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# Effect of Four Pollinating Sources on Nutritional Properties of Medjool Date (*Phoenix dactylifera* L.) Seeds

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**Abstract:** The objective of this study was to evaluate the effect of pollen source on the nutritional composition of seeds of the ‘Medjool’ date (*Phoenix dactylifera* L.) under conditions of northwest Mexico. The content of nutrients (protein, lipids, fiber, and ash), sugars (total, reducing, and non-reducing), and most abundant minerals, were evaluated, as well as antioxidant activities. The pollen of four cultivars (‘Deglet Noor’, ‘Khadrawy’, ‘Medjool’, and ‘Zahidi’) was selected. Four groups of three bunches were pollinated in each of the female palms. For the four treatments, the following ranges were obtained: Seeds had a weight of 1.32–1.41 g and low concentrations of protein (4.44–5.49 g/100 g) and lipids (9.62–10.19 g/100 g). The content of dietary fiber and ash was 59.65–72.59 g/100 g and 1.09–1.42 g/100 g, respectively. Total sugars content was 4.50–6.96 g/100 g, while the insoluble solids were 92.21–96.03 g/100 g. The predominant mineral was potassium with 399.97–459.79 mg/100 g dry weight, followed by sulfur with 135.51–162.67 mg/100 g dry weight. Finally, for antioxidant activities, DPPH (2,2'-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay results were 0.0014–0.0094 IC<sub>50</sub> g/L and 0.176–0.299 IC<sub>50</sub> g/L, respectively. The pollen sources had an effect on the physicochemical properties of the seeds of the ‘Medjool’ cultivar date. However, some of the parameters (diameter, total acidity, pH) did not show statistically significant differences. The pollen sources from the ‘Khadrawy’ and ‘Medjool’ cultivars were the ones that induced differences in physical, proximate, and nutritional parameters, while pollen from the ‘Zahidi’ source induced the largest differences for minerals. The results suggest that selection of the source of pollen has a significant effect on the nutritional properties of the date seed from the cultivar ‘Medjool’, and that its use could be feasible as a functional food or by-product of the date production.

**Keywords:** date palm; *Phoenix dactylifera* L.; medjool; date seed; pollination

## 1. Introduction

The fruit of the date palm (*Phoenix dactylifera* L.) is commercially cultivated in the arid and semi-arid regions of the world, playing an important role in the economic aspects of countries in the Middle East and North Africa [1]. The date is a berry composed of a fleshy mesocarp, covered by a thin epicarp and an endocarp that covers all of its seed. The seed is usually oblong and ventrally grooved, containing a small embryo and a hard endosperm [2]. Date seeds have been studied, but little used, and are still considered as an agricultural waste in many developing countries [3].

The date seed constitutes between 6 and 18% of the total weight of a mature date [4,5], varying according to the variety and agroclimatic conditions of its cultivation. The seed contains sugars, appreciable amounts of minerals, high fiber content, and low contents of protein and fatty acids [6]. Due to its nutritional attributes, it has been used as food for cattle, horses, sheep, goats, camels, and poultry [2]. People also consume coffee-like drinks prepared from the seeds [7], and the use of ground seeds has been suggested in the preparation of certain processed foods [8]. Oil extraction has been one of the main alternative uses of the date seed, with possible applications in the pharmaceutical, food, and cosmetic industries [9]. Options have been explored for its use in the production of biofuels, particularly for the generation of biodiesel [3]. In the medical field, the date seed has great value as an agent with antioxidant, anti-inflammatory and antiviral activities [10].

Date producers are increasingly aware of the need to obtain a higher quality fruit in order to be more competitive in the market. Therefore, they have focused on identifying the male cultivars that have the greatest effects on the quality parameters in the fruit. Recent studies on the fruit of the 'Medjool' date cultivar in Mexico have concluded that there is a significant influence of pollen source on fruit physical and nutritional parameters [11,12].

Every year, interest in the cultivation of date palms increases in Mexico [13]. Much work has been done to improve date cultivation techniques and increase the efficiency of fertilization in order to obtain better yields. In 2018, Mexico produced 8945 tons of dates, of which 94% were the 'Medjool' cultivar [13,14]. The date industry in Mexico has not yet identified a potential use for date seeds, therefore, after the fruit has been consumed, the seeds are discarded. This is possibly due to the lack of knowledge about the nutritional properties of date seeds and the lack of research on this subject. Likewise, the possible use of date seeds as a source of cattle feed has been ruled out by large cattle producers, because a large volume of seeds is required, so its crushing was not affordable for use as a complementary food. In order to value the seed as a by-product, it is necessary to document its nutritional value and to explore possible industrial and medicinal applications.

Several studies have focused on determining the chemical properties of the date seed [1,2,4,10]. However, none has addressed the effect of various pollen sources on the chemical composition of the seed. The objective of this study was to characterize the effect of four different pollen sources on the chemical composition of the seed of the 'Medjool' cultivar under the soil and climatic conditions of the northwestern part of Mexico.

