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ESTIMATION OF THE CONTENT OF LIPIDS AND FATTY ACIDS IN POLLEN OF *PHOENIX DACTYLIFERA* (DATE PALM) FROM BASRAH, IRAQ

ESTIMACIÓN DEL CONTENIDO DE LÍPIDOS Y ÁCIDOS GRASOS EN EL POLEN DE *PHOENIX DACTYLIFERA* (PALMERA DATILERA) DE BASRAH, IRAK

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ABSTRACT

This study aimed to analyze physical and chemical characteristics of pollen got from male spathes varieties grown in Basrah, Iraq. Among the results, the length, width, weight and powder weight of spathes of grains were obtained in the range of 50.3-71.4 cm, 23.8-33.5 cm, 756.3-2314.7 mg and 20.8-40.7 mg, respectively. The composition of the pollen was as follows: moisture 40.94 %, protein 25.89 %, ash 5.19 %, oil 9.19 % and carbohydrates 18.76 %. The fatty acid compositions of the oil were analyzed by GC-MS, where a total of 11-13 fatty acids has been identified. The fatty acids in pollen grains are palmitic acid, oleic acid, and linoleic acid. The results indicated that the percentage of unsaturated and saturated acids were 79.27-76.69 % and 18.06-20.51% respectively. The pollens of male palm can be considered as a good source of protein and oil with having remarkable health nutritional values.

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RESUMEN

***Spanish title**: Estimación del contenido de lípidos y ácidos grasos en el polen de Phoenix dactylifera (palmera datilera) de Basrah, Irak.* El propósito de la presente investigación es analizar las características físicas y químicas del polen obtenido de espátulas masculinas de variedades de palmeras datileras cultivadas en Basrah, Irak. Entre los resultados se determinó la longitud, la anchura, el peso y el peso del polvo de las espátulas de los granos según los rangos 50.3-71.4 cm, 23.8-33.5 cm, 756.3-2314.7 mg y 20.8-40.7 mg, respectivamente. La composición del polen dio: humedad 40.94 %, proteínas 25.89 %, ceniza 5.19 %, aceites 9.19 % y carbohidratos 18.76 %. La composición en ácidos grasos y en aceites fue determinada por análisis de GC-MS, identificándose un total de 11-13 ácidos, siendo los principales en peso: ácido palmítico, ácido oleico y ácido linoleico. Los resultados indican que el porcentaje de ácidos insaturados y saturados fue de 79.27-76.69 % y 18.06-20.51% respectivamente. El polen de DP masculino puede considerarse una buena fuente de proteínas y aceites con elevados valores nutricionales y para la salud en general.

INTRODUCTION

One of the best known and oldest edible fruits is the date-palm (*Phoenix dactylifera L.*), which belongs to the Arecaceae family. The cultivation of the fruits of *P. dactylifera* (the dates) has had a very significant impact on the

