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Directoras:

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Dra. María Dolores de Miguel Gómez

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A mi familia

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GENERAL INDEX

RESUMEN

En el sur de Túnez, el sector de la palmera datilera (*Phoenix dactylifera* L.) representa el principal recurso socioeconómico del oasis y tiene un papel importante en la agricultura tunecina y el desarrollo de la economía nacional. El dátil es el tercer producto agrícola más exportado en Túnez, después del aceite de oliva y los productos del mar. La principal variedad exportada es Deglet Noor, que se produce especialmente en Argelia y Túnez, seguidos por Israel con menos producción. En la última década, Túnez fue el primer productor mundial de Deglet Noor con 190.600 tn en 2012, convirtiéndose en un sector estratégico del país. Sin embargo, la globalización de los mercados ha tenido un enorme impacto en el concepto tradicional de los índices de la ventaja comparada, de lo cual se benefició Túnez en el sector de las exportaciones de dátiles, destacando los aspectos fundamentales para optimar su competitividad en el mercado internacional.

Por otro lado, el sector de la palmera datilera en Túnez se enfrenta a varios problemas técnicos, entre ellos, la infestación en campo y durante el almacenamiento. La polilla del dátil, *Ectomyelois ceratoniae* (Zeller), se considera la principal plaga de los dátiles que causa graves pérdidas económicas durante el almacenamiento y la exportación de los dátiles tunecinos Deglet Noor. El bromuro de metilo ha sido, en varios países, el principal fumigante para la desinsectación de estos frutos, aunque su uso se prohibió en 2015 en los países en vía desarrollo. Por ello, los tratamientos físicos postcosecha se presentan como una alternativa relativamente simple a los fumigantes químicos en general, pudiendo desinsectar los productos perecederos como el dátil.

La presente Tesis Doctoral se divide en dos partes, en la primera se evalúa la ventaja competitiva del sector de la palmera datilera en Túnez en la Cuenca Mediterránea e Irán. En la segunda parte, una vez determinada la posición competitiva

del sector, se estudia el efecto de varios tratamientos físicos postcosecha sobre la desinsectación de los dátiles tunecinos Deglet Noor y los parámetros de calidad del fruto.

Del análisis de la ventaja competitiva del sector de la palmera datilera de Túnez en la Cuenca Mediterránea e Irán en los últimos 20 años se obtuvo que Túnez se haya mantenido como principal proveedor de dátiles a la UE. La variedad Deglet Noor, en particular, sitúa a Túnez por delante de los competidores tradicionales como Argelia e Irán, con promedios de índices de competitividad bastante favorables, alcanzando el índice de especialización de Balassa un valor de 6405.99, el índice de dependencia fue de 17.38, la parte constante de mercado obtuvo un valor de 41.04 y el índice de la balanza comercial fue de 99.50. Todos estos índices fueron más estables en Túnez que en Argelia e Irán durante el período estudiado. Sin embargo, Túnez se enfrenta actualmente a nuevos competidores como Israel y los países re-exportadores como Francia. Nuevas estrategias comerciales como el acondicionamiento, tratamientos físicos postcosecha para la desinsectación, embalaje, la apertura de nuevos mercados, nuevos canales de distribución, etc serían medidas positivas para enfrentar las limitaciones actuales del mercado, la aparición de productores emergentes y las políticas restrictivas de la UE.

En la segunda parte de la presente Tesis Doctoral se ha abordado el estudio de los efectos de tratamientos térmicos, proporcionados por agua o aire caliente, así como, tratamientos de congelación, sobre la mortalidad del *E. ceratoniae* y el mantenimiento de la calidad postcosecha del dátil Deglet Noor.

En cuanto a los tratamientos de agua caliente, se propusieron tres combinaciones de temperatura/tiempo (50 °C 10 min, 55 °C 5 min y 60 °C 3 min) en dátiles Deglet Noor que fueron posteriormente conservados, en atmósfera de aire, durante 30 d a 2 °C, seguidos de un periodo de comercialización de 4 d a 23 °C. Los resultados mostraron que todos los tratamientos utilizados lograron la mortalidad de la larva *E. ceratoniae* y una reducción microbiológica del orden de 3 log ufc g^{-1} para los mesófilos y 4 log ufc g^{-1} para las levaduras y mohos obteniendo unos recuentos finales de <1 log ufc g^{-1} y <2 log ufc g^{-1}, respectivamente. Estos tratamientos de agua caliente indujeron cambios en el color de la piel reduciendo la luminosidad de los dátiles de 36 (testigo) a una media de 35 en los dátiles tratados. Igualmente, se observó una reducción en la actividad antioxidante, pasando de un contenido inicial de 73.8 mg AAE 100 g^{-1} para FRAP y 62.7 mg AAE 100 g^{-1} para DPPH a un descenso del 10 a 15% en FRAP y del 17 a 22% en DPPH. Con respecto a los compuestos fenólicos totales, el contenido inicial fue de 90.1 mg GAE 100 g^{-1} reduciéndose entre un 9 a 14% en los dátiles tratados con agua caliente. La calidad sensorial del fruto disminuyó ligeramente con el tratamiento de 60 °C durante 3 min aunque todos los tratamientos mantuvieron los dátiles por encima del límite de comercialización, sin observarse daños térmicos en el fruto. El periodo de almacenamiento de 30 d a 2 °C seguido por un periodo de comercialización de 4 d a 23 °C, afectó a los parámetros anteriormente mencionados, además se incrementaron los monosacáridos tras la comercialización pasando de 119 g kg^{-1} a 132 g kg^{-1} en glucosa y de 96 g kg^{-1} a 120 g kg^{-1} en fructosa. Tras la comercialización, también se detectó un ligero incremento en el contenido algunos aminoácidos, como alanina, ácido aspártico y prolina, aumentando de 2.2 mg kg^{-1} a 2.8 mg kg^{-1} en alanina, 6.9 mg kg^{-1} a 7.2 mg kg^{-1} en ácido aspártico y de 6.7 mg kg^{-1} a 9.6 mg kg^{-1} en prolina.

Con respecto al estudio de tratamientos de aire caliente, se probaron tres combinaciones de temperatura/tiempo (55 °C 30 min, 60 °C 15 min y 60 °C 20 min) para la desinsectación de los dátiles Deglet Noor, almacenándose en atmósfera de aire, 45 d a 2 °C, seguidos de un período de comercialización de 4 d a 23 °C. Los resultados obtenidos indicaron que todos los tratamientos de aire caliente alcanzaron el 100% de la mortalidad de las larvas de *E. ceratoniae* en dátiles infestados de forma natural. Estos tratamientos no causaron ningún daño superficial en el tejido del fruto y no afectaron a la calidad del dátil, logrando una reducción de 3 log ufc g^{-1} en el crecimiento de bacterias mesófilas, obteniendo recuentos finales de <1 log ufc g^{-1}. Los tratamientos de aire caliente utilizados, especialmente la combinación de alta temperatura y mayor tiempo de aplicación, 60 °C durante 20 min, redujo la actividad antioxidante, pasando de un valor inicial de 42.2 mg AAE 100 g^{-1} determinada por DPPH y 63.7 mg AAE 100 g^{-1} para FRAP, a una reducción del 25% y del 14%, respectivamente. El tiempo de conservación también tuvo un impacto significativo, reduciendo la tonalidad del color (63 a 60 h°), el contenido total de polifenoles (88.7 a 54.1 mg GAE 100 g^{-1}), la firmeza del fruto (12.7 N a 10.6 N) y la calidad sensorial global del fruto (4 puntos a 3.3). No obstante, todos los dátiles tratados con aire caliente mantuvieron la calidad sensorial por encima del límite de comercialización.

Finalmente, se estudió el efecto de la congelación a -18 °C sobre la mortalidad de *E. ceratoniae*. Se estudiaron tres tratamientos de congelación a -18 °C con diferentes tiempos de aplicación (50, 77 y 125 horas). Los resultados mostraron que sometiendo los dátiles a tan sólo 50 h a -18 °C se lograba el 100% de la mortalidad de *E. ceratoniae* (larva, pupa y adulto) en dátiles infestados de forma natural. Se seleccionó este tratamiento de congelación y se analizaron los parámetros de calidad del fruto. Se observó que la congelación inducía un ligero incremento en el contenido de monosacáridos, pasando de 139 g kg^{-1} de glucosa y de 171 g kg^{-1} de fructosa a 162 g kg^{-1} y 209 g kg^{-1}, respectivamente. Hubo una reducción en la actividad antioxidante, del 40 a 45% en FRAP y DPPH, siendo la AAE inicial de 109.0 mg 100 g^{-1} para FRAP y 86.0 mg 100 g^{-1} para DPPH. Sin embargo, otros parámetros como el color, los aminoácidos, polifenoles totales, recuentos microbiológicos y la calidad sensorial no se vieron afectados por dicho tratamiento, manteniéndose el fruto por encima del límite de comercialización y sin observarse daño por congelación.

Mencionar que en esta Tesis también se ha estudiado el uso de atmósferas controladas, evaluando tres combinaciones de O_2 y CO_2: bajo O_2 (2% O_2), bajo O_2 combinado con alto CO_2 (2% O_2 y 90% CO_2) y moderado O_2 combinado con alto CO_2 (10% O_2 y 90% CO_2). Los frutos infectados de forma natural, se mantuvieron 3 días a 23 °C, bajo las combinaciones gaseosas mencionadas, sin lograrse la mortalidad total de la larva de *E. ceratoniae*. Tras la conservación en atmósfera controlada, la actividad antioxidante determinada por DPPH pasó de 86.4 mg AAE 100 g^{-1} a 60.0 mg AAE 100 g^{-1} y en el caso del FRAP, se obtuvo inicialmente 108.8 mg AAE 100 g^{-1} reduciéndose a 84.7 mg AAE 100 g^{-1}. El contenido en polifenoles totales también disminuyó, pasando de 95.7 mg GAE 100 g^{-1} a un contenido medio de 84.3 mg GAE 100 g^{-1}. A pesar de que la calidad sensorial del fruto se mantuvo siempre por encima del límite de comercialización, las combinaciones gaseosas estudiadas, no pudieron ser recomendadas para la desinsectación de *E. ceratoniae.*

Las conclusiones de la presente Tesis Doctoral, se pueden sintetizar en la importante posición comercial que Túnez tiene en el sector datilero en comparación con sus principales competidores de la Cuenca Mediterránea. Sin embargo, se necesitan nuevas estrategias para mantener esta posición competitiva en el futuro. Por otra parte, todos los tratamientos físicos postcosecha propuestos en este trabajo, agua caliente, aire caliente y congelación a -18 °C, fueron eficaces para lograr el 100% de mortalidad de larvas de *E. ceratoniae* y mantener una calidad óptima de la fruta. En particular, el tratamiento de agua caliente a 55 °C durante 10 min, el de aire caliente a 60 °C durante 15 min y la congelación a -18 °C durante 50 horas se recomiendan como alternativas a los tratamientos químicos para el control de la polilla de dátil, permitiendo la exportación del dátil Deglet Noor a mercados internacionales y manteniendo su competitividad.