## 2. Materials and Methods

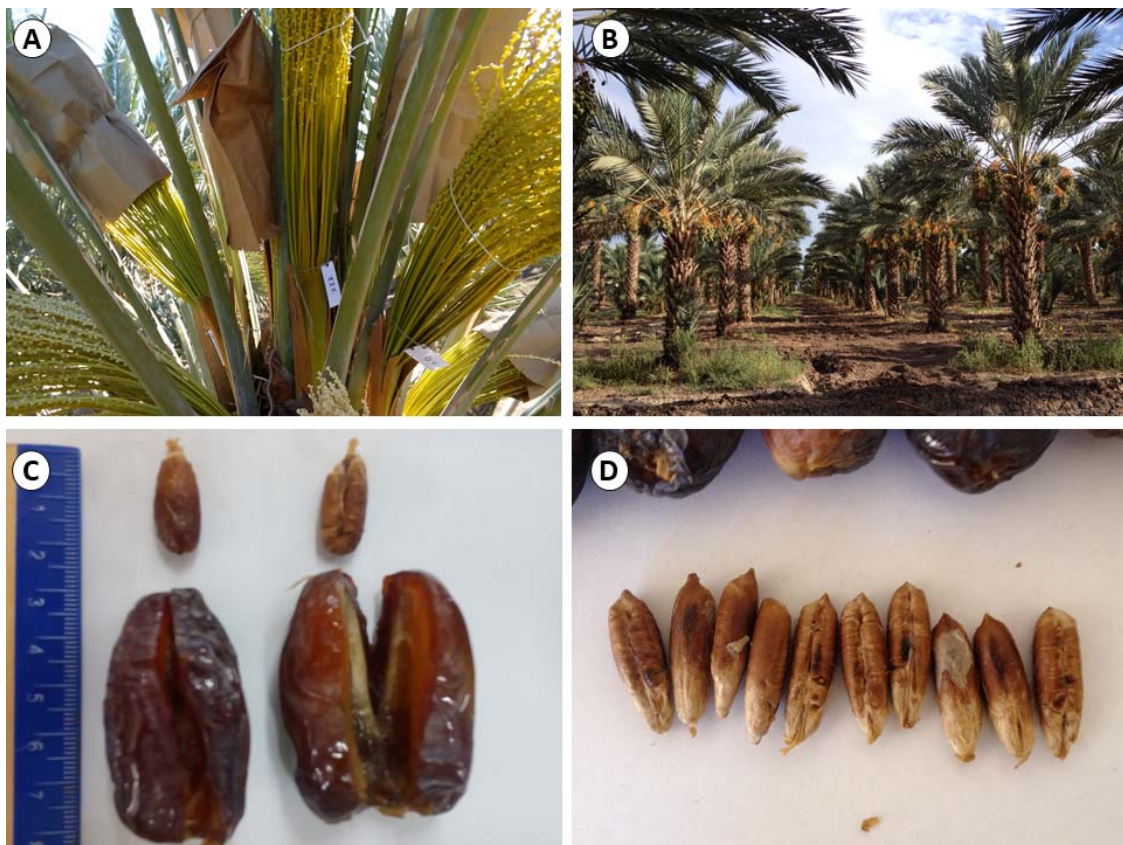
### 2.1. Characterization of the Experimental Area

The experiment was conducted in an orchard of 15 ha with organic certification (Figure 1B) located in "Rancho La Vaca" in the San Luis Rio Colorado Valley of Northwest Mexico (Sonora State) with the geographical coordinates: latitude 32°23'5" N, longitude 114°53'55" W and average altitude of 45 m. The soil is classified as alluvial from the dry bed of the Colorado River. Flood irrigation was used and distributed in six irrigations of 15 cm each during the year.

### 2.2. Plant Materials, Treatments, and Experimental Design

Four vigorous 17-year-old elite male date palms derived from offshoots of the most common cultivars in the area ('Deglet Noor', 'Khadrawy', 'Medjool', and 'Zahidi') were used as pollen sources

for pollination of the ‘Medjool’ cultivar. Once the spathes naturally broke, they were removed and transported to a drying area, where they were hung for a period of 3 days for the extraction of pollen, which was extracted daily on a paper bed. The pollen was stored at 4 °C until pollination time. Four 16-year-old female ‘Medjool’ palms derived from offshoots of the ‘Medjool’ cultivar were randomly selected as recipients. Palms were planted with an 8 × 8 m distance between and within rows. After the natural cracking of their spathes, each spathe was manually opened. Four groups of three bunches (female inflorescences) were selected on each female tree, and each group was pollinated individually only once, using pollen from only one cultivar. Pollination was carried out manually using a brush between the second and fourth day after the spathes opened (Figure 1A). The fresh pollen was diluted with wheat flour in a 1:1 ratio. Additionally, one more plant was selected and managed as a control, using a traditional farmer’s method, consisting of taking pollen from various unknown male palms that are compatible with the ‘Medjool’ female, mixing all the pollen, and diluting it to a 1:1 ratio with commercial wheat flour. Finally, each strand was thinned in all bunches at a rate of 12 dates per thread and 50 strands per bunch. [11]. The experimental design employed was a completely randomized design. In our case, this design was represented by four palms as replicates and four pollen sources. Each pollen treatment was applied to three randomly selected bunches on each palm.



