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Effect of pollen sources on yield oil extraction and fatty acid profile of the date seed (*Phoenix dactylifera* L.) cultivar Medjool from Mexico

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SUMMARY: The present investigation aimed to assess the effect of pollen sources on the mass, dimension, oil content and fatty acid profile of the seeds from female palms of the Medjool date cultivar. The palms were pollinated with Deglet Noor, Khadrawy, Medjool and Zahidi cultivars. In addition, three palms were pollinated as the treatment control. The fatty acids were evaluated by gas chromatography-mass spectrometry. The surface morphology of date seed powder was examined using SEM, before and after n-hexane interaction. The seeds of the Medjool treatment had the greatest mass (1.42 g), but the lowest oil content (5.37% w/w); the control seeds showed smaller mass (1.21 g), but higher oil content (13.57% w/w). The proportion of fatty acids varied significantly among the treatments with respect to the control. The most abundant fatty acids were oleic (C18:1), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), and stearic (C18:0). Together these fatty acids presented a composition between 98.3 and 98.67% for treatments, and 99.0% for the control. The results indicate that the pollen sources from Deglet Noor, Khadrawy, Medjool and Zahidi cultivars had a significant effect on mass, dimension, oil content and fatty acid profile of the seeds of the date cultivar Medjool. The date seed oil could be used as edible oil, in food products, and in pharmaceutical and cosmetic applications.

KEYWORDS*: Date seed; Fatty acid profile; Medjool; Oil; Pollination*

RESUMEN: ***Efecto de las fuentes de polen sobre el rendimiento en la extracción de aceite y perfil de ácidos grasos de las semillas de dátil (*Phoenix dactylifera *L.) cultivar Medjool de México.*** La presente investigación tuvo como objetivo evaluar el efecto de las fuentes de polen, sobre la masa, dimensión, contenido de aceite y el perfil de ácidos grasos de las semillas de dátil del cultivar Medjool. Las palmas hembras del cultivar Medjool fueron polinizadas con cultivares Deglet Noor, Khadrawy, Medjool y Zahidi. Además, tres palmas fueron polinizadas como tratamiento control. Los ácidos grasos se evaluaron por cromatografía de gases-espectrometría de masas. La morfología de la superficie del polvo de semillas de dátil se examinó utilizando un equipo SEM, antes y después de la interacción n-hexano. Las semillas del tratamiento Medjool resultaron con mayor masa (1,42 g), pero menor contenido de aceite (5,37% p/p); la semilla control, mostró una masa más pequeña (1,21 g), pero un mayor contenido de aceite (13,57% p/p). La composición de ácidos grasos varió significativamente entre los tratamientos con respecto al control. Los ácidos grasos mayoritarios fueron: oleico (C18:1), láurico (C12:0), mirístico (C14:0), palmítico (C16:0), linoleico (C18:2) and esteárico (C18:0). En total, estos ácidos grasos alcanzan una composición entre 98.3 y 98.67% para los tratamientos, y 99.0% para el control. Los resultados indican que la fuente de polen de los cultivares Deglet Noor, Khadrawy, Medjool y Zahidi tiene un efecto significativo sobre la masa, dimensión, contenido de aceite y el perfil de ácidos grasos de la semilla del cultivar de dátil Medjool. El aceite de la semilla de dátil, podría usarse como aceite comestible, productos alimenticios, aplicaciones farmacéuticas y cosméticas.

PALABRAS CLAVE*: Aceite; Medjool; Perfil de ácidos grasos; Polinización; Semilla de dátil*

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1. INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is symbolic of warm climates and is cultivated for its edible delicious sweet fruit. The production in México in 2017 was 8,215 tons, harvested from an area of 1,377 ha (SIAP, 2018). A proportion of 95% of the production corresponds to the Medjool cultivar; the rest corresponds to other cultivars such as Deglet Noor, Khadrawy, Zahidi, Bahree, Honey, Hallawy and creole dates.

The date palm plant is unisexual, being either male or female. Flowers grow on inflorescences called spathes, which open naturally when ready to start the pollination process (Rahnama and Rahkhodaei, 2014). The pollination of date palm involves the transfer of the pollen produced by the male palms, until it comes into contact with the flowers of the female palm to fertilize the ovules contained in the flower, and to share half the genetic material required for the production of the date fruit (Georgi, 1982).