history of the ancient Middle East civilization. There exist references of the consumption of the date-palm (DP) fruits that allowed the human development in those deserted territories since 6000 years before to the Christian era [1,2,3,4]. Spread since then in arid and semi-arid territories all over the world, Iraq is thought to be as the the origin of the DPs. More than 2000 varieties of DPs are known worldwide [5,6]. Their fruits are well-reputed as a cheap source of essential nutrients becoming thus in an excelent food [7,8]. The total number of DPs in the world was estimated in about 16.5 million of trees in 2013 [9]. Among the pharmacological properties of dates reported by traditional medicine we count: anti-inflammatory, memory disturbances, fever, paralysis, loss of consciousness and nervous disorders [10]. Pollen of the date-palm was used by folk medicine since immemorial times for improving male and female fertility and for the treatment of sexual weaknesses [11-14]. Pollen is a fine powder holding the male reproductive cells of plant or the so-called male gametes produced by gametogenesis in plants [15]. Pollen grains of DP contain several chemical compounds such as 31- 39 % protein, 28-29 % moisture, 20-31% fat, 13- 20% carbohydrate, 4-6 % ash and 1-2 % crude fiber. Also, they are excellent sources of amino acids, minerals, and vitamins (A, B, C and E) [12,16,17]. Oil from pollen included: palmitic acid (C16:0) 34.45%, linoleic acid (C18:2) 14.24%, 13.33% and myristic acid (C14:0) [16]. Likewise, pollen contents include steroids like estrone, estradiol, β-sitosterol, cholesterol and clionasterol, flavonoids, carotenoids, and all plants' commonest metabolic enzymes like: glutamate pyruvate transaminase, glutamate oxaloacetate transaminases, lactate dehydrogenase and others [18,19,15]. DP pollen grains have a role in reducing the lipid fractions and protect the liver by repair of liver function enzyme behavior [15]. Salihi and his co-workers [20] investigated the impact of pollen grains of the DP, and its isolated flavonoids have hypolipidemic and antiatherosclerotic effects. Several researchers have shown that DP pollen can improve spermatogenesis, enhance sperm count and concentration of testosterone, FSH, and LH. [21]. Vegetal origin oils are important not only for their importance in foods, but also for industrial usages, like the production of pharmaceuticals, cosmetics, and paints, among others. However, very few reports to determine and identify fatty acids in pollen palm varieties are currently available. The present paper is an attempt to determine the oil composition of the pollen grains of DP by means of using GC-MS.

MATERIALS AND METHODS

1. Plant Material

The three different male palm pollens (which are Ghannamy Ahmar, Khikri and Samasmi) were collected from locations with similar soils and climates at the same period (March 2015 early in the day, at Abu Al-Khasseb in Basrah city) and treated equally. Thereafter, samples of male spathes were weighed and their size measured. The pollen grains were dried at room temperature before being utilized. Then, pollen grains were isolated from the flowers using fine sieves (40 mesh). The powder was stored at 5 °C until used.

2. Morphological characteristics

Following the growth characteristics as suggested by Al-Baker [22], we have measured the spathe length (cm), the spathe weight (gm); and the weight (mg) of the flour of the pollen grains.

3. Proximate composition analysis

Moisture and ash were specified according to AOAC. Crude oil was extracted in a Soxhlet extractor using hexane as a solvent. Crude proteins were determined from the nitrogen content by Kjeldahl method using factor 6.25 [23]. Total carbohydrates were calculated by difference as total percent value using the following formula: Total carbohydrates = 100 – (% moisture + % ash + % protein + % fat).

4. Extraction and preparation of fatty acids

Three individual 50 g samples of crushed dry seeds of each samples variety were refluxed with 300 mL of hexane in weighed flasks using a Soxhlet apparatus according to the AOAC, 2000 method. The oil was recovered by distilling the solvent on a rotary evaporator at 45ºC, then dried, collected, weighed, stored in a dark container in a deep-freeze till subsequent analyses. Fatty acids methyl esters were prepared using a solution of KOH 2N was prepared by adding 11.2 g of KOH in 100 ml of methanol as described in Alfekaiki [24].

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5. Gas Chromatography- Mass Spectrometry (GC-MS)

The GC - MS analysis of selected samples was performed with (GC- MS) Shimadzu Model QP2010 Ultra.For the identification of compounds were used the database of the library NIST08 as described by Alfekaiki [24]. GC–MS analysis was run at Chromatography Analyses Laboratory, Food Sciences Department/ Agriculture College/ Basrah University

Method conditions used foi the analysis of samples

Gas Chromatography	**Mass Spectrometer**
Column Oven Temp. :50.0 °C	Ion Source Temp :200.00 °C
Injection Temp. :280.00 °C	Interface Temp. :280.00 °C
Injection Mode: Split	Solvent Cut Time:3.00 min
Flow Control Mode: Linear Velocity	Start Time:3.00min
Pressure :100.1 kPa	End Time :25.00min
Total Flow :55.5 mL/min	ACQ Mode :Scan
Column Flow :1.69 mL/min	Event Time :0.50sec
Linear Velocity :47.2 cm/sec	Scan Speed :1666
Purge Flow :3.0 mL/min	Start m/z :20.00
Split Ratio: 30.	End m/z :800.00

6. Statistical analysis

The data were subjected to One-way analysis of variance (ANOVA) according to Randomized Complete design with three replicate, at the 5% level of significance, using SPSS version 17.0.

