

Research Article

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Amino Acid Profile, Phytochemical Evaluation and Acute Toxicity (LD_{50}) Determination of Five Varieties of Date Palm (*Phoenix Dactylifera*) Seeds

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ABSTRACT

Objective: Amino Acid, phytochemical and Acute Toxicity levels (LD_{50}) of date palm seeds were evaluated in the present study. Seeds of five varieties of date palm fruits at mature stage were manually isolated, soaked in water, oven dried and milled into flour. Five varieties of Deglet Nour, Lulu, Bomaan, Fardh and Barhe were used.

Results: The seeds contained all the essential and non-essential amino acids. Leucine was in highest quantity of (10.10-12.02g/100g protein) while Glutamic acid had the highest amount of 18.62-21.04g/100g protein. Significant amount of oxalate (6.31-8.89 mg/100g), phytate (5.15-8.37mg/100g), Hydrogen Cyanide (3.26-5.34mg/100g), Tannin (3.40-6.31mg/100g), Saponin (2.29-4.36mg/100g) were found in all the five varieties. The LD_{50} tests outcome suggests that the samples were completely free from any form of toxicity since all treated experimental animals showed no sign of toxicity, rather looked physically healthy and emotionally stable all through the period, even at 5000mg/kg body weight of the samples.

Conclusion: The seeds were found to be edible and safe. They can be used in formulating diets. Conventional processing methods will ensure the elimination of the anti-nutrients. The seed flour can therefore be used as composite with other flours and hence find application in food industries such as bakery industry.

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Introduction

Date palm is one of the oldest fruit in the world. It is a flowering plant species in the palm family-Arecaceae, cultivated for its edible sweet fruit. It plays an important role in the economic and social life of the people in the date producing countries such as Egypt, Saudi Arabia, Iraq, Iran, Algeria. Date seeds constitute 10-15% of the date fruit weight. The seed characteristics of date varieties vary according to variety and environmental conditions. The length, width and weight of the seed range from 1.2cm, 3.6cm and 0.6-1.3g, respectively. The World production of dates was 7.5 million tons in 2011, meaning that approximately 750, 000 tons of date seeds were produced during that year. A large number of date seeds are generated from the date processing industries as waste products annually. Presently however, very little use is made of these seeds after removing the pulp. This by-product of date processing industries could be regarded as an excellent source of food ingredients. It could also be used as an important source of dietary fiber and protein [1-6]. Hence, the need for amino acid and phytochemical evaluation.

Malnutrition is still widespread in Nigeria due to the decline in protein intake occasioned by the scarcity and unaffordable price of animal protein food sources such as milk, egg, meat and fish [7,8]. Therefore, to meet the challenges of protein inadequacy and price increase from animal sources, the development of new, sustainable and inexpensive proteins sources (non-animal sources) is of great importance. Date palm seeds could be one of these sources. These seeds are considered a waste and a major problem to the date palm industry.

Materials and Methods

Five varieties of mature date palm fruits (*Phoenix dactylifera* L.) were purchased from Dilimi local market situated at city of Jos, the capital of Plateau State, North Central of Nigeria. Date seeds were manually extracted from 20kg of whole Date fruits. The extracted seeds were soaked in water and washed to remove any remaining date flesh. They were then oven dried at 50°C for 24h using Electrothermal Oven (Model: DGH 9023A). Date palm seeds were milled using a hammer mill to produce flour that passed through 2 mm screens. The seed flour obtained was packaged and stored at ambient temperature prior to analysis.