**Figure 1. Experimental area and seed extraction.** (A) Female inflorescences covered with paper bags after pollination to avoid contamination with other pollen sources. (B) Panoramic view of the plantation 50 days after pollination. (C) Removing the seed of the ripe fruit. (D) Sample of seeds extracted from the pollinated fruit with the ‘Zahidi’ pollen source.

### 2.3. Physical Properties

For each treatment (pollen source) in each palm, 15 fruit were collected from among its three bunches (60 fruit per treatment per palm), resulting in a total of 240 fruit among the four palms. From the control palm, 60 fruit were taken randomly from among its bunches. The fruit were weighed,

then the seeds were removed (Figure 1C,D) and weighed with an electronic scale. Length and diameter were measured as well.

#### 2.4. Proximate Analysis

Derived from the sampling performed in Section 2.3, nine samples (seeds) were taken randomly from each treatment of the four palms and the control, for a total of 45 samples; proximate analysis and three replicate measurements from each sample were made. All seeds (fresh) were incubated at 105 °C for 24 h to determine the moisture content. The samples were then incinerated at 550 °C for 8 h in a muffle oven and the ash percentage was calculated by weight difference [15]. The crude protein was obtained using a chemical digestion process (Kjeldahl method) to determine total nitrogen followed by conversion to protein percentage [15] using a conversion factor of 6.25 [16]. Total lipids were determined according to the method of Folch et al., (1957) [17]. Determination of dietary fiber was carried out using the AOAC enzymatic-gravimetric official method (Method 991.43) [18]. The pH was taken from a homogeneous sample at 20 °C using a MP 744 pH meter (Metrohm AG, Herisau, Switzerland). Total insoluble and soluble solids were determined using a refractometer to obtain °Brix [19]. The total acidity was calculated titrating against 0.1 N NaOH and was expressed as malic acid percentage. All the elements were expressed in g/100 g dry weight.

#### 2.5. Sugars Determination

From the samples in Section 2.3, five seeds from each treatment and control were taken (that is, 25 seeds in total) and three replicate measurements of sugars content were made for each sample. Total sugars and reducing sugars were determined using the Lane and Eynon volumetric method [20]. Non-reducing sugars were calculated as the difference between total sugars and reducing sugars.

#### 2.6. Minerals Analysis

The mineral content was determined using standard methods [18]. From the samples in Section 2.3, three seeds from each treatment and control were taken (that is, 15 seeds in total) and three replicates from each sample were used for the mineral analysis. Each seed was converted to ash at 550 °C for 24 h. Then 1 g of ash from each sample was dissolved in 5 mL of analytical grade hydrochloric acid (20%) and the solution was transferred to a 50 mL volumetric flask. The final volume was completed using deionized water. Total minerals were determined using an atomic absorption spectrophotometer (model 4200 MPAES, Agilent Technologies, Santa Clara, CA, USA) and were expressed in mg/100 g dry weight basis.

#### 2.7. Antioxidant Activities and Total Phenols

From the samples in Section 2.3, five seeds from each treatment and control were taken (that is, 25 seeds in total) and three replicates for each were used. The radical scavenging profile from each date seed was determined by three methods: DPPH scavenging activity [21], ABTS radical cation decolorization assay [22,23], and oxidation of  $\beta$ -carotene and linoleic acid system [24]. DPPH and ABTS are expressed as IC<sub>50</sub> g/L (half maximal inhibitory concentration), which means how much extract is needed to reduce 50% of the compound. Total phenols were determined using the Folin-Ciocalteu method [25]. The results were expressed as milligrams of equivalent gallic acid per 100g dry weight (mg GAE/100 g dw).

#### 2.8. Energy Value Determination

The energy values of the seed for each treatment were calculated using the percentages of crude protein, fat, and carbohydrate: Energy value (kcal/100 g) = (2.62 × % protein) + (8.37 × % fat) + (4.2 × % carbohydrates) [26].

### 2.9. Statistical Analysis

The data were statistically analyzed using one-way ANOVA test in a balanced design. The means of the treatments were compared according to the least significant differences (LSD) test, at a significance level of 5% [27]. The variance analysis was calculated using version 3.6.1 of Statistical Software R [28,29]. The final results were defined as mean values  $\pm$  standard deviation of three separate determinations by sample.

## 3. Results

### 3.1. Physical Characteristics of Seed

Table 1 shows the significant variations in the physical characteristics of the ‘Medjool’ date seed. The highest average seed weight was obtained with ‘Medjool’ pollen. This average weight does not show a significant difference compared to the rest of the treatments, except for ‘Deglet Noor’, which had the lowest average. Seeds from the control had an average weight significantly lower than any of the other treatments. The maximum seed length was obtained by the ‘Khadrawy’ treatment. The shortest seed lengths were obtained by the ‘Deglet Noor’ treatment and control. The average diameter of the seed did not show significant differences among the four pollen sources. However, the control had a significantly lower average diameter than that induced by the other pollen sources used in this study. The seed represents a small percentage of the total mass of the date fruit. In this study, the highest percentage of seed mass in the fruit was induced by the pollen of the ‘Medjool’ cultivar, while the lowest value corresponded to the ‘Khadrawy’ cultivar. The seed mass of the control was significantly smaller than any of the treatments. The results indicate that the pollen source has a significant effect on the physical characteristics of date seeds and all of the treatments significantly increase its weight as compared to the pollen used by the farmer.

