^1$ CFU/g)
RADPF	1.5 \pm 0.1	0.5 \pm 0.3
NDPF	11.3 \pm 0.2	13.0 \pm 0.1
BADPF	5.5 \pm 0.1	2.0 \pm 0.2

RADPF- Refined Arabian Date Palm Fruit

NDPF- Nigeria Date Palm Fruit

BADPF- Black Arabian Date Palm Fruit

Phytochemical Composition of the date palm fruit samples

Table 2 shows the phytochemical compounds present in the date palm fruit samples. Saponins, flavonoids, anthraquinones, terpenoids, and alkaloids were present in all the samples while tannins, cardiac glycosides, steroids, and phenol were absent. However, saponins, flavonoids, and alkaloids were abundant in RADPF and BADPF than NDPF.

Martin-Sanchez *et al.* (2014) reported the presence of phenolic acids, isoflavons, lignans, and flavonoids, tannins, and sterols in date palm fruit co-products of the two varieties from Elche, Alicante, Spain. Al-Shwyeh (2019) also reported the presence of the phenolic compounds, in date palm fruits from the Kingdom Saudi Arabia.

Table 2: Phytochemical Composition of the date palm fruit samples

Test	RADPF	NDPF	BADPF
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Saponins	++	+	++
Tannins	-	-	-
Flavonoids	++	+	++
Cardiac glycosides	-	-	-
Anthraquinones	+	+	+
Terpenoids	+	+	+
Steroids	-	-	-
Alkaloids	++	+	++
Phenol	-	-	-

-: Absent

+: Present

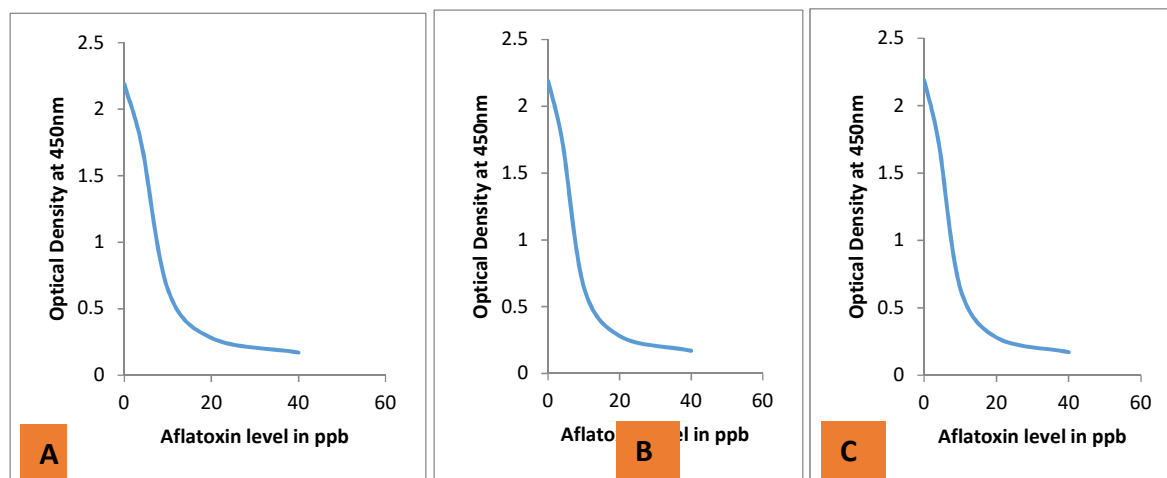
RADPF- Refined Arabian Date Palm Fruit

NDPF- Nigeria Date Palm Fruit

BADPF- Black Arabian Date Palm Fruit

Total Aflatoxin Content of the date palm fruit samples

Figures 1A, 1B and 1C show the aflatoxin levels in the RADPF, NDPF, and BADPF respectively. By extrapolation from the standard curve, value of total aflatoxin level in RADPF, NDPF, and BADPF is 4.3ppb, 3.9ppb, and 2.00ppb respectively. Although all the three date palm fruits have some level of aflatoxin, the value is within the permissible level of aflatoxin. Aslamet *al.* (2021) also found some level of aflatoxin B1, B2, G1, and G2 in date palm fruits from Pakistan, Iran and Saudi Arabia.



Figures 1a-c: Total aflatoxin levels in a) RADPF, b) NDPF, and c) BADPF samples

Antioxidant Activities of the date palm fruit samples

Table 3 shows the DPPH scavenging activities of the date palm fruit samples. At a concentration of 1000 $\mu\text{g/mL}$ NDPF had the highest percentage DPPH scavenging activity of 77.83% while the percentage DPPH scavenging activity of RADPF and BADPF were 53.40% and 56.14% respectively.

Results showed that all the date pal fruit samples exhibited concentration-dependent DPPH scavenging activities. The percentage DPPH scavenging activities were higher at 1000 µg/mL. This finding is in agreement with Al-Mamary *et al.* (2014) which reported higher DPPH scavenging activities (75.78-88.72%) at concentration of 1000 µg/mL in Iraqi and Saudi date palm fruits. Contrarily, Shahdadi *et al.* (2013) reported lower DPPH scavenging activities (29.7-42.9%) from Iranian date palm fruits.