ABSTRACT

In South of Tunisia, the palm dates sector (*Phoenix dactylifera* L.) is the main socioeconomic resource of oases and has a major role in Tunisian agriculture and the development of the national economy. Tunisian dates occupy the third place in agricultural exports after olive oil and seafood. The major exported cultivar is Deglet Noor, which is mainly produced in by Algeria and Tunisia, with Israel producing a somewhat lesser amount. Tunisia is the world's leading producer of Deglet Noor, with 190,600 metric tons in 2012, making it a strategic sector within the country. However, the market globalization has had a large impact on the traditional concept of comparative advantage indexes enjoyed by Tunisia with date exports, highlighting the fundamental aspects needed to increase competitiveness in the international scenario.

On the other hand, the palm date sector in Tunisia faces several technical problems including insect infestation caused by *Ectomyelois ceratoniae* or carob moth, which is one of the main postharvest disease pests of date fruits that causes serious economic losses during storage and export of Tunisian palm date fruit Deglet Noor cv. Methyl bromide is the most widely used fumigant on stored dates in several countries, although by 2015 it had been withdrawn in developing countries. Physical postharvest treatments are currently a relatively simple, non-chemical alternative that can kill quarantine pests in perishable commodities such as palm date fruit.

The current PhD Thesis is divided into two parts; the first is focused on the analysis of the competitive advantage of the Tunisian date sector in the Mediterranean basin and Iran. In the second part, once the competitive position of the sector is studied, the Thesis investigates the efficacy of various physical postharvest treatments for sanitizing palm date fruit (Deglet Noor) and their effects on fruit quality parameters.

The results of the analysis of the competitive advantage of the Tunisian date industry in the Mediterranean basin and Iran over the last 20 years shows that Tunisia is still the main supplier of dates to the EU. The Deglet Noor cv., in particular, places Tunisia ahead of traditional competitors such as Algeria and Iran, with averages of competitiveness indices such as the Balassa Index of Specialization (BIS) 6405.99; Dependency Index (DI) 17.38, Constant Market Share (CMS) 41.04 and Trade Balance Index (TBI) 99.50. For Tunisia, these indices were more stable than those countries during the studied period. However, it is currently facing new competition from countries such as Israel and re-exporting countries such as France. New business strategies such as conditioning, new non-chemical treatments, packaging, opening new markets, new distribution channels, etc. would be positive measures that could be used to tackle current market limitations, the emergence of new producers and restrictive EU policies.

In the second part of the current Thesis, a study of the effects of physical treatment provided by hot water treatments (HWT), hot air treatments (HAT) and freezing treatments on the *Ectomyelois ceratoniae* mortality and maintenance of fruit quality parameters was performed.

Regarding HWT, three combinations of temperature/time were studied (50 °C 10 min, 55 °C 5 min and 60 °C 3 min) in Deglet Noor date fruits. After HWT, date fruits were stored for 30 d at 2 °C followed by a retail period of 4 d at 23 °C. Results showed that all the HWTs led to 100% *E. ceratoniae* larvae mortality, reduced the microbial growth to about 3 log cfu g^{-1} for mesophilic bacteria and 4 log cfu g^{-1} for yeasts and molds, obtaining final microbial counts of <1 log cfu g^{-1} for mesophilic bacteria and < 2 log cfu g^{-1} for yeast and molds. HWTs induced a slight change in skin color, reducing the luminosity from 36 (control) to an average of 35. The antioxidant activity was also

decreased from an initial content of around 73.8 mg AAE 100 g^{-1} for FRAP and 62.7 mg AAE 100 g^{-1} for DPPH, reducing it about 10 to 15% and 17 to 22%, respectively. The initial content of total phenolic compounds was about 90.1 mg GAE 100 g^{-1}, decreasing 9 to 14% with HWTs. Overall quality was slightly reduced when using 60 °C for 3 min, although all the treatments the fruit remained above the limit of marketability as there was no heat damage. The storage time of 30 d at 2 °C followed by a retail period of 4 d at 23 °C also affected the parameters mentioned above and increased the concentration of monosaccharides from 119 g kg^{-1} to 132 g kg^{-1} for glucose and from 96 g kg^{-1} to 120 g kg^{-1} for fructose. The concentrations of some amino acids such as alanine, aspartic acid and proline increased during the final retail period, from an initial content of about 2.2 mg kg^{-1} to 2.8 mg kg^{-1} for alanine, from 6.9 mg kg^{-1} to 7.2 mg kg^{-1} for aspartic acid and from 6.7 mg kg^{-1} to 9.6 mg kg^{-1} for proline.

Three combinations of HATs (temperature/time) were studied (55 °C for 30 min, 60 °C for 15 min, 60 °C for 20 min) for disinfesting Deglet Noor dates. Fruits were stored for 45 d at 2 °C followed by a retail period of 4 d at 23 °C. The results showed that the use of HATs resulted in 100% *E. ceratoniae* larvae mortality in naturally-infested date. These HATs did not cause any damage to fruit quality and reduced mesophilic bacterial counts to about 3 log cfu g^{-1} obtaining at final counts of < 1 log cfu g^{-1}. These HATs, especially the combination of highest temperature and longer time of application (60 °C for 20 min), decreased the antioxidant activity from 42.2 mg AAE 100 g^{-1} for DPPH and 63.7 mg AAE 100 g^{-1} for FRAP in control samples to 25% in and 14% in HATs, respectively. Storage time also had a significant impact, reducing color (63 to 60 h°), the total phenolic content of date fruits (88.7 to 54.1 mg GAE 100 g^{-1}), firmness (12.7 N to 10.6 N) and overall quality (score 4 to 3.3). Nevertheless, all date

fruits from HATs and control treatments maintained their marketability quality during the shelf-life period.

Lastly, the effect of freezing treatments at -18 °C on the *E. ceratoniae* mortality and fruit quality parameters of Deglet Noor dates was studied, by the application of three different treatment times (50 , 77, and 125 hours). Results showed that the use of freezing at -18 °C for 50 h resulted in to 100% of *E. ceratoniae* mortality (larva, pupa and adult) in naturally-infested date. Fruit quality was also examined under this selected sanitizing freezing treatment. Results showed that this freezing treatment induced an increase on monosaccharide concentration from 139 g kg^{-1} of glucose and 171 g kg^{-1} of fructose to 162 g kg^{-1} and 209 g kg^{-1}, respectively. A 40 to 45% reduction in FRAP and DPPH of antioxidant activity was detected, with an initial content of 109.0 mg 100 g^{-1} for FRAP and 86.0 mg 100 g^{-1} for DPPH. However, other parameters as color, amino acids, total phenolic, microbial and sensorial quality were not affected by that freezing treatment. All samples remained above the limit of marketability as there was no cold damage. This treatment can be recommended as an alternative to chemical treatment to control carob moth, as it yielded optimum-quality Deglet Noor date fruits.

In the current Thesis, the use of controlled atmospheres (CA), with three combinations of O_2 and CO_2: low O_2 (2% O_2), low O_2 combined with high CO_2 (2% O_2 and 90% CO_2) and moderate O_2 combined high CO_2 (10% O_2 and 90% CO_2) was also studied. Naturally infested fruits were kept for 3 days at 23 °C under these CA, without achieving total mortality of the *E. ceratoniae* larvae. After CA storage, the antioxidant activity estimated by DPPH decreased from 86.4 mg AAE 100 g^{-1} to 60.0 mg AAE 100 g^{-1} and as measured by FRAP from 108.8 mg AAE 100 g^{-1} to 84.7 mg AAE 100 g^{-1}. The total polyphenol content also decreased from 95.7 mg GAE 100 g^{-1} to an average content of about 84.3 mg GAE 100 g^{-1}. Although the sensory quality of the fruit was

maintained above the limit of marketability, the gaseous combinations studied could not be recommended for disinfestation of *E. ceratoniae*.

As the main conclusions of current PhD Thesis we can state that Tunisia has a highly important trade position in the palm date fruit sector as compared to the rest of the Mediterranean basin. Nevertheless, new business strategies are needed to maintain this competitive position in the future. Moreover, all the suggested physical postharvest treatments studied in this Thesis were effective in achieving 100% *E. ceratoniae* larvae mortality while maintaining a suitable fruit quality parameter. Specifically, HWT of 55 °C 10 min, HAT of 60 °C 15 min and a freezing treatment of 50 hours at -18 °C are recommended as an alternative to chemical treatments to control carob moth, as these yielded optimum-quality of Deglet Noor date fruits that could be exported to the international markets, so that Tunisia maintains its position of competitiveness.

LIST OF ABBREVIATIONS

Ala: Alanine.

ANOVA: Analysis of Variance.

APHIS: Animal and Plant Health Inspection Service.

APIA: Agence de Promotion des Investissements Agricoles de la Tunisie.

Arg: Arginine.

BIS: Balassa Index of Specialization

CMS: Constant Market Share.

DI: Dependency Index.

DR: Dependency Ratio.

EFTA: European Free Trade Association.

ES: Standard Error.

EU: European Union.

GAFTA: Great Arab Free Trade Area.

GI-Fruit: Groupement Interporfessionel des Fruits de Tunis.

ICP: Competitive Price Index.

ICP_{ex}: Export Competitive Price Index.

LT: Lethal Time.

LT_{99}: Lethal Time for 99% insect mortality.

O_3: Ozone.

OPP: Micro-perforated Oriented Polypropylene Film.

RC: Revealed Competitiveness.

RCA: Balassa's Revealed Comparative Advantage Index.

SD: Standard Deviation.

TBI: Trade Balance Index.

U.S. EPA: United States Environmental Protection Agency.

UNEP: United Nations Environment Program.

USDA: United States Department of Agriculture.

UV-C: Ultra Violet radiation.

WTO: World Trade Organization.

Pro: Proline.

Met: Methionine.

Glu: Glutamic Acid.

Asp: Aspartic Acid.

IC: Ion Chromatography.

PAD: Pulsed Amperometric Detection.

h: hours.

AA: Ascorbic Acid.

AAE: Ascorbic Acid Equivalent.

TPC: Total phenolic content.

w/v: weight/volume.

GA: Gallic Acid.

cfu: colony forming units.

OQ: Overall Quality.

AfDB: African Development Bank.

UN CONTRADE: The United Nations Commodity Trade Statistic Database.

Fig.: Figure.

CA: Controlled atmosphere

cv.: cultivar

d: Days.

FAO: Food and Agriculture Organization of the United Nations.

FAOSTAT: Statistics of Food and Agriculture Organization of the United Nations.

FRAP: Ferric reducing ability of plasma.

fw: Fresh weight.

GAE: Gallic acid equivalents.

ºh: Hue angle.

HAT: Hot Air Treatment.

HWT: Hot Water Treatment.