**Table 1.** Physical characteristics of ‘Medjool’ date seeds obtained from fruit resulting from pollination using pollen from ‘Deglet Noor’, ‘Khadrawy’, ‘Medjool’, and ‘Zahidi’ cultivars. Control is a pollen mixture from random date palm male plants used by the grower.

Parameter	‘Deglet Noor’	‘Khadrawy’	‘Medjool’	‘Zahidi’	‘Control’
Weight (g)	1.32 $\pm$ 0.079b	1.38 $\pm$ 0.074ab	1.41 $\pm$ 0.076a	1.34 $\pm$ 0.054ab	1.22 $\pm$ 0.113c
Length (cm)	2.53 $\pm$ 0.128c	2.71 $\pm$ 0.085a	2.64 $\pm$ 0.15ab	2.57 $\pm$ 0.067bc	2.51 $\pm$ 0.131c
Diameter (cm)	0.88 $\pm$ 0.029a	0.89 $\pm$ 0.029a	0.91 $\pm$ 0.019a	0.88 $\pm$ 0.034a	0.84 $\pm$ 0.043b
% of mass in the fruit	5.61 $\pm$ 0.3c	5.36 $\pm$ 0.8c	6.16 $\pm$ 0.5a	5.88 $\pm$ 0.4b	5.06 $\pm$ 0.3d

Values are mean  $\pm$  SD of 60 samples per treatment and control. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level.

### 3.2. Proximate Composition of the Seed

The proximate composition of the seeds is summarized in Table 2. A significantly higher moisture content was observed in the ‘Medjool’ treatment. The lowest moisture content was from the ‘Deglet Noor’ treatment, which was significantly lower than the ‘Khadrawy’ treatment and control. The protein content in the seed was significantly higher for the ‘Khadrawy’ treatment. The ‘Deglet Noor’ and ‘Zahidi’ treatments had protein contents significantly lower than the control and that of ‘Medjool’ treatment. Significant differences among the four cultivars were observed for the lipid concentration. The highest average was for the ‘Medjool’ treatment, whereas the lowest was for the ‘Zahidi’ and ‘Deglet Noor’ treatments. The control showed the lowest lipid concentration of all treatments. The influence of pollen sources had a significant effect on the dietary fiber content. The highest average was for the ‘Khadrawy’ treatment and the lowest average value was for the ‘Zahidi’ treatment. The control resulted in an average significantly lower than all other treatments. The highest ash content was for the ‘Deglet Noor’ treatment, being significantly higher than the rest of the treatments. The lowest ash

content was obtained from the 'Medjool' treatment. Finally, the ash content in the control was not significantly different compared to 'Medjool' treatment.

**Table 2.** Proximate composition of 'Medjool' date seeds obtained from fruit resulting from pollination using pollen from 'Deglet Noor', 'Khadrawy', 'Medjool', and 'Zahidi' cultivars. Control is a pollen mixture from random date palm male plants used by the grower.

Parameter	'Deglet Noor'	'Khadrawy'	'Medjool'	'Zahidi'	'Control'
Moisture (%) <sup>a</sup>	4.90 ± 0.22c	5.89 ± 0.23ab	6.36 ± 0.32a	5.40 ± 0.40bc	5.69 ± 0.12b
Protein (g/100 g) <sup>b</sup>	4.44 ± 0.12c	5.49 ± 0.14a	4.99 ± 0.15b	4.50 ± 0.14c	4.82 ± 0.05b
Lipids (g/100 g) <sup>b</sup>	9.63 ± 0.07c	9.98 ± 0.18b	10.19 ± 0.14a	9.62 ± 0.08c	9.32 ± 0.11d
Dietary fiber (g/100 g) <sup>b</sup>	69.37 ± 5.42ab	72.59 ± 1.74a	65.56 ± 2.41b	59.65 ± 4.19c	52.31 ± 4.32d
Ash (g/100 g) <sup>b</sup>	1.42 ± 0.27a	1.27 ± 0.28ab	1.09 ± 0.40bc	1.20 ± 0.04ab	0.96 ± 0.02c

Values are mean ± SD of nine samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level. <sup>a</sup> Expressed on fresh weight basis. <sup>b</sup> Expressed on dry weight basis.

### 3.3. Content of Total Soluble Solids, Insoluble Solids, Total Acidity, and pH.

The averages of the total soluble solids, insoluble solids, total acidity and pH, are shown in Table 3. A significantly higher average of total soluble solids (TSS) was observed in the 'Zahidi' treatment. The lowest significant TSS content was for 'Medjool' and 'Khadrawy' treatments. The control was not significantly different than these last two treatments. The highest significant content of insoluble solids was for the 'Khadrawy' and 'Medjool' treatments, and control, compared to the other cultivars, but was not significant among them. The 'Zahidi' treatment showed the lowest insoluble solids content, differing from the rest of the treatments. The sources of pollen did not have a significant effect on the total acidity and pH. No studies were found that described these same parameters for the 'Medjool' cultivar grown in other countries.