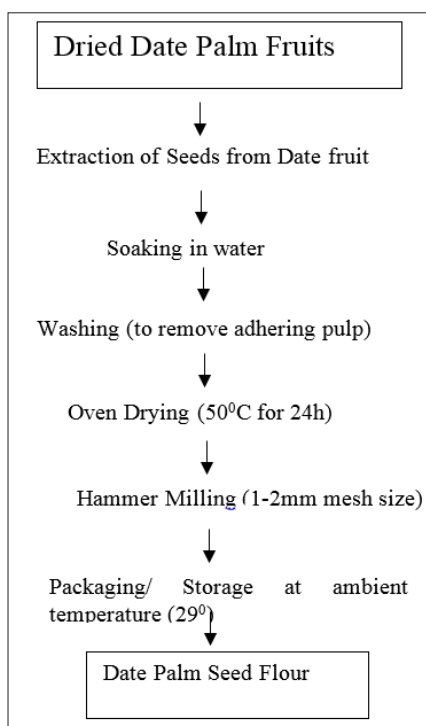


Figure 1: Flow chart for the production of date palm seed flour

Extraction

For each variety of the seed flour, fifty (50) grams of the flour was introduced into the extraction chamber of the soxhlet extractor and extraction was carried out using ethanol. Extraction temperature

was maintained at 60°C for 48 hours. New Lorke's method was adopted for toxicity evaluation as described by Orieko et al (2019). Each extract, administration was done on two test phases and was carried out on a total of 18 mice of both sexes. In the first phase, 9 rats divided into 3 groups were administered 10, 100 and 1000 mg/kg of the extract respectively. In the second phase, 9 mice also divided into 3 groups of 3 mice each were administered 2900 mg/kg and 5000 mg/kg respectively while the highest dose used (5000 mg/kg) was repeated on the last group. All treatments were single dose and via the oral route. Observed was made on the mice for toxicity for 24 hours of treatment and a further 7 days (for possible delayed toxicity). The Phytochemical content was determined using the method of Association of Analytical Chemist and Amino Acid profile in the known sample was determined using methods described [9,10].

Results and Discussions

Phytochemicals

The results of the phytochemical content of date palm seed are shown.

Tannin Content

The Fardh date variety had the highest content of tannin (6.31mg/100g) while Barhe variety had the lowest value of 3.40mg/100g. Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. They are found in most plants and they react to cause enzymatic browning on the cut or bruised surfaces of fresh fruits and vegetables. Tannins inhibits virtually digestive enzyme and reduces the bioavailability of iron and vitamin B₁₂ [11].

Table 1: The Phytochemical Composition of the Date Palm Seed samples

Parameters							
Varieties	Tannin (mg/100g)	Phytate (mg/100g)	Steroid (mg/100g)	HCN (mg/100g)	Alkaloid (mg/100g)	Saponin (mg/100g)	Oxalate (mg/100g)
Deglet Nour	3.71 ^b ±0.02	7.77 ^c ±0.17	0.82 ^c ±0.11	4.52 ^b ±0.18	0.13 ^c ±0.01	4.36 ^c ±0.01	8.55 ^c ±0.11
Lulu	4.89 ^c ±0.00	7.68 ^c ±0.06	0.20 ^a ±0.04	3.26 ^a ±0.13	0.09 ^b ±0.01	3.93 ^b ±0.06	8.89 ^c ±0.04
Bomaan	4.69 ^c ±0.09	8.37 ^d ±0.00	0.63 ^b ±0.01	5.34 ^c ±0.17	0.06 ^a ±0.01	2.29 ^a ±0.21	7.33 ^a ±0.01
Fardh	6.31 ^d ±0.25	5.70 ^b ±0.38	0.29 ^a ±0.01	4.46 ^b ±0.11	0.17 ^d ±0.02	3.86 ^b ±0.01	6.31 ^a ±0.38
Barhe	3.40 ^a ±0.00	5.15 ^a ±0.09	0.21 ^a ±0.01	4.49 ^b ±0.11	0.11 ^{bc} ±0.01	4.20 ^{bc} ±0.22	7.04 ^b ±0.21

Values are means ± standard deviation of duplicate determination.

^{a-d}Means with similar superscript in the same column are not significantly different (P>0.05)

KEY: HCN : Hydrogen Cyanide.