DPPH: 2,2-diphenyl-1-picrylhydrazyl radical.

HPLC: High-Performance Liquid Chromatography.

log: Logarithm.

LSD: Least significant difference.

min: Minute.

NS: Non significant.

ppm: parts per million.

RH: Relative humidity.

TPTZ: 2,4,6-tripyridyl-s-triazine.

UPCT: Universidad Politécnica de Cartagena.

USA: United States of America.

v/v: Volume/volume.

vs.: Versus.

INTRODUCTION

1. INTRODUCTION

1.1 Palm date fruit

1.1.1. Origin and history

The date palm is a dioecious species belonging to the palm family (Palmae or Arecaceae, subfamily Coryphoideae, tribe Phoeniceae) (Dransfield et al., 2008). The date palm *(Phoenix dactylifera L.)* (Figure 1) is the emblematic species of the oasis agro-system, providing an essential source of food with a high nutritive value in the cultivation areas and neighboring regions. It was one of the first perennial plants to be domesticated (Zohary and Spiegel-Roy, 1975), and has been cultivated in North Africa and the Middle East for at least 5,000 years (Zohary and Hopf, 2000).

The exact origin of the date palm *(Phoenix dactylifera* L.) is considered to be lost in Antiquity. However, it most likely originated from the ancient Mesopotamia area (southern Iraq) or western India (Wrigley, 1995). It is probably the most ancient cultivated tree in the world. It could be safely assumed that the reason for mentioning dates and date palms in the Jewish, Christian, and Islamic religions was due mainly to the influence of the Prophet Abraham, who was born and raised in the old city of Ur, where date palms were grown (Zaid and Wet, 2002).

The old world of date palm stretches from east to west (± 8,000 km) and from north to south (± 2,000 km). According to Dowson (1982), date palm covers 3% of the world's cultivated surface.

From its center of origin, date cultivation spread throughout the Arabian Peninsula, North Africa, and the Middle East. Date culture had apparently spread into Egypt by the middle of the second millennium BCE (Chao and Krueger, 2007). The extension of date cultivation later accompanied the expansion of Islam and reached southern Spain and Pakistan. The Spanish were the first to introduce date palms the

Arabian Peninsula, North Africa, and the Middle East/South Asia, carrying them to America (Nixon, 1951).

Phoenix dactylifera is a widely distributed species occurring in diverse geographic, soil and climatic areas (El Hadrami et al., 2011), growing in hot and arid areas, and are relatively tolerant of salty and alkaline soils, as long as ground water, which roots are able to reach in deep horizons, is not limiting (Terral et al., 2012). Date palms require a long, intensely hot summer with little rain and very low humidity during the period from pollination to harvest, but with abundant underground water near the surface or irrigation (Chao and Krueger, 2007).

Figure 1. *Phoenix dactylifera* L.

These agro-ecological requirements explain in part the actual distribution area of date palm which spreads from Spain to India. However, the date palm is also strongly present in sub-Saharan Africa and was introduced principally to California, and other places. By propagating it over long distances and providing favorable growing conditions, but human activities have substantially altered its natural distribution, which remains unknown (Barrow, 1998).

In conclusion, date palm is found in both the Old World (Near East and North Africa) and the New World (American continent) where dates are grown commercially in large quantities. The date belt stretches from the Indus Valley in the east to the Atlantic Ocean in the west (Zaid and Wet, 2002).

In the last 25 years, it has been a slight change in the geographical distribution of date palm, with an important concentration in the countries located in the southern area of the Mediterranean Sea having approximately 35 million palms (35% of world total). Based on 200 palms/ha, they have a date palm superficies of about 175,000 ha (FAOSTAT, 2015).

Into the Mediterranean basin, date palm grows principally in Egypt, Algeria, Tunisia, Israel, Jordan, Morocco, Libya, Turkey and Spain.

Tunisian oasis of Gabes occupies an area of about 7,000 ha. This is the largest littoral oasis group of Mediterranean. The palm of Gabes is the largest of them. It covers 700 hectares with 300,000 date palms, spread over ten sites (including Chenini Nahal, Jara, Menzel, Chott Essalam, Bou Chemma Zrig, Menara and Téboulbou) (Bensalah, 2011).

1.1.2. Fruit characteristics, varieties of palm date fruits and nutritional importance

Fruit characteristics: Dates, fruit of the palm date, are a berry, usually elongated or rounded. It is composed of a stone with a hard consistency, surrounded by flesh as shown in figure 2.

The edible part of the date, named flesh or pulp, consists of:

- A pericarp or fine cellulose covering named skin

- A mesocarp usually fleshy, with a variable consistency according to its sugar content

- An endocarp with a clearer tone (hue) and fiber texture; sometimes reduced to a parchment membrane surrounding the stone (Espiard, 2002).

The date dimensions are very variable, from 2 to 8 cm in length and a weight ranging between 2 and 8 grams depending on the variety. The color ranges from yellowish white to black through the amber, red, brown more or less dark (Djerbi, 1994).

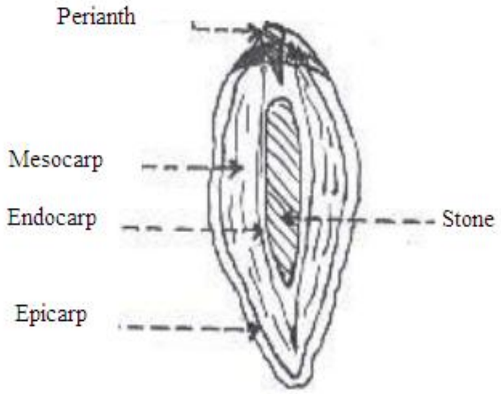

Figure 2. Longitudinal section of a date

The date fruit undergoes several external and internal changes during its various stages of growth and development (Al-Hooti et al., 1997). Palm date is one of climacteric fruits for which ripening are characterized by softening of flesh, an increase in the sugar: acid ratio, enhanced color development, and increases in respiratory activity and ethylene production (Lurie, 1998).

Since pollination, dates successively pass through five ripeness stages. Kader and Hussein (2009) reported that maturity stages of dates include “hababouk”, “khimri”, “khalal”, “rutab” and “tamar”.

- ***Hababouk*** (earliest stage of development): This stage starts just after fecundation and lasts about five weeks. At this stage, the fruit is completely covered by the perianth and is characterized by a growth slow (Djerbi, 1994).

- ***Khimri:*** Dates fruit are not edible at Khimri stage when all cultivars are green in color (Kader and Hussein, 2009). It is characterized a fast fruit enlargement, an increase in the concentration of tannins and starch, a slight increase of total sugars and the dry matter. This stage lasts nine to fourteen weeks.

- ***Khalal:*** A few cultivars, are harvested at the "Khalal" stage (partially-ripe) having yellow or red color (depending on cultivar), but there are very astringent (due to high tannin content) (Kader and Hussein, 2009). This stage is marked by a rapid increase of the total sugar content, active acidity, in contrast the water content decreases. It lasts three to five weeks (Djerbi, 1994).

- ***Rutab and Tamar:*** Most dates are harvested at the full maturity stage "Rutab" (light-brown and soft) and "Tamar" (dark brown) stages, when they have much greater levels of sugars, lower contents of moisture and tannins (disappearance of astringency), and are softer than the "Khalal" stage dates. Increased sweetness with ripening of dates results from the increase in total sugars and in soft cultivars the conversion of sucrose to fructose and glucose (Kader and Hussein, 2009).

Varieties of palm date fruits: At present, more than 2,000 different cultivars of date palm are known to exist all over the world, but only a few important ones have been evaluated for their agronomic performance and fruit quality (Al-Hooti et al., 1997). They differ in flavor, texture, form, color, weight and dimensions (Djerbi, 1994; Buelguedj, 2001). The main varieties of palm date fruits, harvested in the Mediterranean basin, according to their origin, with their characteristics are shown on the Table. 1