**Table 3.** Content of total soluble solids, insoluble solids, total acidity, and pH of 'Medjool' date seeds obtained from fruit resulting from pollination using pollen from 'Deglet Noor', 'Khadrawy', 'Medjool', and 'Zahidi' cultivars. Control is a pollen mixture from random date palm male plants used by the grower. All parameters are expressed in g/100 g dry weight.

Parameter	'Deglet Noor'	'Khadrawy'	'Medjool'	'Zahidi'	'Control'
Total soluble solids	6.00 ± 0.05b	4.13 ± 0.23c	4.03 ± 0.08c	7.94 ± 0.05a	4.08 ± 0.13c
Insoluble solids	94.35 ± 0.29b	96.03 ± 0.05a	95.69 ± 0.57a	92.21 ± 0.31c	96.13 ± 0.21a
Total acidity	0.052 ± 0.03a	0.053 ± 0.04a	0.052 ± 0.07a	0.052 ± 0.02a	0.053 ± 0.02a
pH	6.98 ± 0.02a	6.96 ± 0.04a	7.00 ± 0.02a	6.95 ± 0.05a	7.02 ± 0.03a

Values are mean ± SD of nine samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level.

### 3.4. Sugar Content

The effect of the four treatments on the average sugar content was statistically significant (Table 4). Total sugar content was significantly higher for the 'Deglet Noor' treatment, while the 'Zahidi' treatment was the lowest, being significantly less than the control. For reducing sugars, the highest significant percentage was reached by the 'Khadrawy' treatment, whereas the lowest was for the 'Zahidi' treatment. The control showed the second highest average. Non-reducing sugars were significantly affected by the different pollen sources. The highest percentage was obtained in 'Deglet Noor'-pollinated fruit, while the lowest was obtained from the 'Zahidi' treatment, which was lower than the control. Unlike the high sugar content in the date pulp, the seed has a very low sugar content. Finally, the highest energy content corresponded to the 'Medjool' cultivar, while the lowest was from the 'Zahidi' treatment.

**Table 4.** Sugar content and energy content of ‘Medjool’ date seeds obtained from fruit resulting from pollination using pollen from ‘Deglet Noor’, ‘Khadrawy’, ‘Medjool’, and ‘Zahidi’ cultivars. Control is a pollen mixture from random date palm male plants used by the grower. All parameters are expressed in g/100 g dry weight.

Parameter	‘Deglet Noor’	‘Khadrawy’	‘Medjool’	‘Zahidi’	‘Control’
Total sugars	6.96 ± 0.21a	5.75 ± 0.23c	6.22 ± 0.12b	4.50 ± 0.11e	5.42 ± 0.07d
Reducing sugars	4.24 ± 0.03c	5.06 ± 0.02a	4.20 ± 0.08c	4.08 ± 0.05d	4.58 ± 0.11b
Non-Reducing sugars	1.72 ± 0.03a	1.51 ± 0.11b	1.50 ± 0.14b	1.07 ± 0.15d	1.30 ± 0.14c
Energetic value <sup>a</sup>	122.32 ± 0.78b	121.96 ± 1.66b	125.26 ± 0.76a	112.40 ± 0.96d	114.03 ± 0.59c

Values are mean ± SD of five samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level. <sup>a</sup> Expressed in Kcal/100 g.

### 3.5. Macronutrient Content

Table 5 shows the macronutrient content of date seeds, where significant differences were observed among the treatments. The potassium content was the highest and was followed in descending order by sulfur, phosphorus, calcium, magnesium, and sodium. ‘Zahidi’ pollen induced the highest content of sulfur, phosphorus, magnesium, and sodium, while ‘Khadrawy’ and control induced it for potassium content, and ‘Medjool’ for calcium content. The lowest significant content for sulfur, phosphorus and magnesium resulted from the ‘Medjool’ treatment, for potassium and calcium from the ‘Zahidi’ treatment, and for sodium from the ‘Khadrawy’ treatment. The control produced the highest average value for potassium and sulfur, but the lowest for calcium, compared to the other treatments.

**Table 5.** Total macrominerals content for four pollen source treatments and control for a recipient ‘Medjool’ cultivar. Control is a pollen mixture from random date palm male plants, used by the grower. All parameters are expressed in mg/100 g dry weight.

Parameter	‘Deglet Noor’	‘Khadrawy’	‘Medjool’	‘Zahidi’	‘Control’
Potassium	425.87 ± 13.6c	459.79 ± 16.9b	414.21 ± 15.2d	399.97 ± 12.6d	461.06 ± 11.1a
Sulfur	147.21 ± 2.8c	160.05 ± 2.4b	135.51 ± 4.8c	162.67 ± 1.5b	190.64 ± 2.9a
Phosphorus	87.70 ± 2.6c	92.84 ± 2.06ab	79.43 ± 2.1bc	96.71 ± 2.4a	93.44 ± 2.3ab
Calcium	56.75 ± 1.3b	53.94 ± 0.9c	62.16 ± 1.10a	51.35 ± 1.06d	47.02 ± 1.5e
Magnesium	35.21 ± 1.9b	36.72 ± 2.1b	31.85 ± 2.03c	40.71 ± 3.1a	35.02 ± 2.5b
Sodium	32.10 ± 0.9c	28.60 ± 0.5d	33.50 ± 1.6b	42.36 ± 0.7a	33.33 ± 1.3b

Values are mean ± SD of three samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level.