Table 4 shows the ferric reducing antioxidant power of the date palm fruits. All the date palm fruits have higher FRAP value at 100 µg/mL. RADPF had the highest FRAP value of 4.765 µg/mL while NDPF had the least FRAP value (2.516 µg/mL). In a similar study, Odeh *et al.* (2014) also reported higher FRAP values (2.2-12.6 µg/mL) from seven cultivars of date palm fruits in Palestine. Qiao *et al.* (2009) reported a direct correlation between antioxidant activity and the ferric ion reducing power.

Table 5 shows the total phenolic content of the date palm fruit samples. RADPF had the highest total phenolic content of 1.615 mg GAE/mL while BADPF had the least total phenolic content, 1.465 mg GAE/mL. A higher total phenolic content (8.8 mg GAE/100 g) was reported by Odeh *et al.* (2014) from Palestinian date palm fruits. Studies have established that total phenolic contents of compounds are associated with their antioxidant activities. This was attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Adedapo *et al.*, 2008; Neo *et al.* 2010).

Table 3: DPPH scavenging activities of the date palm fruit samples

Concentration (µg/mL)	DPPH Scavenging Activity (%)			
	RADPF	NDPF	BADPF	Standard
200	26.02%	38.43%	31.08%	95.6
400	28.67%	43.37%	41.81%	95.7
600	38.67%	52.05%	47.35%	95.7
800	46.51%	63.25%	54.34%	95.8
1000	53.40%	77.83%	56.14%	96.9

Table 4: Ferric Reducing Antioxidant Power of the date palm fruit samples

Concentration (µg/mL)	FRAP (µM/µg)
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	RADPF	NDPF	BADPF
40	2.217	1.901	2.174
60	3.141	2.395	2.437
80	4.056	2.490	2.580
100	4.765	2.516	2.667

Table5: Total Phenolic Content of the date palm fruit samples

Concentration (µg/mL)	Total phenolic content (mg GAE/mL)		
	RADPF	NDPF	BADPF
400	1.500	1.431	1.441
600	1.574	1.446	1.451
800	1.605	1.480	1.460
1000	1.615	1.502	1.465

Proximate and Mineral Contents of the Date Palm Fruit Samples

Table 6 shows the proximate and mineral contents of the date palm fruit samples. Results showed that moisture content (16.98%) was significantly higher ($p < 0.05$) in RADPF, while ash (3.37%) and carbohydrate (84.6%) were significantly higher in NDPF. Calcium (99.05 mg/kg), Potassium (19.60 mg/kg) and Phosphorus (170.30 mg/kg) were significantly higher in RADPF.

Similarly, Shaba *et al.* (2015) reported that date palm fruits sold in Minna, Nigeria contain some percentage crude protein ($1.21 \pm 0.02\%$), crude fat ($1.73 \pm 0.46\%$), crude fibre ($2.26 \pm 0.07\%$), ash ($1.88 \pm 0.03\%$), moisture contents ($1.16 \pm 0.16\%$), and carbohydrate ($91.76 \pm 0.06\%$).

Table 6: Proximate and mineral contents of the date palm fruit samples

Parameters	RADPF	NDPF	BADPF
Moisture (%)	16.98 ± 0.02^a	9.91 ± 0.02^c	16.71 ± 0.02^b

Crude protein (%)	3.36±0.07 ^a	3.43±0.07 ^a	3.61±0.07 ^a
Crude fat (%)	0.010±0.0 ^a	0.006±0.03 ^a	0.010±0.05 ^a
Crude fibre (%)	1.39±0.01 ^b	1.79±0.02 ^a	1.78±0.01 ^a
Ash (%)	2.65±0.03 ^b	2.65±0.01 ^b	3.37±0.01 ^a
Carbohydrate (%)	76.70±0.05 ^a	84.6±0.02 ^b	76.30±0.07 ^a
Calcium (mg/kg)	99.05±0.55 ^a	80.00±1.02 ^b	61.00±1.00 ^b
Potassium(mg/kg)	19.60±0.60 ^a	18.15±0.65 ^{ab}	16.50±0.50 ^b
Iron(mg/kg)	0.49±0.04 ^b	0.59±0.01 ^b	0.12±0.05 ^a
Phosphorus(mg/kg)	170.50±2.50 ^a	155.00±3.0 ^b	142.00±4.0 ^c

Values are mean ± standard error. Means with different superscript within a row are significantly different

4. CONCLUSION

This study revealed that NDPF is much contaminated by both bacteria and fungi. All the three date palm fruits sampled have some level of aflatoxin and significant mineral contents. The date palm fruits also have substantial antioxidant activities as indicated by the DPPH scavenging activity, ferric reducing antioxidant power and total phenolic content.

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