Egypt	Iran	Algeria	Tunisia	Libya	Morocco	Israel	Spain
Hayani: They have a soft texture, high moisture content (over 50%). The majority of their sugars are inverted. They are usually of an oblong shape and consumed fresh (Riad, 1993).	**Mazafati:** Their color is brown to black, with an extra soft. Natural soft caramel and chocolate reminder could be a description of the taste of this delicious fruit.	**Deglet Noor:** Commercial variety par excellence, with semi-soft texture and translucent yellow color. (Bacha, 2008)	**Deglet Noor:** It is the queen of dates, elongated, a translucent yellow color and very sweet (GIFruit, 2008).	**Abel:** Sweet dates but astringent taste with yellowish brown, medium fruit size and sensitive to pests. They considered with extra quality (Libyan Dates, 2015).	**Mejhoul o Madjool:** They are the noblest variety with high price. They have a semi-soft texture, strong-smelling and very sweet (Sedra, 2003).	**Madjool:** The most cultivated variety, considered the noblest one with high price, They have a semi-soft texture, strong-smelling and very sweet (Bernstein, 1993).	**Caqui 2:** Fresh dates very appreciated and reproduced by seed, so each is unique and unrepeatable. They are the only ones in the world that are consumed fresh, in autumn and winter. (Amorós et al., 2007)
Sewi o saidi: With half dry texture and high content of inverted sugar. It can be preserved for long periods because of their high content of soluble solids and low moisture content.(Riad, 1993)	**Sayer:** They have a semi dried texture with dark brown color and round around size. They have a shelf life of 18 months at room temperature, can be consumed fresh or processed.	**Ghars:** Common date with 4-5 cm of long. They have a floury texture tender, sweet, marron dark color; they are used principally for pastry. (Sayah and Ould El Hadj, 2010).	**Allig:** More elongated than Deglet Noor, sweet, dark reddish brown, abundant pulp with semi-soft texture (GIFruit, 2008).	**Bamour:** Variety not widespread with medium quality, semi soft texture, honey or red color and cylindrical shape. Part of them is intended to animal feed (Libyan Dates, 2015).	**Boufeggous:** Fragrant and lightly caramelized, highly appreciated quality. They have a semi-soft consistency; they are intended to industry (Sedra, 2003).	**Hayani:** The second most cultivated variety with soft texture, oblong shape and high moisture content. They are consumed fresh (Bernstein, 1993).	**Caqui 4:** Fresh dates very appreciated and reproduced by seed, so each is unique and unrepeatable. They are the only ones in the world that are consumed fresh, in autumn and winter. (Amorós et al., 2007)
Samani: With soft texture and high content of soluble solids and carotenoids. They have a marron yellowish color, It is used in animal feed also.(Riad, 1993)	**Zahidi:** Date is dry fruit with yellow color, meaty and so delicious which its moisture is low. They have a shelf life of 18 months at room temperature.	**Degla Beida:** Common date with dry texture. There have white color and flat. They can serve to prepare the meal date, highly appreciated in West Africa. (Sayah. and Ouled El Hadj. 2010).	**Khouet Allig**: Texture, color and flavor similar to Allig, from which it derived its name; "sister Allig" is slightly thinner and less sweet (GIFruit, 2008).	**Berni**: Soft dates with cylindrical shape, amber honey color. There are consumed fresh or paste. They considered with extra quality (Libyan Dates, 2015).	**Aguellid:** Early ripening variety. They have a golden color with soft consistency and sweet taste having high-value (Sedra, 2003).	**Deglet Noor:** Commercial variety par excellence with semi-soft texture. They are considered the best in appearance and taste (Bacha, 2008).	**Caqui 29:** Fresh dates very appreciated and reproduced by seed, so each is unique and unrepeatable. They are the only ones in the world that are consumed fresh, in autumn and winter. (Amorós et al., 2007)
Zagloul: They are similar to Hayani, only differs by their darker color and their thermal requirements. (Riad, 1993)	**Kabkab**: They are round in size, and the color varies from light brown to dark brown. It is soft in nature with moisture under 18%. With short shelf life, they are used for direct consumption and industrial purposes.	**Mech Degla:** Common date with dry texture, of 1.8 to 3.5 cm long, they have a light brown color, very pronounced, very digestible, with wrinkled and bit bright epicarp (Sayah. and Ouled El Hadj. 2010).	**Kenta:** Light golden color, is an early maturing variety, less sweet than other varieties with semi-dry texture (GIFruit, 2008).	**Bestian:** cylindrical shape, honey color with low sugar content, variety recommended for diabetics, they have a medium quality. They are also used for animal feed (Libyan Dates, 2015).	**Bouskri:** Semi dry variety with agreeable taste and very sweet (Sedra, 2003).	**Bahri:** Consumed fresh with less widespread, low consumption in Europe. They have a particular flavor and bitterness is not beneficial to their marketing, taking very special consumers (Bernstein,. 1993).	**Los Olmos 2** Fresh dates very appreciated and reproduced by seed, so each is unique and unrepeatable. They are the only ones in the world that are consumed fresh, in autumn and winter. (Amorós et al., 2007)
El barakaoui: They are a dry date variety with high content of sugars, in which sucrose represents a significant part. They can be kept for a very long time under normal ambient temperature. (Riad, 1993)	**Rabby:** They are semi dry and meaty. Their color is dark brown with round shape. They can be consumed fresh or for industry.	**Tadala:** Common date with soft texture. There are consumed fresh with medium quality (Asad Allah, 2004).	**Kentichi:** Dry variety, characterized by the high sucrose content and low content of reducing sugars and rich in dietary fiber (GIFruit, 2008).	**Deglet Noor**: Commercial variety par excellence with semi-soft texture. They are considered the best in appearance and taste (Bacha A. 2008).	**Najda:** Date of appreciated quality, with significant commercial potential for resistance to Fusarium. They have a semi-soft consistency (Sedra, 2003).	**Deri**: Dry variety, with a brilliant dark color and caramel flavor. (Bernstein,. 1993).	**Los Olmos 8:** Fresh dates very appreciated and reproduced by seed, so each is unique and unrepeatable. They are the only ones in the world that are consumed fresh, in autumn and winter. (Amorós et al., 2007)

Table 1. The main varieties of palm date fruits, harvested in the Mediterranean basin, according to their origin and their characteristics

According to Espiard (2002), the date consistency is variable and based on this characteristic; dates are divided into three categories (Figure 3):

1- Soft dates: Hayani, Mazafri and Berni.

2- Semi-soft or semi-dry dates: Deglet Noor, Medjoul and Sayer.

3- Dry dates with firm consistency: Degla Beida, Mech Degla and El barakaoui.

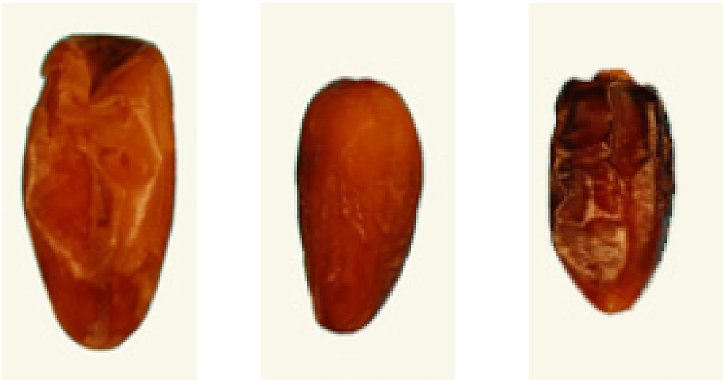

Figure 3. Various consistencies of palm date fruits.

<u>*Nutritional importance:*</u> Dates represent an important nutritional element in the diet of local populations where the trees are grown. The fruit also becomes a part of the daily intake of residents in countries importing this fruit (El Hadrami and El Hadrami, 2009). In fact, date fruits are rich in nutrients and compared to other fruits and foods (apricot: 520 calories/kg; banana: 970 calories/kg; orange: 480 calories/kg; cooked rice: 1,800 calories/kg; wheat bread: 2,295 calories/kg; meat (without fat): 2,245 calories/kg), dates give more than 3,000 calories per kilogram (Zaid and Wet, 2002).

Dates provide an excellent source of rapid energy due to their high carbohydrate content (70-80%). Most of the carbohydrates in dates are in the form of fructose and glucose, which are easily absorbed by the human body (Ahmed et al., 1995; Myhara et al., 1999). The good nutritional value of dates is also based on their dietary fiber content, which makes them suitable for the preparation of fiber-based foods and dietary supplements (Al-Farsi et al., 2005). Dietary fiber has important therapeutic implications for certain conditions such as diabetes, hyperlipidemia, and obesity and may exhibit a protective effect against hypertension, coronary heart disease (CHD), cholesterol, colorectal and prostate cancers, and intestinal disorders (Tariq et al., 2000). Dates have also been reported to serve as a good source of essential minerals (Al-Shahib and Marshall, 2003). Dayang et al., (2014) also confirmed this reporting that date is an important font of protein (2.3-5.6%), fat (0.2-9.3%), essential salts and minerals, vitamins and an elevated proportion of dietary fiber (6.4-11.5%). They also contain oil in the flesh (0.2-0.5%) and the seed (7.7-9.7%). The seed represents 5.6-14.2% of the entire fruit weight.

1.1.3. Socioeconomic importance

Worldwide date production FAO estimates that the harvested area of date growing was 1.1 million ha in 2012 (FAOSTAT, 2015). This area had increased by 0.03 million ha over the previous decade. The largest area (833,351 ha) is located on the Asian continent, which includes the Middle Eastern producing countries, followed by Africa (416,695 ha), North Africa (392,200 ha). In the Americas and Europe the production covers only a few thousand hectares. The worldwide production of dates was estimated in 2012 to 7.6 million t, which represents an increase of over 1 million t in a decade. The production mirrors the area of producing groves with production shared between Middle Eastern and North African countries (2.7 million t).

According to FAO statistics (2015), the world's largest producer over the last five years (2008-2012) was Egypt with an average of 1.35 million t followed by Iran (just over 1 million t), Saudi Arabia (around 1 million t), Algeria (662,490 t), United Arab Emirates (566,200 t), Pakistan (555,782 t) and Sudan (411,580 t).

Other significant producing countries are Iraq, Oman, Tunisia Libya, Morocco, Yemen, Israel, Mauritania, USA, Bahrain, Qatar, Spain and Kuwait.

In terms of annual yields during 2008-2012, the USA leads with an estimated average of 83,560 hg/ha. Next is Africa especially the Northern parts, producing 76,763 hg/ha on average. In the Middle Eastern countries, although a large area of producing groves, yields are below the world average (64,139 hg/ha).

The date palm is currently grown for fruit production intended for local consumption, trade and export in more than 100 countries around the world in 2011.

In the last 10 years the Mediterranean basin could lead trade in palm date fruit, ensuring more than 50 % of their exports worldwide as shown in Figure 4 (FAOSTAT, 2015).

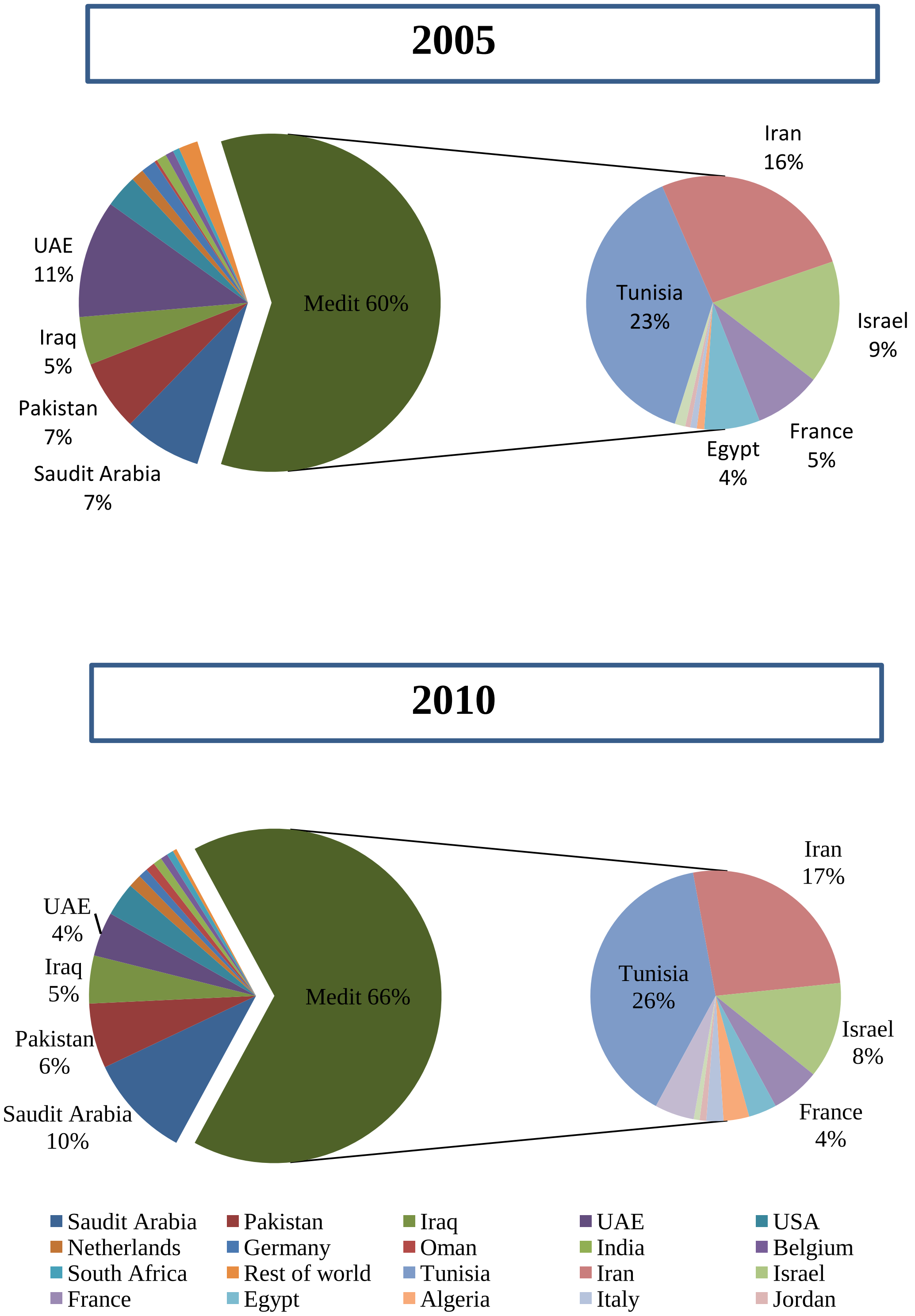

Figure 4. Distribution of palm date fruits exports worldwide and in the Mediterranean Basin in 2005 and 2010 (FAOSTAT, 2015).