The natural form of pollination of the date palm is mainly due to the action of the wind and bees. However, for commercial production, it is necessary to carry out artificial pollination in order to increase the percentages of fruit. The pollen obtained from a single male palm can pollinate from 50 to 100 female date palms. Pollen has extensive effects on the chemical, quality and quantity characteristics of date fruit and seeds (Salomon-Torres *et al.*, 2017). Therefore it is important to choose proper pollen for pollination because this specification affects the size of the fruit and seed, development rate and time of fruit ripening (Hussein *et al.*, 1979; Swingle, 1928).

When the date is in its ripeness stage (Tamar), the harvest begins, and usually, the seed is discarded as waste once the flesh is removed, since the last part is the edible part. The industrial exploitation of date seeds in Mexico is null, due to various factors such as lack of knowledge on the part of producers for the valorization of the seed, as well as the lack of infrastructure and specialized supplies.

The date seed, depending on variety and quality grade, contains between 6–12% of the total fruit weight in its ripe stage (Barreveld, 1993). An alternative use for the date seed is through the exploitation of its oil content (Aljuhaimi *et al.*, 2012), which can be extracted by several physical and chemical techniques, the most common by organic solvents. Some applications of date seed oil can replace portions of other fruit oils such as palm or coconut oil in food and toilet product formulations, among others.

The aim of the present study was to assess the effect of pollen sources, on the mass, dimension, oil content and fatty acid profile of the Medjool date cultivar seed under the conditions of the Northwest of Mexico.

2. MATERIALS AND METHODS

2.1. Pollination

The studies were carried out in a plantation of female recipients of the Medjool cultivar of 17 years of age and vigor, located in the San Luis Rio Colorado Valley, in Northwest Mexico (Latitude: 32°23'5", Longitude: 114°53'55"). The pollen was extracted from male creole palms of the most common cultivars of the agricultural area, which are Medjool, Deglet Noor, Khadrawy, and Zahidi cultivars. These were used as the sources of pollen for the pollination of the Medjool cultivar. Male inflorescences were collected once the spathes were naturally broken; they were then transported to a drying area, the inflorescences were hung separately during a 3-day period and the pollen was collected daily on a paper bed (Abdeloauhhab and Arias-Jimenez, 1999). The pollen was stored at 4 °C until pollination time. The interaction of pollination is exemplified in Figure 1.

Pollen preparation for the four treatments and control consisted of mixing with commercial wheat flour in a 1:1 ratio, according to the traditional method of Mexican farmers to prepare pollination mixtures. The pollen from the control

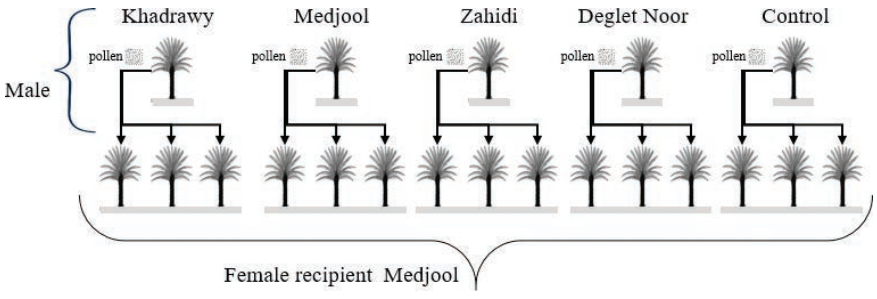

FIGURE 1. Design of pollination process in female Medjool recipients.

FIGURE 2. Manual pollination using a plastic squeeze bottle as container for the pollination mixture on female Medjool inflorescences.

treatment was composed of a mixture of pollen from different sources in unknown proportions, which is the traditional method for manual pollination (Figure 2).

The pollination process was carried out manually using a plastic squeeze bottle as container for the pollination mixture on female inflorescences, between the third and seventh day after the spathe had broken, according to Salomon-Torres *et al*., (2017).