RESULTS AND DISCUSSION

Morphological characters of male spathes

Table 1 summarizes the morphological measurements, for the spathes variety utilized in this study:

***Table 1**: Morphological characteristics of male spathes.*

Species	Weight (mg)	Width (cm)	Length (cm)	Weigh of grains (mg)
Ghannamy Ahmar	1281.6b	29.8ab	53.6ab	20.8b
Khikri	2314.7a	33.5a	71.4a	40.7a
Samasmi	756.3ab	23.8b	50.3b	23.7ab

Spathes length and width: Data indicated that there were significant differences ($p \leq 0.05$), in spathes length and width between DP cultivars Khikar and Samasmi. It was observed (50.3 -71.4 cm) and (23.8 -33.5) cm in length and width respectively These results were in agreement with those estimated by [25] for male spathes. Spathe Weight: The weight of the spathes ranged from 756.3 – 2314.7 g in the variety of males. So these values are confirmed by the findings of [26, 25]. Pollen Grain Weight: The values obtained for weight grains were (20.8 – 40.7 g). This value was within the range assessed by [25] in several male DP cultivars. Spathe flour: Weight was significantly different in the species males Khikri and Ghannamy Ahmar. The pollen grains vary significantly in their size, and the characteristics of spathes, as well as in differences in agriculture treatments and environmental conditions. Researchers also found large differences between DP's male tested regarding the viability and compatibility of pollen [25,26].

Proximate compositions of palm pollens are presented in Figure 2: The moisture content of pollen grains was (39.09- 44.88%). They were within the range of the values (45.37 – 56.4%) obtained by [16] but higher than the values (28.80% & 29.0%) achieved by [16,17] respectively. The total ash content (4.61 - 5.68 %) for the grains is close to that reported by [16,17,27], where there were significant differences ($p > 0.05$) in moisture and ash of pollens samples between Khikri and Samasmi. The palm pollen grains contained a high percentage of crude fat (11.13 - 7.20

%). Similar values for oil contents of the pollen grain was reported by [27] and was quite smaller than that observed by [16,17].