As previously mentioned, the major exported cultivar is Deglet Noor, which is produced principally by Algeria and Tunisia, then with significant less production is situated Israel.

In Tunisia, date palms occur in the southern part of the country (North latitude 34°25'), where the conditions are most favorable for the production and ripening of such valuable "dessert date" varieties as Deglet Noor (El-Juhany, 2010). This variety accounts for 74% of the total production (INS, 2015).Tunisia is the world leading producer of Deglet Noor (FAOSTAT, 2015), making it a strategic sector in the country.

The economic importance of the phoeniciculture in Tunisia, is reflected first in the cultivation area of dates, which extends over 51,000 hectares in 2011, this area has been growing steadily over the past 20 years, doubling between 1990 and 2010 (FASOSTAT, 2015), being the sixth most widespread area in the world, after Saudi Arabia, Algeria, Iran, Iraq and Morocco and also the continued growth of its production and exports comparing to its competitors as shown in Figure 5.

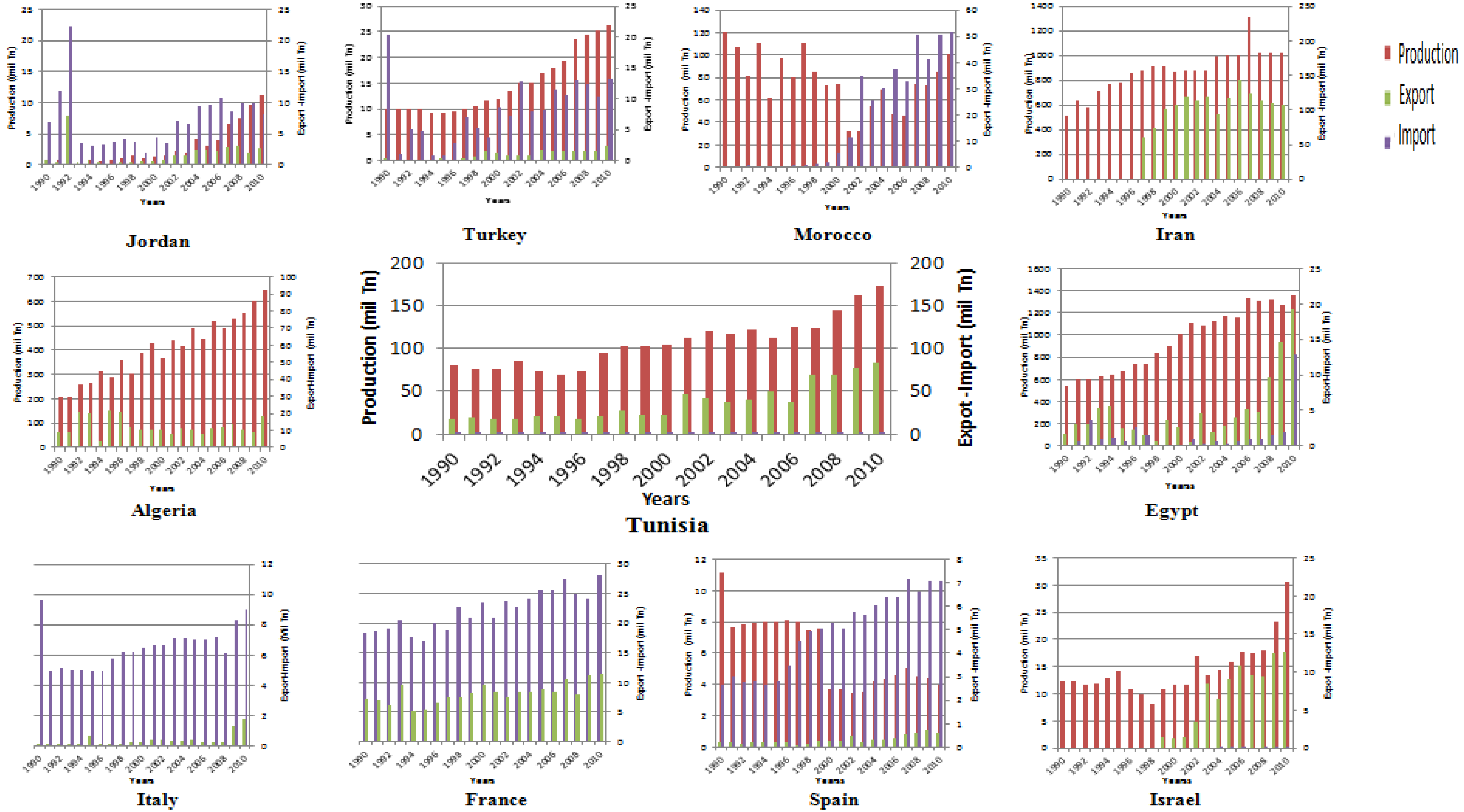

Figure 5. Evolution of production, import and export of pal date fruits in the major countries of the Mediterranean basin (FASOSTAT, 2015).

Although Tunisia represents only about 2.5 % of the world's dates production in 2012, it is the leader in terms of value of exports with 24% of the world's export total and it provides Europe with more than half of its Deglet Noor dates. Tunisian date palm plantations are characterized by the prevalence of this variety in spite of their large genetic diversity. It is occupies approximately 60% of the Tunisian palm plantations and continues to be multiplied (Bouguedoura et al., 2008). In 2012, Tunisia’s fruiting date palms were estimated at 5,400,000 and produced 190,600 tons of dates (CTD, 2015). The date palm sector in Tunisia comes in third place at its domestic farm product exports, after olive oil and seafood products (GiFruit, 2009). Tunisian dates are placed fourth in the world's ranking in terms of quantities exported for 2011 after Iraq, Pakistan and Iran (FAOSTAT, 2015). The date producers aim mainly to improve the quality and marketing methods to face the competition of other countries, like Algeria and Morocco (El-Juhany, 2010).

According to the Tunisian Date technologic Center (CTD, 2015) palm date sector represents 6.6% of the Tunisian agricultural production where they exercise more than 60,000 farmers and contributes with 16% in its exports after oil olive in 2011.

GID counts about thirty conditioning enterprises for exports dates in Tunisia in 2012, generally the packaging of dates is carried in the same way for all units and the same steps of the manipulating is therefore the similar.

For exports of palm date fruit, the principal steps for packaging and expedition followed by collecting and exporting enterprises in Tunisia are presented in the following simplified flux (Figure 6).

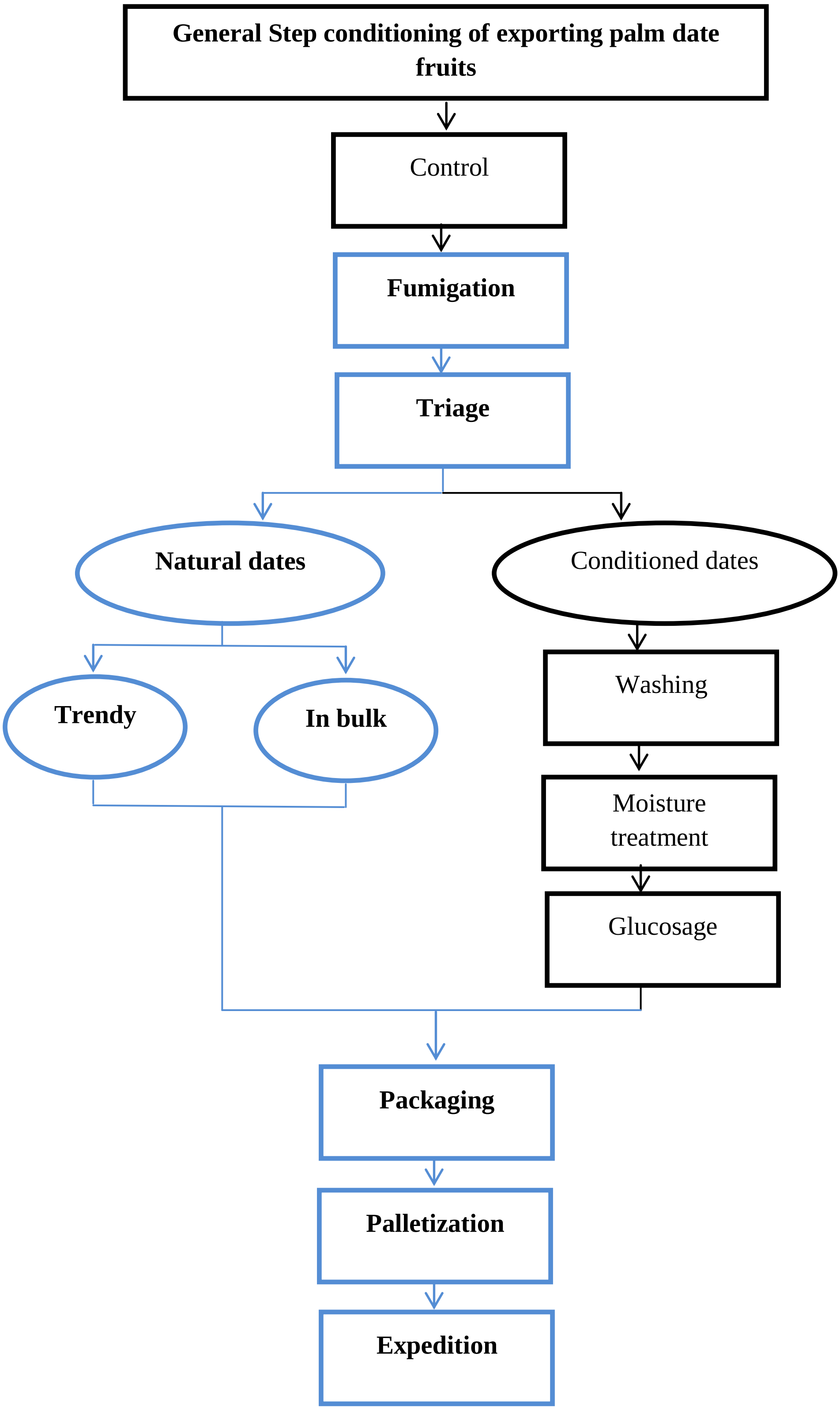

Figure 6. Flux of principal step for conditioning exports palm date fruit.