2.2. Sampling and sample preparation

During the 2017 harvest season 100 dates were collected from each treatment. The seeds were removed from the date flesh and soaked in clean water to free of any adhering date flesh and then were air-dried at 40 °C. Date seeds from each treatment were separately milled in a heavy-duty grinder to pass 1–2 mm screens and then preserved at 20 °C for further analyses.

2.3. Physical properties

A hundred samples were taken randomly from each treatment to determine date seed weight and dimension (length and diameter). The weight was determined using an analytical balance, and the length and width of the seeds were then measured using a caliper micrometer.

2.4. Seed oil extraction

Oil extraction was performed according to the method described by AOAC 963.15 (2005). Briefly, 10 g of date powder were extracted with 200 mL of n-hexane in a Soxhlet extractor for 6 h. The n-hexane was removed by evaporation using a rotary evaporator at 80 °C in a water bath. The extracted oil was kept in glass vials and stored at −4 °C until fatty acid methyl ester (FAME) preparation. All extraction processes were performed in triplicate and the yield was expressed as percent of oil (w/w) obtained based on the weight of date seed powder used.

2.5. Date seed powder surface morphology

The date seed powder was analyzed using a Scanning Electron Microscope (SEM) JEOL JSM- 6360 to examine the external surface of the date seed powder and to identify the morphology changes occurring on the surfaces due to the effect of contact with n-hexane. The conditions were constant in the two images, such as acceleration voltage of 15 kV and a vacuum of 50 Pa due to the non-conductive nature of the sample.

2.6. Fatty acid methyl ester preparation

The evaluation of fatty acids in date seeds requires conversion into FAME in order to improve volatility and reduce tailing in the chromatogram's peak with good precision and reproducibility (Cert *et al.*, 2000). In this study, the AOCS method Ce 2-66 (1997) was used for FAME preparation, according to the process clearly illustrated by Akbari *et al*., (2012).

2.7. GC/MS analysis

The analyses were performed using an Agilent 7890A GC coupled to 5975C Mass detector Agilent Technologies, equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 micron) Agilent Technologies, Inc. An Agilent Technologies 7693 auto sampler was used to inject 1 µL of solution sample. The ionization energy was 70 eV with a mass range of 30 to 800 m/z. The initial temperature of the column at 125 °C, held for 0.5 min, ramped at 25 °C/min to 150 ºC, held for 2 min, then up to 200 °C with a 50 °C/min rate. The temperature of the injector was set at 255 °C and the detector to 270 °C. The flow rate of the carrier gas (Helium) was 1.0 mL/min injected with a gas dilution of 1:50. Identification of the individual components was based on comparison with the mass spectra library (NIST98). All determinations were carried out in triplicate.

2.8. Statistical analysis

The collected data were subjected to the one-way analysis of variance (ANOVA). The means of the results were compared by multiple comparisons of means by the least significant differences (LSD) test, at a significance level of 5% (Steel and Torrie, 1980). The statistical analysis was calculated using version 3.5.0 of Statistical Software R (Kabacoff, 2011; R Core Team, 2015). Finally, the results of fatty acid profile were expressed as mean values ± standard deviation of the three separate determinations per sample.

3. RESULTS

3.1. Date seed physical properties

The diameter, length and weight of the seeds were measured. Means followed by the same letter(s) in a column do not differ significantly at 0.05 probability level. These parameters are commonly used by farmers to evaluate the quality of date seeds. The results are depicted in Figure 3.

The dimensions of the seeds of the treatments were measured for diameter and length and were on average 0.89 and 2.63 cm, respectively. The seeds with the smallest dimensions were from the treatment with Deglet Noor pollen showing values of 0.88 and 2.55 cm for diameter and length, respectively. The seeds with the greatest dimensions were from the treatment Medjool with 0.91 cm in diameter and Khadrawy with 2.71 cm in length. While the dimensions for the control seeds were 0.84 and 2.47 cm.

The weight of the seeds obtained from the treatments showed an average of 11% more mass than the control; whereas the lowest values corresponded to the Deglet Noor treatment, which was 9% heavier than the control. The largest seed mass was induced by the Medjool pollen, and was 17% higher than the control treatment, which averaged 1.21 g.

3.2. Oil yield

The oil contents in the date seeds, expressed as percentages, are shown in Figure 4. The oil contents in date seed treatments were the highest for Zahidi (11.09%) and lowest for Medjool (5.37%). The control treatment showed the highest content (13.5%).