Table 2: The Amino Acid Profile of the Date Palm Seed Samples

Amino Acid (g/100g protein)	Deglet Nour	Lulu	Bomaan	Fardh	Barhe	FAO/WHO (1991 Ref. Pattern) (g/100g protein)
Essential Amino Acids						
Leucine	11.62	11.91	11.79	10.10	12.02	1.10
Isoleucine	4.32	4.45	4.39	3.73	4.58	2.80
Lysine	2.33	2.17	2.28	2.01	2.39	5.80
Phenylalanine	5.14	5.50	5.32	4.43	5.41	6.30
Threonine	3.11	3.33	3.22	2.83	3.27	3.40
Valine	5.44	5.49	5.49	5.03	5.55	5.00
Histidine	2.24	2.30	2.24	2.04	2.30	2.80
Methionine	1.98	2.14	2.03	1.50	2.19	2.50
Tryptophan	1.26	1.33	1.21	1.10	1.31	1.10
Non-essential Amino Acids						
Alanine	8.72	9.33	8.95	7.89	9.10	6.10
Arginine	4.64	4.99	4.82	3.96	4.82	5.20
Aspartic Acid	7.38	8.00	7.88	6.76	7.88	7.70
Glutamic Acid	19.99	21.04	20.74	18.62	20.76	14.70
Glycine	3.23	3.42	3.47	2.99	3.18	2.20
Serine	4.27	4.37	4.32	3.83	4.37	7.00
Cysteine	1.82	1.83	2.06	1.33	1.82	3.00
Trysine	4.13	4.13	3.96	3.78	4.30	1.10
Proline	8.93	8.32	8.93	8.43	8.93	10.70

Acute toxicity of Date Palm Seeds.

Acute toxicity values of samples Deglet Nour, Lulu, Bomaan, Fardh and Barhe date palm varieties

No death was recorded within 24 hours and further 7 days of acute toxicity observation, even at the highest dose of 5000 mg/kg body weight. The mice had normal disposition and were emotionally stable all through the 7 – day period of observation. (Tables 3.1-3.2).

Table 3.1: Phase 1 result of acute toxicity evaluation of sample Deglet Nour

Group	Dose (mg/kg)	No. of death	Observation
1	10, 100 and 1000	0/3	Animals remained active and physically stable

Table 3.2: Phase 2 result of acute toxicity evaluation of sample Deglet Nour

Group	Dose (mg/kg)	No. of death	Observation
2	2900	0/3	Animals were active and physically stable
3	5000	0/3	Animals clustered together but soon regained their physical activities
4	5000	0/3	Animals clustered together but soon regained their physical activities

Key: LD₅₀ = √(A x B)

A= Maximum dose that produced no mortality

B= Minimum dose that killed all animals in a group

LD₅₀ is therefore >5000 mg/kg body weight

This method was repeated for all the rest of the samples – Lulu, Bomaan, Fardh, and Barhe and the same results were obtained for all the varieties.

The LD50 tests outcome suggest that the samples of the date palm seeds were non – toxic as all animals treated with the extract showed no sign of toxicity but rather looked physically healthy and emotionally stable. This conclusion is based on the recommendation of the Organization for Economic Co-operation and Development (OECD, 2001) guideline for acute toxicity studies [22].

OECD (2001) had reported that mortality is the expected end point of acute toxicity and non-observation of mortality within a population treated with a dose range at which mortality is expected indicates tolerance or lack of acute toxicity. Similar conclusions were drawn in other acute toxicity investigations involving plant materials [23]

Conclusion

The following conclusions emanate from this study:

- (i) The seeds contain significant amount of anti-nutrients such as phytate, oxalate and tannins.
- (ii) The seeds were found to be non-toxic with an LD₅₀ of > 5000mg/kg body weight.
- (iii) The seeds contained a good profile of amino acids when compared with the WHO/FAO 1991 Reference pattern except for lysine which was significantly low in all the five varieties. Plant proteins are known to be deficient in most of the essential amino acids but this result on date seeds had a contrary result. This is very significant of the date seeds; therefore, a little complementation with other plant proteins can help in meeting up with amino acid requirement in the body.

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