Due to this growing importance of the palm date sector in Tunisian economy structure, it is interest to analyze the competitive position of this strategic sector in Tunisia.

- **Analysis of palm date Competitiveness**

To measure the economic importance of a determinate sector in a particular country pertaining to a given geographic area, various economic indices have been developed. Those most employed in the agricultural sector:

- *Analysis of Balassa's Revealed Comparative Advantage Index (RCAI):* Balassa (1965) defined the revealed comparative advantage or specialization index as the ratio between exports of certain products of a country and total exports of this country to the rest of the world (or the geographical reference area), and world exports (or geographical reference area) of the same product *j* of the total world exports (or of the geographical reference area) (Vollrath, 1991; Bojnec, 2001). It was employed to analyze the competitiveness of olive oil sector in Tunisia (Boudiche et al., 2003), almond, apple, hazelnut, walnut and orange in Iran (Bakhshinejad and Zadeh, 2012), and fruit and vegetable industry in Turkey (Serin and Civan, 2008).
- *Dependency Ratio (DR):* Parallel to the Balassa index of specialization, the DR for the imports of sector *i* from country *j* is the ratio between the imports of this sector with respect to total imports, considering this relationship with the ratio between the imports of that sector and total imports of the reference area (Martínez-Sánchez, 1994), also called the relative advantage of imports by Vollrath (1991). It was applied to analyze the competitiveness of Algeria in meat and poultry sector (Kaci and Cheriet, 2013).
- *Constant Market Share (CMS):* is an approach that analyzes trade patterns and trends in order to formulate policies. The technique identifies the factors

underlying the results of comparative export of a country (Ahmadi-Esfahani, 2006). It was used to evaluate the position of olive oil sector in Tunisia (Boudiche et al., 2003), Thai natural rubber in Thailand (Poramacom, 2002).

- *Trade Balance Index (TBI):* Defined by Lafay (1992) and revised by Widodo (2009), TBI is the ratio between total output and export of traded goods (coupled exports and imports). The index reveals whether a country a net exporter or net importer.

The TBI value indicates a qualitative structure of product export and import and trade flows. It was used as an important index to analyze the competitiveness of Hungarian in agri-food sectors (Fertö and Hubbard, 2002) and to evaluate the position of Russian federation in the international market of agricultural and foodstuff products (Ishchukova, 2013).

- *Competitive Price Index (CPI):* This index of price competitiveness and export performance, also known as the trade-weighted currency index, attempts to measure trends or competitiveness of product price from a particular country worldwide. These indices incorporate information on developments in domestic currency, price of the products exported by the country, as well as trends in the exchange rate. It was applied to evaluate the impact of food scares on price adjustment in the UK beef market (Lloyd et al., 2001) and to analyze the position of olive oil sector in Tunisia (Boudiche et al., 2003).

1.1.4. Problematic of the palm date sector

Similarly, to other plants, date palms are in danger of dying out because of human activity. Although date palm cultivation has a long history, yet the efforts expended for the development of this important crop, although significant, yet still insufficient and fall below expectations (El-Juhany, 2010). On the whole, the product quality is still low, the field and post-harvest losses are quite high and the date products and byproducts can no doubt be improved (Sawaya, 2000).

Date palm production is fronting serious problems, such as low yields due to the lack of investigation, the spread of pests, as well as marketing constraints (El-Juhany, 2010). During the last decade, productivity has decreased in the traditional growing regions. Pests and diseases have affected significantly the date production in the world.

Disease such as Bayoud which is caused by fungus threatens the date palms in North Africa except Tunisia, has affected nearly all Moroccan palm groves as well as those of western and central Algerian Sahara. It has killed more than 12 million in Morocco and 3 million in Algeria and has accelerated desertification (Djerbi, 1998). Unfortunately, pests and diseases spread increasingly with the expansion of trade and travel in the globalizing world system (UN Press Release, 2004). In North Africa (Africa except Tunisia), date palm cultivation and production are suffering from different problems but, the main problem is Bayoud disease (*Fuzarium oxysporum f.sp. albedinis*), which killed in less than a century more than 13 million trees in Morocco and Algeria (El-Juhany, 2010).

Kader and Hussein (2009) reported that insects infestation and damage caused by insect feeding on the dates is one of the primary causes of postharvest losses in quality and quantity. Dates can be infested with some of the stored-products insects (such as *Rhynchophorus ferrugineus, Oryzaephilus surinamensis, Oryzaephilus mercator, Tribolium confusum, Plodia interpunctella*, *Cryptolestes ferrugineus*, and *Cadra spp*.)

The most important pest of the date palm in the world *Rhynchophorus ferrugineus Olivier* known as Red Palm Weevil, it is native and has been detected in many countries of southern Asia. Since the 1980s it has rapidly expanded its geographical range westwards and reached many countries in the Middle East and Europe. It is present in Egypt, Jordan, Iraq, Palestinian Authority Territories, Kuwait, Bahrain, Oman, Qatar, Saudi Arabia, United Arab Emirates, Spain, France, Italy, Greece and Turkey (Malumphy and Moran, 2009).

Moreover, high infestation rates by pyralid moth pests, namely the carob moth *Ectomyelois ceratoniae* (Zeller) (picture 3), the Mediterranean flour moth *Ephestia kuehniella* (Zeller) and the almond moth *Ephestia cautella* (Walker), are causing significant economic losses during storage in North Africa specially Algeria and Tunisia. These insects are reported as major pests of stored dates in Tunisia (Dhouibi, 1989; Jarraya, 2003). These devastating insects cause loss of weight and downgrading of the commercial value of the fruit (Dhouibi, 2000).

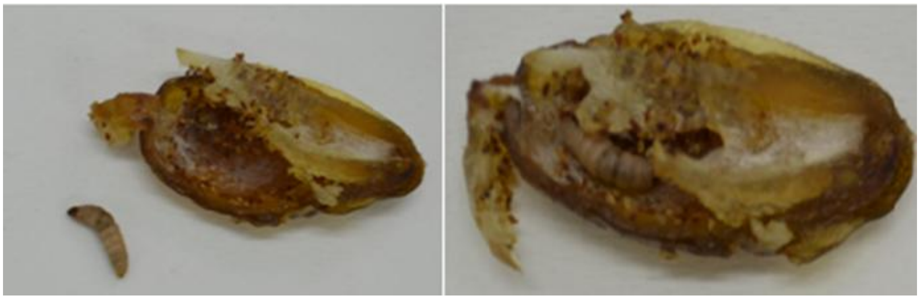

Figure 7.- *Ectomyelois ceratoniae* (Zeller).

The carob moth, *Ectomyelois ceratoniae* (Zeller), is another important economically pest species, which infests 10–40% of the harvestable crop annually in the world (Farrar, 2000). In Tunisia, the date moth *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) is the major insect pest of dates both in field and in storage (Dhouibi, 1989). Date moth infests 20% of the harvestable crop annually (GI-fruit, 2009). This devastating insect degrades the stored dates and causes weight loss and downgrading of the commercial value of the fruit (Haouel et al., 2010). Date infestation by *E. ceratoniae* in the field and in packing and storage houses enormously depreciates the marketable quality of dates and risks to compromise exports in particular those of the variety Deglet Noor.

Therefore, harvested dates must be fumigated with an approved fumigant for disinfestation followed by packaging in insect proof containers. Methyl bromide at 30 g/m^3 (30 ppm) for 12 to 24 h at temperatures above 16 °C is very effective in insect disinfestation (Kader and Hussein, 2009). Nevertheless, the use of this fumigant is being restricted because of its health and environmental damages (Bell, 2000). Moreover, the Montreal Protocol of the United Nations Environment Program (UNEP, 1995) recommends the phasing out of methyl bromide by 2005 in developed countries and by 2015 in developing countries (MBTOC, 1998).

Other fumigant which could be used is sulphuryl fluoride at 34 g/m^3 for 24 hh at 20-25 °C, which was recently registered by the USEPA (Kader and Hussein, 2009). Nevertheless, treatments with sulphuryl fluoride can last very long, up to 72 h, and are still not effective against eggs of insects, most likely due to low permeability of the chorion to this gas (Bell et al., 1998).

A potential other alternative could be the phosphine which is an approved and effective fumigant, but the treatment takes 3 to 5 days at 20 °C and 60% relative humidity (Kader and Hussein, 2009), which is considered the drawback to this treatment (Neven, 2010).

Whereby, these chemicals fumigants, by their nature, are harmful to either the environment or human health. Alternative treatments are being developed to be more environmentally friendly and have less impact on human health such as heat treatments (hot air, hot water), ozonated water, electrolyzed water, UV-C treatments, essential oil, freezing treatments and controlled atmosphere, which are providing good rate of disinfestation, and could be named under physical or non-chemical treatments.

1.2 Physical treatments

1.2.1 Heat treatments

1.2.1.1 Hot water

Hot water dips are perhaps the oldest and most widely used heat treatments for fresh fruits and vegetables (Sharp, 1994). Most of the commodities treated with hot water dips are tropical and sub-tropical fruits and vegetables. Hot water revealed effective disinfestation in oranges (Schirra et al., 2005), mangoes (Jacobi et al., 2001), guavas (McGuire, 1997), grapefruit (Hallman, 1996), limes (Gould and McGuire, 2000), bananas (Wall, 2004) and apples (Smith and Lay-Yee, 2000). The advantage of hot water dips is the speed at which the target pulp temperatures are attained and the short duration of the treatment. There is more efficient heat transfer medium than hot air, and when properly circulated through a load of fruit, a more uniform temperature profile is established (Shellie and Mangan, 1994).

1.2.1.2 Hot Air

Hot air treatments can be provided by hot-forced air or vapor heat treatments. Microwave and radio frequency are also forms of hot air treatments (Follet and Neven, 2006). Hot air treatments were first developed for disinfestation of tropical fruits such as papaya (Tsang and Fujii, 1992), mangoes (Heather et al., 1997; Jacobi et al., 2001), palm date fruit (Barreveld, 1993; Navarro, 2006) and citrus (Schirra et al., 2005). Precisely, in dates, hot air at 60 °C during 33 min has been efficient to reach 100% mortality of *Cadra cautella* (Al-Azawi et al., 1983) in palm date fruit. Other combination of temperature/time was effective to achieve total mortality of larval stage of *Carpophilus hemipterus* L. (Finkelman et al., 2006.) in dates. However, several varieties of dates not tolerate high temperatures, for example, the Madjool variety tolerate moderate temperatures between 45 °C and 55 °C (Finkelman et al., 2006).