The results of oil content vary considerably according to the source of the pollen on the female recipient Medjool cultivar. The treatments 6.22 and 8.56% for Khadrawy and Deglet Noor, respectively, showed higher oil contents than the Medjool treatment, but lower oil extraction yields than the control (13.57%).

3.3. Date seed powder surface morphology

Representative micrographs of powdered date seed surface before and after interaction with n-hexane were obtained through SEM and are presented in Figure 5. The images are representative of all treatments and control, and were taken at a magnification of 200X.

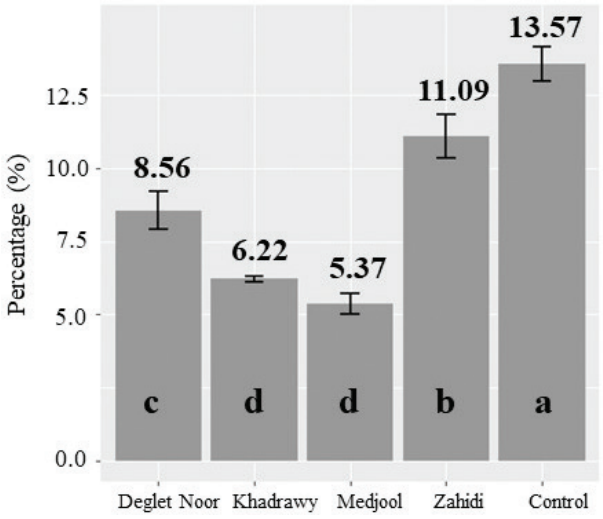

FIGURE 4. Date seed treatment oil content. Values are mean ± SD of three independent determinations. The collected data were submitted to analysis of variance (ANOVA), and were compared by a multiple comparison of means according to the least significant differences (LSD) test at a significance level of 5%. Means followed by same letter(s) in a column do not differ significantly.

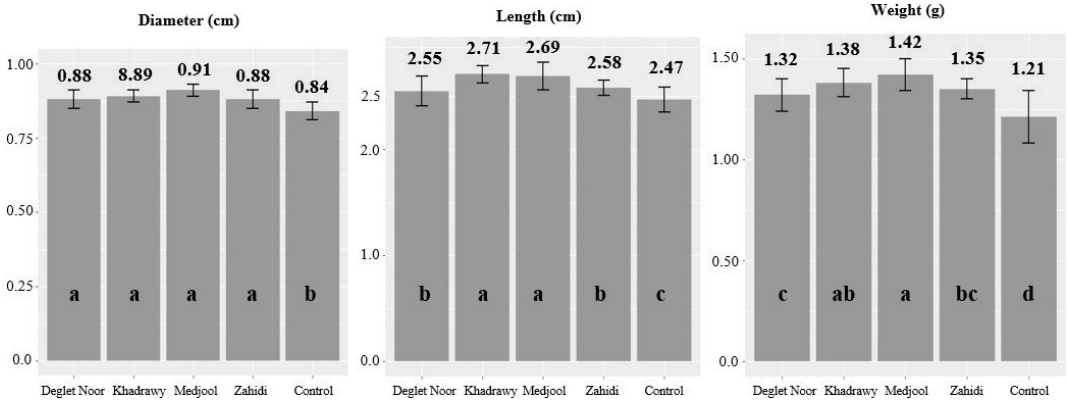

FIGURE 3. Date seed treatment physical characterization (diameter, length and weight). Values are mean ± SD of 100 samples. The collected data were submitted to analysis of variance (ANOVA), and were compared by a multiple comparison of means according to the least significant differences (LSD) test at a significance level of 5%. Means followed by the same letter(s) in a column do not differ significantly.

In Figure 5a, a pattern of irregularly shaped and randomly distributed cells was identified to have a width between 30–50 µm, and length between 30 and 60 µm. In Fig. 5b, no pattern of cells was identified, only a solid mixture of different sizes in disorder was observed, caused by the interaction of the organic solvent used to remove the oil contained in the date seed powder. The aggressive effect of the organic solvent on the surface of the date seed powder can be observed clearly.