### 3.6. Micronutrient Content

Table 6 shows the micronutrient contents found in date seeds, where significant differences were observed among the four treatments. The selenium content was the highest, followed in descending order by iron, silicon, zinc, copper, manganese, strontium, and aluminum. ‘Zahidi’ pollen induced the highest significant content for iron, zinc, copper, strontium, and aluminum. ‘Medjool’ pollen induced the highest levels of selenium and manganese, and ‘Deglet Noor’ pollen the highest silicon level. The lowest significant contents of iron, manganese, strontium, and aluminum resulted from ‘Deglet Noor’ pollen, and selenium and silicon from ‘Zahidi’ pollen. The lowest zinc and copper contents were associated with the ‘Medjool’ treatment. The control had the highest average contents of iron, zinc, manganese, and aluminum, but the lowest of strontium, compared to the rest of the treatments.

**Table 6.** Total microminerals content for four pollen source treatments and control for a recipient ‘Medjool’ cultivar. Control is a pollen mixture from random date palm male plants, used by the grower. All parameters are expressed in mg/100 g dry weight.

Parameter	‘Deglet Noor’	‘Khadrawy’	‘Medjool’	‘Zahidi’	‘Control’
Selenium	3.91 ± 0.11b	4.14 ± 0.04a	4.33 ± 0.12a	3.77 ± 0.08b	4.08 ± 0.08a
Iron	1.05 ± 0.01c	1.10 ± 0.01c	1.30 ± 0.03b	1.45 ± 0.02b	1.68 ± 0.03a
Silicon	0.85 ± 0.06a	0.80 ± 0.09a	0.75 ± 0.01b	0.62 ± 0.08c	0.77 ± 0.09b
Zinc	0.78 ± 0.01d	1.01 ± 0.07c	0.70 ± 0.04d	1.18 ± 0.04b	1.37 ± 0.03a
Copper	0.77 ± 0.05c	0.75 ± 0.06c	0.67 ± 0.03d	0.91 ± 0.10a	0.88 ± 0.08b
Manganese	0.56 ± 0.01d	0.63 ± 0.03cd	0.91 ± 0.01b	0.79 ± 0.02c	1.16 ± 0.03a
Strontium	0.25 ± 0.01bc	0.26 ± 0.02b	0.28 ± 0.02a	0.29 ± 0.01a	0.21 ± 0.01d
Aluminum	0.18 ± 0.01d	0.25 ± 0.01b	0.20 ± 0.01c	0.36 ± 0.01a	0.37 ± 0.01a

Values are mean ± SD of three samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level.

### 3.7. Antioxidant Activity and Phytochemical Evaluation

Significant differences were observed among the four treatments for total phenolic content and antioxidant activities (Table 7). The maximum significant  $\beta$ -Carotene percent was induced by pollen of the ‘Deglet Noor’ cultivar, whereas the lowest value was induced by the ‘Medjool’ pollen. The control resulted in the second highest percentage compared to the rest of the treatments. DPPH results had the highest significant value for the ‘Zahidi’ treatment, while the lowest significant value was for the ‘Medjool’ treatment. The control resulted in the third highest value of DPPH with respect to the four treatments. The highest value of the ABTS assay resulted from the ‘Khadrawy’ treatment, and the lowest from the ‘Medjool’ treatment. The control showed the third highest result with respect to the four treatments and was not significantly different from the ‘Khadrawy’ and ‘Zahidi’ treatments. Regarding the total phenolic content, the ‘Khadrawy’ pollen treatment induced the highest content, whereas the lowest significant total phenolic content was obtained from the ‘Deglet Noor’ treatment. However, the control had the third highest content with respect to the four treatments.

**Table 7.** Total phenolic content and antioxidants activities for ‘Medjool’ date seeds, obtained from fruit resulting from pollination using pollen from ‘Deglet Noor’, ‘Khadrawy’, ‘Medjool’, and ‘Zahidi’ cultivars. Control is a pollen mixture from random date palm male plants used by the grower.

Parameter	‘Deglet Noor’	‘Khadrawy’	‘Medjool’	‘Zahidi’	‘Control’
$\beta$ -Carotene (%)	62.33 ± 3.52a	46.01 ± 5.19b	33.66 ± 3.78b	50.58 ± 5.50a	52.25 ± 7.66a
DPPH (IC <sub>50</sub> g/L)	0.0023 ± 0.0008c	0.0055 ± 0.0004b	0.0014 ± 0.0003c	0.0094 ± 0.0021a	0.0032 ± 0.0006bc
ABTS (IC <sub>50</sub> g/L)	0.199 ± 0.016ab	0.299 ± 0.005a	0.176 ± 0.072b	0.278 ± 0.003a	0.257 ± 0.057a
Total phenolic contentppppp(mg GAE/100 g, DW)	11.44 ± 0.36d	18.26 ± 0.50ab	16.03 ± 0.75bc	12.75 ± 0.16d	14.25 ± 0.80b

Values are mean ± SD of five samples per treatment and control with three independent determinations per sample. Means followed by same letter(s) in a row do not differ significantly at 0.05 probability level.