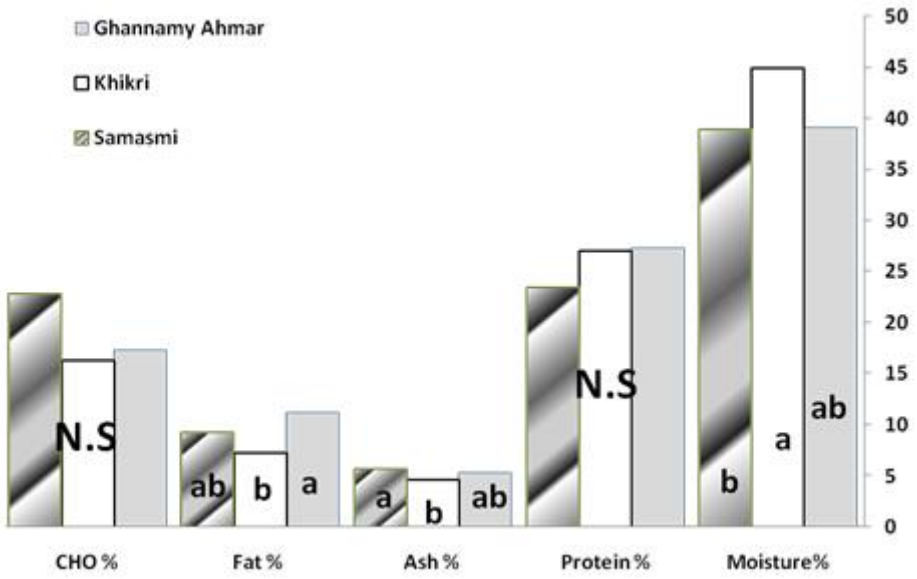

Figure 1: *Proximate analysis of male spathes.*

Oil showed significant differences ($p > 0.05$) among Ghannamy Ahmar and Khikri varieties of DP pollen tested. Crude protein content of palm pollen grains was (27.24 - 23.42%). The value of crude protein content was within the range estimated by [27] and was quite smaller than that published by [16,22]. The total carbohydrate of the grains was (16.27 - 22.78 %), similar to that reported by [16,17,28]. No significant differences in protein and carbohydrate content were observed among all varieties. The protein and fat content makes the palm pollen grains a potential commercial source of vegetable oil and protein. The variations in the chemical composition of pollen grains show differences according to the species, in function of their environmental conditions during the plant's maturation, age, and vigor [29]. The analysis of Fatty acid methyl esters composition of pollen grains oils was determined by GS-MS. Table 2 summarizes the results from the palm pollen grains oil by Gas Chromatography-Mass Spectrographic Analysis (CMSA). Data showed that the oil fraction of pollen includes 11-13 methyl esters of fatty acids and a number of unidentified secondary peaks. The predominant fatty acids were: oleic acid, palmitic acid, and linoleic acid, which were almost similar to the Egyptian palm pollen [17]. The total amount of saturated fat rose up to 18.06 – 20.51% which was less than the value reported by [17]. The saturated fatty acid present in all investigated samples in a significant amount is palmitic acid. The amount of total unsaturated fatty acids were in a percent of 76.69- 79.25% where the most predominant were oleic acid in a 36.01 -44.14% percent and linoleic acid in a 29.53 - 38.22% percent.

CONCLUSIONS

The pollen grains can be considered as a good source of protein and oil with having exceptional healthy nutritional value. This study advises more researches in the biochemical, nutritional and pharmacological fields on pollens of date-palm male.

Table 2: *Composition of pollen grains oils in methyl estesr of fatty acids determined by GC-MS technique.*

Name	Formula	Mol. Weight	Ghannamy Ahmar		Khikri		Samasmi	
			Similarity Index	Area%	Similarity Index	Area%	Similarity Index	Area%
Myristic acid, methyl ester	C15H30O2	242	95	0.11	95	0.11	95	0.10
Palmitoleic acid, methyl ester	C17H32O2	268	97	1.90	97	3.25	97	2.21
Palmitic acid, methyl ester	C17H34O2	270	95	11.17	96	10.44	95	10.59
Linoleic acid, methyl ester	C19H34O2	294	93	38.22	94	29.53	93	33.79
Oleic acid, methyl ester	C19H36O2	296	93	36.01	94	44.14	93	40.61
Stearic acid, methyl ester	C19H38O2	298	96	5.43	96	4.60	96	5.19
Oxiraneoctanoic acid, 3-octyl-, methyl ester	C19H36O3	312	-	-	-	-	92	0.43
Methyl 2-octylcyclopropene-1-octanoate	C20H36O2	308	67	0.08	71	0.09	69	0.34
cis-11-Eicosenoic acid, methyl ester	C21H40O2	324	95	0.48	95	0.94	94	0.87
Arachidic acid methyl ester	C21H42O2	326	90	0.94	94	0.97	94	0.92
Erucic acid methyl este	C23H44O2	352	-	-	88	0.12	88	0.10
Behenic acid, methyl ester	C23H46O2	354	92	2.42	92	2.49	92	2.18
Tricosanoic acid, methyl ester	C24H48O2	368	94	0.19	86	0.22	-	-
Tetracosanoic acid, methyl ester	C25H50O2	382	-	-	90	1.68	-	-
Total Fatty Acids %			96.95		98.58		97.33	
Total Saturated %			20.26		20.51		18.06	
Total Unsaturated %			76.69		78.07		79.27	

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