3.4. Date seed powder surface morphology

The oil composition of the date seed was identified and quantified as FAME. The chromatogram is shown in Figure 6, and is presented as general for all treatments and the control, since the retention times of the FAME's were the same. Thirteen peaks were identified for Deglet Noor, Medjool and Zahidi; while for the Khadrawy and the control only twelve peaks were detected.

The relative concentrations of the fatty acids of date seed oil in the treatments and control were as follows: oleic (C18:1), > lauric (C12:0), > myristic (C14:0), > palmitic (C16:0), > linoleic (C18:2), > stearic (C18:0). Taken together these fatty acids composed of around 98.3 - 98.67% for the treatments, and 99.0% for the control. The following compounds were found in amounts less than 0.5% for all treatments and control: caprylic (C8:0), capric (C10:0), palmitoleic (C16:1), margaric (C17:0), arachidic (C20:0), gondoic (C20:1), *trans*-gondoic (C20:1). The complete fatty acid compositions of the date seed oil for treatments and control, detected throughout the study are listed in Table 1.

Lauric acid (C12:0) was found to be the predominant SFA in the date seed oil treatments studied (17.06 - 17.6%) and the control date seed oil (17.24%). Palmitic (C16:0) and myristic acids (C14:0) followed with very close values for the treatments (10.36 - 10.86%), while for the control

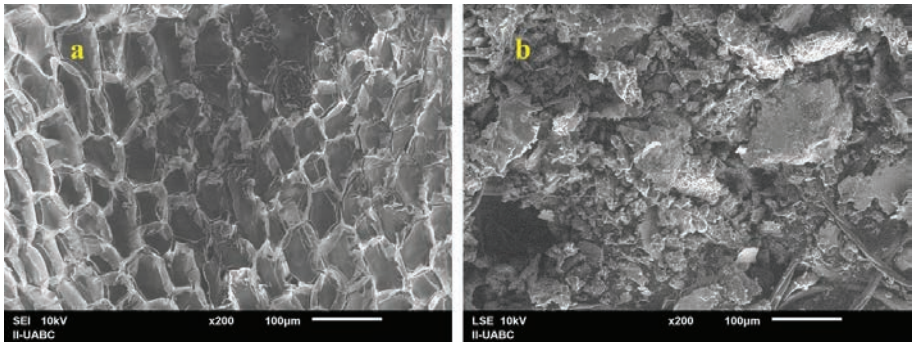

FIGURE 5. Scanning electron microscope micrograph of date seed powder at 200x before (a) and after n-hexane interaction by Soxhlet (b).

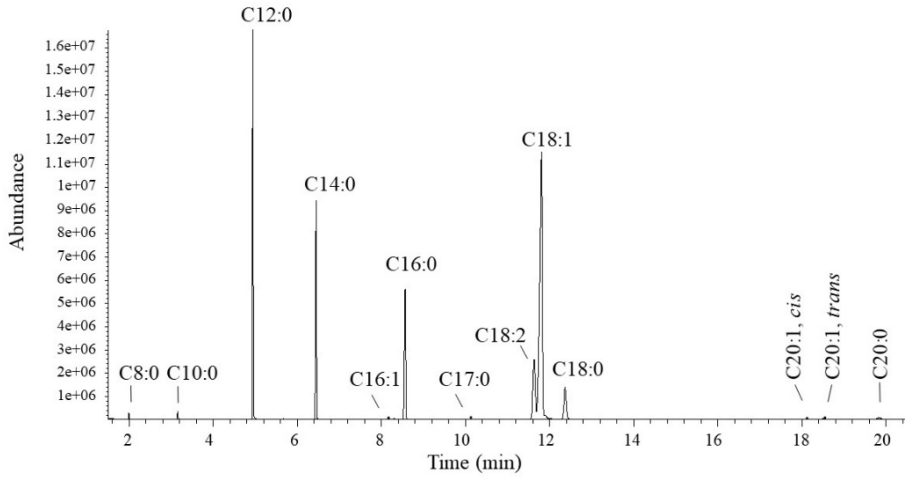

FIGURE 6. GC-MS general for fatty acid methyl esters from date seed oil for all treatments and control; (C8:0) Caprylic acid, (C10:0) Capric acid, (C12:0) Lauric acid, (C14:0) Myristic acid, (C16:0) Palmitic acid, (C16:1) Palmitoleic acid, (C17:0) Margaric acid, (C18:0) Stearic acid, (C18:1) Oleic acid, (C18:2) Linoleic acid, (C20:1) Gondoic acid, (C20:1) trans-gondoic acid, (C20:0) Arachidic acid.