## 4. Discussion

The pollen sources selected for this study had an effect on the physicochemical properties of the seeds of the ‘Medjool’ date cultivar. However, some of the parameters (diameter, total acidity, pH) did not show statistically significant differences. In previous studies carried out in Mexico, under the same conditions, it was determined that the pollen from the ‘Khadrawy’ cultivar influenced most of the analyzed physico-chemical characteristics of ‘Medjool’ fruit [11,12].

Previous studies in Morocco [30] and the United Arab Emirates [31] quantified the physical properties of seeds of the date cultivar ‘Medjool’. The variations in seed characteristics reported from these studies and ours can be attributed to various factors in addition to pollens, such as environment, irrigation, climate, and fertilization, among others.

A high range of dietary fiber content (52.31–72.59 g/100 g) for all treatments in this study was identified in the seed of the ‘Medjool’ date (Table 2). Therefore, date seeds could potentially be used as an ingredient in the production of food for human consumption in which there is a need to increase



the fiber content, such as wheat flour-based products [31]. This would stimulate solid waste transit in the body, to possibly prevent constipation and some gastrointestinal diseases [10,30].

The date seed is characterized by having a low protein content, which can vary between 4 and 6 g/100 g according to the seed variety [30,31]. In this study, its average value was 4.5 g/100 g (Table 2) for the seed of 'Medjool'. Although low, the seed protein content is higher than the content of protein in the pulp of the fruit [12].

Likewise, the seed contains a low lipid content (Table 2), resulting in little commercial potential for industrial oil extraction [9]. However, a great advantage for its exploitation is that this material, being considered an agricultural waste, can be acquired at a very low cost or without any cost. The percentage of the most abundant fatty acids in the seed of the 'Medjool' date was reported in a study from Israel [9] as 44.92% oleic and 21.8% lauric, while a study from Morocco [30] reported 44.92% oleic and 20.34% lauric. Oleic and lauric acids were followed by myristic, palmitic, and linoleic acids in lower proportions. Date seed oil has already been used to replace portions of other vegetable oils in products such as body creams, shampoos, bar soaps, and shaving creams [9]. Another study reported that date seed oil used in mayonnaise production produced a superior sensory characteristic compared to another vegetable oil [32]. Based on last year's date production in Mexico [14] and the percentage of lipids in the date seed reported in this study (Table 2), almost 894 tons of date seed oil could be produced. Therefore, the operation of a small oil extraction plant may be feasible in Mexico. Table 8 shows the average values of the physical parameters and proximal composition of 'Medjool' date seeds obtained from the four treatments (pollen sources: 'Deglet Noor', 'Khadrawy', 'Medjool', and 'Zahidi') in this study compared to those reported by studies carried out in Morocco [30] and United Arab Emirates [31].

**Table 8.** Comparative analysis of the physical characteristics and proximal composition of the average obtained from the four treatments used in this study, against studies developed in the Medjool seeds in Morocco and the United Arab Emirates.

Parameter	Average for This Study <sup>a</sup>	Morocco [30]	United Arab Emirates [31]
Weight (g)	1.36	1.10	–
Length (cm)	2.61	2.09	2.75
Diameter (cm)	0.89	0.93	1.15
% of mass in the fruit	5.75	7.48	7.29
Moisture (%) <sup>b</sup>	5.63	9.79	8.25
Protein (g/100 g) <sup>c</sup>	4.85	5.55	6.14
Lipids (g/100 g) <sup>c</sup>	9.85	6.52	5.66
Dietary fiber (g/100 g) <sup>c</sup>	66.79	19.90	73.36
Ash (g/100 g) <sup>c</sup>	1.24	1.14	1.09

<sup>a</sup> The average was obtained by averaging all treatments (pollen sources) from this experiment. <sup>b</sup> Expressed on fresh weight basis. <sup>c</sup> Expressed on dry weight basis.

The insoluble solids content on average for this study was 94.64 g/100 g for the seed (Table 3), much higher than the pulp content of the same fruit (15.70 g/100 g) reported in another study under the same production conditions [12]. The average of the four treatments in total soluble solids and total acidity of 'Medjool' seeds were lower than the results reported for fruit by the study mentioned above; however, the pH turned out to be very similar to that of the 'Medjool' date fruit [12].

The study carried out in Morocco [30] reported a content of total sugars of 9.54 g/100 g dw and an energetic value of 103.58 Kcal/100 g dw for the seed of 'Medjool'. In this study, an average of 5.85 g/100 g dw and 120.48 Kcal/100 g dw for total sugars and energy value, respectively, are reported among the four pollen sources (Table 4). No other studies were found that reported the reducing and non-reducing sugar averages of seeds of the 'Medjool' cultivar. The sugar content in the seed of the 'Medjool' cultivar date is very small compared to that in the pulp of the same fruit (Table 4) [12].