1.2.1.3 Other Heat Treatments

Microwave and radio frequency are more rapid methods of heating horticultural commodities (Neven, 2003). Microwave has been successful for treating logs (Nzokou et al., 2008), rice (Zhao et al., 2007), stored products (Shayesteh and Barthakur, 1996) and even in palm date fruit Deglet Noor variety reaching total mortality of *E. ceratoniae* (Zouba et al., 2009). Moreover, radio frequency treatments have been very successful in treating dried fruits and nuts (Wang and Tang, 2001) against pests. The advantage of microwave and radio frequency treatments is principally that treatment times can be very short, normally in the span of a few minutes (Neven, 2010).

1.2.2 Cold and Freezing treatments

Many tropical pests cannot withstand extended exposures to above freezing, low temperatures, but the most successful cold treatments are those that require freezing (Neven, 2010). Most insects require only a short exposure at very low temperatures (-10 °C or below) to ensure control (Chauvin and Vannier, 1991; Fields, 1992). Johnson and Valero (2003) reported that freezing was effective for garbanzo beans against cowpea weevil (*Callosobruchus maculates)*. Freezing at -15 °C for at least 48 h has also been reported to be effective in the control of Indian meal moth in dried fruits and nuts where diapausing larvae are potentially present (Johnson, 2007). In palm date fruits (Dhouibi, 2013) showed 100% mortality of *E. ceratoniae* larvae using only freezing treatments at -18 °C for conventional treatments of dates. Kader and Hussein (2009) reported that freezing at -18 ºC or lower for at least 48 h (from the time when the fruit temperature reaches -18 °C or lower) is enough to kill all life stages of stored products insects.

1.2.3 Controlled atmosphere

Controlled atmosphere (CA) means the alteration of the levels of atmospheric gases beyond those levels found at standard temperature and pressure (Neven et al., 2009). CA has been shown to be very effective for fresh fruits (Neven, 2005). It was shown that very low O_2 and very high CO_2 atmospheres have an insecticidal effect and that the time required to achieve 100% mortality depend of the insect species and its developmental stage, temperatures, O_2 and CO_2 levels and relative humidity (Kader and Ke, 1994).

CA could be combined with low temperature (Neven et al., 2009) and hot temperature (Neven and Rehfield-Ray, 2006), improving efficiency of these treatment. Other research indicates that the duration of the high temperature combined with CA treatment can be shortened if it is followed by cold storage (Chervin and al., 1999).

In palm date fruit, CA was only used as a tool to improve quality and stability of the fruit (Baloch et al., 2006), and the effectiveness of high CO_2 treatment on quality response of date fruit (Al-Redhaiman, 2005; Dehghan-Shoar et al., 2010). However, Kader and Hussein (2009) reported that storage in low-oxygen (<0.5%) atmospheres prevents insect activity in palm date fruit.

1.2.4 Other physical treatments

As a quarantine treatment, irradiation is easy to apply, quick, and generally safe. Most of the original research on the effects of radiation on insects was focused on the development of the sterile insect technique (IAEA, 2005). Irradiation was effective as a sanitizer in many fruits and vegetable as shown in palm date fruits (Auda and Al-

Wandawi, 1980; Azelmat et al., 2005). Although radiation treatments of horticultural products do not render the commodity radioactive, organic producers, regulators and some consumers are reluctant to accept this treatment (Neven, 2010) and has received a less than favorable reputation among those supporting ‘pure foods’. Additionally, the sheer volume of product that needs to be treated and cost has proven to be inhibitory to its application (Aegerter and Folwell, 2001).

Moreover, essential oils (Haouel et al., 2010, Ben Jemaa et al., 2013), vacuum and modified atmosphere packaging (Achour et al., 2003), have proven efficiency against same pest, especially in palm date fruits.

Other sanitizer treatments such as UV-C radiation, ozonated water and alkaline and neutral electrolyzed water are considered as emergent technical for disinfestation, but recent study shown that only obtained mortality percentages between 8 to 15% in palm date fruits (Abo-El-Saad et al., 2011; Jemni et al., 2014).

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INTERESTS AND OBJECTIVES

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Date fruit is an important commercial crop in the Middle East, Mediterranean basin and Arab countries. The main exporting area of palm date fruit is the Mediterranean basin, with about 66% of the worldwide date exports in term of volume in 2010, with 26% of these exports coming from Tunisia. Hence, in Tunisia, this sector constitutes an integral part of the agricultural economy, and contributes significantly to the national GDP. In addition to its contribution to the national economy, dates provide a major source of livelihood to the majority of the population living in the South of the country. Unfortunately, the date palm industry in Tunisia, as in many date producing areas, is confronted by several technical problems.

Firstly, Tunisia needs to maintain its competitive position as compared to the neighboring countries of the Mediterranean basin. In this line, various economic indices have been developed to analyze the position of competitiveness of a determinate sector found in a particular country given a geographic area.

Besides competition from neighboring countries, there other factors that can greatly affect the date palm industry such as insect damage to the fruit. The carob moth *Ectomyelois ceratoniae* (Zeller) is a major insect pest of dates, and causes great economics losses. It vastly decreases the marketable quality of dates and risks compromising exports due to price depreciation. Therefore, there is an urgent need to create *E. ceratoniae* disinfestation measures for Deglet Noor cv. dates, other than using methyl bromide and other chemical fumigants, to be able to continue exporting to international markets, since the Montreal Protocol of the United Nations Environment Program has recommended the phasing out of methyl bromide by 2005 in developed countries and by 2015 in developing countries.

In this sense, there has been a growing interest in the use of physical postharvest treatments for the control of pests and insects as alternative to chemical fumigants.

For all of the above reasons, the specific objectives in this Thesis are:

1. To analyze the Tunisian competitive position of the palm date sector in relation to its main competitors in the Euro-Mediterranean area and Iran, insomuch as more than 88% of the production and 70% of world trade take place there. This analysis examines competitiveness in terms of price and non-price competitiveness, determined by the degree of specialization and dependence, trade balance, analysis of market share, quality, national efforts and product differentiation.

2. To study the efficacy of postharvest hot water treatments (HWTs: 50 ºC 10 min, 55 ºC 5 min and 60 ºC 3 min) in Tunisian date fruits, Deglet Noor cv., as a possible treatment to control the carob moth *E. ceratoniae* (Lepidoptera: Pyralidae).

3. To assess the effects of HWTs and storage time, used as disinfestation treatment in Deglet Noor date fruits, on the physico-chemical, microbiological, functional and sensory parameters.

4. To study the efficacy of postharvest hot air treatments (HATs: 50 ºC 30 min, 60 ºC 15 min and 60 ºC 20 min) in Tunisian dates, Deglet Noor cv., as a possible treatment to control the carob moth *E. ceratoniae*.

5. To assess the effects of HATs and storage time used as a disinfestation treatment in Deglet Noor date fruits, on the physico-chemical, microbiological, functional and sensory parameters.

6. To study the efficacy of freezing treatments at – 18 °C (50, 77 and 125 hours) in Tunisian dates, Deglet Noor cv., as a possible treatment to control the carob moth *E. ceratoniae*.

7. To assess the effects of a selected freezing treatment (50 hours at -18 °C) used as disinfestation treatment in Deglet Noor date fruits, on the physico-chemical, microbiological, functional and sensory parameters.

CHAPTER I:

The competitive advantage of the Tunisian palm date sector in the Mediterranean region.

CHAPTER I

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Abstract

In Tunisia, date-palm cultivation and production are of clear strategic importance in terms of economic, social and environmental development. However, the globalization of markets has had a huge impact on the traditional concept of the comparative advantage enjoyed by Tunisia in date exports, highlighting the necessary determinants for competitiveness in the international scenario. In fact, an analysis of the competitive advantage of the Tunisian date industry in the Mediterranean area and Iran over the last 20 years shows that Tunisia is still the main supplier of dates to the EU. The Deglet Noor variety, in particular, puts Tunisia ahead of traditional competitors such as Algeria and Iran, with average of competitiveness indices as BIS 6405.99, DI 17.38, CMS 41.04 and TBI 99.50 are more stable than those countries during the studied period. But it is currently facing new competitors like Israel and re-exporting countries like France. New business strategies (conditioning, new non-chemical treatments, packing, opening new markets, new distribution channels) would be positive responses to tackle current market limitations, the emergence of new producers and restrictive EU policies.

Additional key words: competitiveness indices; date-producing sector; varieties; export-import; Tunisia.

Abbreviations used: BIS (Balassa index of specialization); CMS (constant market share); CPI (competitive price index); CPI_{ex} (export competitive price index);

DR (dependency ratio); EU (European Union); RC (revealed competitiveness); RCA (Balassa's revealed comparative advantage index); TBI (trade balance index)

Introduction

The foreign trade policy in Tunisia has been marked by two events: accession to membership of the World Trade Organization (WTO); and the Free Trade Agreement with the European Union (EU) in 1995. Within this context, the food industry has been facing new challenges arising from free trade and greater access of the Tunisian economy to the rest of the world. This has led companies in this sector to improve both their performance and competitiveness.

The agricultural and food policy in Tunisia has focused, on the one hand, on intensifying agriculture, improving irrigation, water infrastructure and water supplies and, on the other, on intensive use of inputs (equipment, chemical supplies, seeds and improved varieties, etc.). These efforts have focused on the maximization of production, thus agricultural activities were first carried out according to national guidelines and objectives of self-sufficiency in terms of food, and subsequently food safety, by supporting production prices and subsidizing most agricultural inputs (AfDB, 2012).

In their work Laajimi *et al.* (2012) explained that the approach to liberalization of the economy has not achieved the desired impact in either terms of institutions or behavior of the key characteristics of the Tunisian economy. Also, a simple analysis of export distribution shows that the main target is the Euro-Mediterranean area, especially the EU. In fact, more than ¾ of total Tunisian agricultural exports are destined to Italy, Spain, France and Germany. This situation has been achieved due to the multitude of trade agreements signed by Tunisia (EU in 1995; WTO in 1994; Great Arab Free Trade Area (GAFTA) in 1995; European Free Trade Association (EFTA) in 2005; Agadir in

2007). Clear examples are the case of olive oil, seafood and dates, which are considered strategic sectors of the national economy.

Globalization has made companies worldwide more competitive. Omoregie & Thomson (2001) informed that competitiveness is a relative concept; therefore there is a need for a measurement framework that will help to systematically evaluate all comparable factors thought to be relevant in the pertinent economic activities. This concept can be analyzed through competitive advantage, in this line; this work proposes to study the competitive position of a strategic sector in the Tunisian economy as palm date sector.

In 2011, seven million tons of dates were produced worldwide (compared to 5 million in 1999) of which 2.5% correspond to Tunisia, where production reached 180,000 tons. This makes Tunisia the ninth largest producer in the world, and the third in the Mediterranean after Egypt (the largest producer in the world with 25%), and Algeria.

The economic importance of date-growing in Tunisia is reflected in the date-growing area, which covered over 51,000 hectares in 2011 (Siddiq *et al.*, 2013). This extension has been steadily increasing over the past 20 years, doubling between 1990 and 2010, and is the sixth most widespread area in the world, after Saudi Arabia, Algeria, Iran, Iraq and Morocco (Fig.1a).