Table 1. Fatty acid profiles of date seed oil, expressed in percent of the total fatty acids.

Fatty acid	Deglet Noor	Khadrawy	Medjool	Zahidi	Control
(C8:0) Caprylic	0.28 ± 0.00^{a}	0.27 ± 0.00^{a}	0.27 ± 0.01^{b}	0.27 ± 0.00^{b}	0.22 ± 0.00^{c}
(C10:0) Capric	0.36 ± 0.01^{b}	0.34 ± 0.01^{c}	0.35 ± 0.00^{b}	0.37 ± 0.00^{a}	0.29 ± 0.00^{d}
(C12:0) Lauric	17.44 ± 0.46^{a}	17.60 ± 0.72^{a}	17.06 ± 0.11^{a}	17.21 ± 0.05^{a}	17.24 ± 0.12^{a}
(C14:0) Myristic	10.76 ± 0.13^{a}	10.49 ± 0.12^{b}	10.44 ± 0.04^{b}	10.86 ± 0.05^{a}	10.42 ± 0.01^{b}
(C16:0) Palmitic	10.81 ± 0.04^{ab}	10.36 ± 0.27^{c}	10.61 ± 0.04^{b}	10.86 ± 0.03^{a}	9.96 ± 0.01^{d}
(C16:1) Palmitoleic	0.06 ± 0.00^{a}	0.05 ± 0.01^{b}	0.06 ± 0.00^{a}	0.06 ± 0.00^{a}	0.04 ± 0.01^{b}
(C17:0) Margaric	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.00 ± 0.00^{b}
(C18:0) Stearic	4.83 ± 0.06^{ab}	4.62 ± 0.22^{c}	4.65 ± 0.01^{bc}	4.88 ± 0.01^{a}	4.20 ± 0.04^{d}
(C18:1) Oleic	45.43 ± 0.49^{c}	46.98 ± 0.29^{b}	46.69 ± 0.06^{b}	45.63 ± 0.04^{c}	49.58 ± 0.18^{a}
(C18:2) Linoleic	9.06 ± 0.05^{a}	8.62 ± 0.37^{b}	9.04 ± 0.01^{a}	9.06 ± 0.06^{a}	7.60 ± 0.03^{c}
(C20:1) Gondoic	0.07 ± 0.01^{a}	0.00 ± 0.00^{b}	0.07 ± 0.00^{a}	0.05 ± 0.01^{b}	0.08 ± 0.01^{a}
(C20:1) *trans*-gondoic	0.23 ± 0.01^{a}	0.16 ± 0.01^{b}	0.21 ± 0.01^{a}	0.19 ± 0.01^{ab}	0.06 ± 0.03^{c}
(C20:0) Arachidic	0.47 ± 0.02^{a}	0.38 ± 0.01^{c}	0.44 ± 0.01^{b}	0.45 ± 0.01^{ab}	0.24 ± 0.01^{d}
ΣSFA	45.14	44.16	43.90	44.98	42.60
ΣMUFA	45.80	47.21	47.05	45.96	49.79
ΣPUFA	9.06	8.63	9.05	9.07	7.61
TU	54.86	55.84	56.01	55.03	57.40
TU/SFA index	1.21	1.26	1.27	1.22	1.34

ΣSFA: saturated fatty acid; ΣMUFA: monounsaturated fatty acid; ΣPUFA: polyunsaturated fatty acid; TU: total unsaturated fatty acid; TU/SFA index: total unsaturated fatty acid/saturated fatty acid index. Determinations were made in triplicate and the data are reported as the mean. The collected data were submitted to analysis of variance (ANOVA), and were compared by a multiple comparison of means according to the least significant differences (LSD) test, at a significance level of 5%. Means followed by same letter(s) in each row do not differ significantly.

treatment, myristic acid was found in a greater proportion (10.42%) than palmitic acid (9.96%). The composition of total SFA in the treatments averaged 44.54%, and 42.60% in the control.