The seeds of the 'Medjool' date are a good source of minerals, providing a greater amount of sulfur, sodium, iron, manganese, and zinc (Tables 5 and 6) than the pulp of the same fruit [12]. However,

the contents of potassium, magnesium, calcium, and phosphorus in the seed (Table 5) are not as high as the contents found in the fruit [12]. Variations in the mineral content between studies conducted in Morocco [30] and the United Arab Emirates [31] compared to those reported in the present study can be attributed to factors involved in crop nutrition, such as the level of fertility in the soil and the amount and type of fertilizers applied to the palms [33]. Minerals are micronutrients that the body requires to develop various vital functions such as bone formation, hormone production, and metabolic processes. The intake of 100 g of date seed is capable of providing 14% of the recommended daily diet of iron, manganese, copper, and magnesium, as well as 7% and 4% of zinc and calcium, respectively [30]. Table 9 shows the average of the four treatments (pollen sources: ‘Deglet Noor’, ‘Khadrawy’, ‘Medjool’, and ‘Zahidi’) for most of the mineral contents reported in this study, compared them against those reported in studies carried out in Morocco [30], United Arab Emirates [31], and India [34].

**Table 9.** Comparative analysis minerals content of the average obtained from the four treatments used in this study, against studies developed in the Medjool seeds in Morocco, United Arab Emirates and India. All expressed in mg/100 g dry weight.

Parameter	Average for This Study <sup>a</sup>	Morocco [30]	United Arab Emirates [31]	India [34]
Potassium	424.96	296.71	240.33	181.33
Phosphorus	89.17	-	146.50	121.33
Calcium	56.05	22.70	23.00	39.49
Magnesium	36.12	82.76	74.92	28.00
Sodium	34.14	10.80	15.42	-
Iron	1.22	0.55	5.04	1.83
Zinc	0.91	1.47	1.62	1.46
Copper	0.77	0.83	0.21	-
Manganese	0.72	0.55	0.55	-
Strontium	0.27	-	0.88	-
Aluminum	0.24	-	1.88	-

<sup>a</sup> The average was obtained by averaging all treatments (pollen sources) from this experiment.

Another characteristic of the date seed is its high phenolic content and antioxidant activity. The average total phenolic content in the four treatments for ‘Medjool’ seed was found to be higher than that reported in the pulp of the same fruit [12]. The results of the antioxidant capacity reported by this study using the DPPH and ABTS tests for the seed were found to be lower against those reported in the pulp of the same fruit by another study [35]. DPPH results gave an average of 0.0046 IC<sub>50</sub> g/L for this study (Table 7), while the study carried out in Morocco [30] reported 0.133 IC<sub>50</sub> g/L for ‘Medjool’ seeds, showing a lower degree of antioxidant activity than in our study. An average of 14.62 mg GAE/100 g in total phenolic content was found in this study, while the study in Morocco reported 30.78 mg GAE/100 g. The average of  $\beta$ -Carotene and ABTS were 48.14% and 0.238 IC<sub>50</sub> g/L, respectively, for the four treatments in this study (Table 7). No studies were found reporting the  $\beta$ -Carotene percentage, nor the concentration providing 50% inhibition (IC<sub>50</sub>) for the ABTS assay in ‘Medjool’ seeds.

The functional components in food are nutrient materials of plant and microbial origin that stimulate human health. Dietary fiber, essential vitamins, and minerals are functional components that are characterized by antimicrobial, anticancer, antioxidant, and other activities [36]. The great nutritional potential of the date seed is mainly related to the significant amount of fiber content and its high polyphenol content, which are far superior to those contained in the pulp of the same fruit. Possible health benefits, such as the prevention of chronic diseases and the strengthening of the oxidative system of the human body, have been suggested to be derived from its antioxidant properties [4].

## 5. Conclusions

Considering the results obtained in this study, it could be concluded that pollen sources from the 'Khadrawy' and 'Medjool' cultivars had the most significant effects on the nutritional characteristics of the seed of the 'Medjool' date cultivar, compared to the other pollen sources. The analysis presented revealed that the 'Khadrawy' pollen source increased the dietary fiber content by 28%, phenols by 22% and protein by 13%, whereas pollen of 'Medjool' increased lipid content by 9% compared to the traditional pollination practices of the farmer. Therefore, it may be recommended to use the pollen of 'Khadrawy' or 'Medjool' cultivars, mixed or separately, in order to obtain the best contents of fiber, minerals, lipids, and antioxidants in the seeds.

These nutritional components can give a high added value to the by-products that could be generated from this seed. This could stimulate the interest in its exploitation in Mexico, for its use in some functional foods for human consumption, as nutritional supplements in food for cattle and birds, and other formulations in the industries such as cosmetics and pharmaceuticals. This work is the first of its kind to characterize the effect that pollen from various sources has on the seed of the 'Medjool' date.

**Author Contributions:** R.S.-T., N.O.-U. and J.A.S.-U. conceived and designed the study, completed the fieldwork and wrote the paper; B.V.-S., C.G.-G. and S.N.-P. performed the experiments, contributed reagents/materials/analysis tools. D.H.-B., J.P.G.-V. and R.K. co-wrote the paper. All authors contributed to its editing and approved the final draft. All authors have read and agreed to the published version of the manuscript.

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