All the date seed oil treatments and the control exhibited higher amounts of TU than SFA. The date seed oil from the treatments contained total unsaturated fatty acids in the range of 54.86 - 56.01% for the Deglet Noor and Medjool treatments, and for the control it was higher at 57.40%. The TU for the treatments were composed on average of 83.56% MUFA and 16.44% PUFA, and 86.74 and 13.25% for the control.

The most prevalent MUFA among the oils from the treatments was oleic acid (C18:1), which ranged from 45.43–46.98%, and control contained a higher amount at 49.58%, although palmitoleic (C16:1), gondoic (C20:1) and *trans*-gondoic (C20:1) were found in trace amounts. All MUFA compounds were consistent in the treatments and control, except in the Khadrawy treatment, where gondoic (C20:1) was not found.

The date seed oil content is low in PUFA, and linoleic acid (C18:2) was the only one present in treatment and control. For the treatments it was detected at 8.63 - 9.07%, but in the control it was lower than 7.61%. In general, a lower degree in polyunsaturated fatty acids would reduce the susceptibility to oxidative deterioration, a characteristic which contributes to its stability during storage (Saafi *et al.*, 2008).

4. DISCUSSION

The dimensions of the control seeds were lower than the treatment seeds, on average by 5.61 and 6.08% for length and diameter, respectively. The diameter of the treatment and seeds was lower than that reported by Bouhlali *et al.*, (2017) by about 1.15 cm, but in relation to the length of 2.75 cm, the value was close for the Khadrawy treatment with 2.71 cm, and for the other treatments this value moves away. For the control seeds, the result was even farther at 2.47 cm.

The weight of the control seeds had lower mass compared to the mass of the treatment seeds, which was 8.33% below the lowest mass, which corresponded to Deglet Noor and 14.78% of the highest value, which corresponded to Medjool. The mass for the Medjool treatment reported in the present study was 1.42 g, which was very close to the mass reported by Zaid and Wet (2002) of 1.5 g. For the rest of the treatments, the weight was between 1.32 - 1.38 g and the control presented the lowest value of 1.21 g.

The oil content of treatment seeds, varied between 5.37 - 11.09% w/w, for Medjool and Zahidi, respectively. For control seeds the oil content was the highest at 13.59% w/w, on average 1.7 times greater than the treatments. The source of pollen from the Medjool cultivar on the female recipient palm of the same cultivar showed the lowest oil yield. This finding matched the results reported by Bouhlali *et al*., (2017) and Aljuhaimi *et al*., (2018), however, the source of pollen and pollination technique were not reported. Another challenge is the oil extraction process from date seeds since the seeds did not provide a high oil yield (Golshan-Tafti *et al.,* 2017).

The Fatty acid profiles were consistent in the treatments with slight percentage variations. Margaric acid (C17:0), was found as trace (less than 0.058%) in all treatments, but was not found in the control treatment.

The level of total unsaturated fatty acid content of date seed oil using the TU/SFA index showed that the control had a 34% higher content of unsaturation; while for the treatment the values were between 21 and 27%. The high amount of oleic acid was very close (44.92%) to that reported by Bouhlali *et al*., (2017) for the Medjool cultivar.

5. CONCLUSIONS

This study demonstrates that the mass, dimension, oil content and fatty acid profile of date seeds are influenced by pollen source. Here, the Deglet Noor, Khadrawy, Medjool and Zahidi cultivars were used to pollinate female palms of the Medjool cultivar, and the results were compared with control pollen, as used traditionally by Mexican farmers. The effect of the pollen source in the treatment was manifested by an increase in the size and weight of the seeds, but a reduction in the oil content compared to the control seeds was also observed.

The traditional method of pollination of the date palm of the Medjool cultivar, presented a greater yield of oil extraction from the date seed and a greater proportion of oleic acid compared to pollination with Medjool, Deglet Noor, Khadrawy or Zahidi cultivars. These results may contribute to the Mexican date industry in order to take advantage of the added value of date seeds as a source of good quality oil with high relative percentages of oleic acids and good shelf-life.

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