Date palm sector in Tunisia is of clear socio-economic importance, as the sector forms an important part of the food trade balance. It is also a source of income for farmers in southern Tunisia and plays a strategic social role in balancing production systems in the oasis. In Tunisia, the date-growing sector accounts for 4% of total agricultural production, 7% of plant production and 12% of agricultural exports. There are about 5.4 million date palm-trees and approximately 50,000 farmers are employed, spreading over four production areas: Tozeur, Kebili, Gafsa and Gabès (GI Fruit, 2008).

Tunisia is considered leader in the production and export of cv. Deglet Noor (APIA, 2008), which has specific organoleptic characteristics (flavor, color, texture...), with over 73% of its production, and over 85% of exports. This cultivar is *par excellence* the most marketed in Europe, since about 90% of dates imported to the EU are ˈDeglet Noorˈ, and 90% of these are imported from Tunisia and Algeria. The latter is Tunisia's main competitor, with the remaining 10% being supplied by Israel and the USA, emerging producers of this variety.

APIA (2008) divides the competing countries of Tunisia into three categories: (i) non-traditional producers that have developed an integrated agro-industry, such as Israel, Palestine and the USA; (ii) re-exporters, which add value to the low-cost imported product, basically France, Italy, Spain, Netherlands, Germany, United Kingdom and Switzerland; and (iii) traditional producers of ´Deglet Noor´, like Algeria.

Tunisia is in the best position in terms of quantity of exports and related income. But this is not so in product valuation, as it is positioned after the re-exporting countries, such as USA and Israel or Palestine (APIA, 2008).

FAO (2000) reports that countries wishing to develop their ´Deglet Noor´ exports to Europe come into competition with well-established and strong suppliers. As already mentioned Tunisia has the highest market share and is the undisputed leader. Despite this strong appearance, there are structural weaknesses, including the disruption of its trading system and the inconsistent quality of the packaged product, as well as a high rate of product infestation (APIA, 2008; Jemni *et al.,* 2014).

This is noteworthy, given the importance of this sector in the Tunisian economy in terms of export incomes as dates represent the second flagship product after the olive oil (FAO, 2000). However, it should be noted that there are currently various technical problems including pests and diseases, which are a major threat to the sector. The carob moth, *Ectomyelois ceratoniae*, is by far the most important problem faced by Tunisian ´Deglet Noor´ production and export. For decades, methyl bromide has been the only fumigant for quarantine pests. Because of its harmful effect on human health and the environment –identified as harmful to the ozone layer by the Montreal Protocol (PNUMA, 1992)– its use is restricted, thus there is an urgent need for new treatments and alternative techniques (Ahmed, 2001; Likhayo *et al.,* 2014).

Given the importance of palm date sector in the Tunisian economy and the different technical problems that limit its trading system, we analyzed Tunisian competitive position of this sector in relation to its main competitors in the Euro-Mediterranean area and Iran, insomuch as more than 88% of production and 70% of world trade take place there. This analysis examines competitiveness in terms of price and competitiveness not price, determined by the degree of specialization and dependence, trade balance, analysis of market share, quality, national efforts and product differentiation.

Methodology

Asche *et al*. (2005) reported that on microeconomic theory one assumes that there exists a market constituted by a group of commodities. The commodities compete in the same market when the goods are substitutable for the consumer or the producer, which is the case of palm date fruit, in the Mediterranean area.

Economic competitiveness indices:

— *Balassa's revealed comparative advantage index (RCA).* Liesner (1958) was the first to use RCA, but it was Balassa (1965) who improved it, and its subsequent dissemination in 1989 made it the most commonly used and, thus, it became known as the "Balassa index". This index basically measures normalized export shares of a country *i*, compared to exports of the same industry in a group of reference countries. The concept of "revealed comparative advantage" is widely used to determine the weak and strong sectors of a country. Porter (1990) used a Balassa index >1 (100), in some cases reinforced with a Balassa index >2 (200), to identify the strongest sectors of a country's economy. Meanwhile, Vollrath (1991) suggested three alternative ways of measuring the RCA of a country: (i) the relative trade advantage, which considers both imports and exports, (ii) the simple logarithm of the relative export advantage (lnRXA); and (iii) the revealed competitiveness (RC), defined as the difference between the lnRXA and the logarithm of the relative import advantage (Fretö & Hubbard, 2003).

Balassa (1965) defined the RCA or specialization index as the ratio between exports of certain product *j* of a country and total exports of this country to the rest of the world (or the geographical area taken as a reference), and world exports (or geographical reference area) of the same product *j* of the total world exports (or of the geographical reference area) (Vollrath, 1991; Bojnec, 2001). Among other studies, the RCA of Blázquez-Lidoy *et al*. (2006), is named the Balassa Index of Specialization (BIS), measuring the degree of specialization of country *i* for product *j*.

In this research we used the classic model of Balassa, to analyze the commercial development of dates from the viewpoint of specialization through the comparative advantage in exporting. This decision is in accordance with Chudnovsky & Porta (1990), who recommend using it, simply as an indicator of an economy's, specialization in international trade at any given time.

The Balassa RCA is defined as:

$$RCA_{ij} \ or \ BIS_{ij} = \frac{\left({x_{ij}}/{\sum x_j} \right)}{\left({x_j}/{\sum x_j} \right)} \quad [1]$$

where $\boldsymbol{x_{ij}}$ represents exports from country *i* for product *j*; $\sum \boldsymbol{x_j}$, represents exports of all products of country *i*; $\boldsymbol{X_j}$ represents exports of product *j* in a reference area; and $\sum \boldsymbol{X_j}$, represents exports of all products of the reference area.

This index varies between 0 and + ∞, values <200 indicate that country *i* is relatively less specialized in the sector *j* than the reference area, or is at a disadvantage compared to the reference area. For values >200, we can say that participation of the sector *j* in the structure of exports from country *i* is higher than that observed in the reference area, whereby one can state that the said country is strongly specialized in this sector.

— *Dependency ratio (DR).* Parallel to the BIS, the DR for the imports of sector *i* from country *j* is the ratio between the imports of this sector with respect to total imports, considering this relationship with the ratio between the imports of that sector and total imports of the reference area, also called the relative advantage of imports by Vollrath (1991).

Thus, one can compare the structure of imports of a country within the reference area. If it is >100 for sector *i*, one can say that participation in the said sector in the structure of imports of country *j* is higher than that observed in the reference area.

$$DR_{ij} = \frac{\left({m_{ij}}/{\sum m_j}\right)}{\left({M_j}/{\sum M_j}\right)} \times 100 \quad [2]$$

where $\boldsymbol{m_{ij}}$, represents imports from country *i* for product *j*; $\sum \boldsymbol{m_j}$, represents imports of all products from country *i*; $\boldsymbol{M_j}$, represents imports of product *j* in the reference area; and $\sum \boldsymbol{M_j}$, represents imports of all products from the reference area.

This index does not have to be correlated with the specialization index of RCA, since there is no relationship between the two indices (Martínez-Sánchez, 1994).

— *Constant market share (CMS).* Is an approach that analyzes trade patterns and trends in order to formulate policies, the technique identifies the factors underlying the results of comparative export of a country (Ahmadi-Esfahani, 2006). This method disaggregates trade data of the countries surveyed and compares trade flows around the world.

The traditional CMS model was first applied to studying international trade by Tyszynski (1951). Other studies continued using this model despite the well-documented problems (*e.g* Richardson, 1971a,b; Jepma, 1986; Oldersma & Van Bergeijk, 1993).

$$CMS_{ij} \ or \ PM_{ij} = \left(\frac{x_{ij}}{x_m}\right) \times 100 \qquad [3]$$

where $\boldsymbol{X_{ij}}$, represents exports from country *j* of product *i*; $\boldsymbol{X_m}$, represents exports of the geographical area taken as a reference of a product *i*.

— *Competitive price index (CPI).* This index of price competitiveness and export performance, also known as the trade-weighted currency *index,* attempts to measure trends or competitiveness of product price from a particular country worldwide. These indices incorporate information on developments in domestic currency, price of the products exported by the country, as well as trends in the exchange rate.

Since these indices compare prices from one country to others, one must select the area for which they are calculated. In general, it is advisable to select an area that is representative of the true competitors of the country.

The same index is applied to the prices paid to the farmer and is called the producer's CPI, so the competitive price index of country *i* over a competitor *j* is defined as price, but in this study we only analyze the producer's CPI:

$$CPI_{ij} = \left(\frac{e_j}{e_i}\right) \times \left(\frac{PX_i}{PX_j}\right) \qquad [4]$$

where CPI_{ij}, is the competitive price index of country *i* over competitor *j*; $\boldsymbol{e_j}$, the exchange rate of country *j* to the US dollar; $\boldsymbol{e_i}$, the exchange rate of country *i* to the dollar; $\boldsymbol{PX_i}$, the price of reference country *i* of a product; and $\boldsymbol{PX_j}$, the price of the competing country *j* of the same product.

In this study, we work with data of updated FAO prices in a single currency (US$), from which the CPI are calculated from the producer and exporter price given by FAO, without applying the exchange rate. Thus is defined the:

— *Export competitive price index (CPIex).* Relating export price of the reference country to export prices of its competitors converted to a common currency and converted to 100 as the reference year.

Whereby, in this case, the above equation is reduced to the following equation:

$$CPIex_{ij} = \left(\frac{PX_i}{PX_j}\right) \quad [5]$$

According to the formula used to construct the indices, an increase (or decrease) of the same indicates a real appreciation (or depreciation) of the currency of the country under study and, therefore, a deterioration (or improvement) of the said country’s external competitiveness with respect to the geographical area of reference.

This index of price competitiveness and export performance relates the export price of country *i* for a given product, with the export price of its competitors for the same product, but first converts these prices into a common currency and on the basis of 100 in a given year. To calculate this CPIex, more countries must be included in the analysis, such as the re-exporting countries and non-producers.

— *Trade balance index (TBI).* It is employed to analyze whether a country has specialization in export (as net-exporter) or in import (as net-importer) for a specific group of products. Lafay (1992) used TBI to measure RCA. More recently, in 2010, TBI was used by Widodo (2009) as one of the crucial variables for analyzing the catching-up economies comparative advantage.

The TBI value indicates a qualitative structure of product export and import and trade flows. It is formulated as follows:

$$TBI_{ij} = \left[\left(\frac{X_{ij} - M_{ij}}{X_{ij} + M_{ij}}\right)\right] \times 100 \qquad [6]$$

where $\boldsymbol{X_{ij}}$ and $\mathbf{M}_{ij}$ represent exports and imports, respectively, of country *i* for product *j*.

The TBI value varies between –100 (if a country only imports) and 100 (if a country only exports). Any value within –100 and +100 implies that the country exports and imports a commodity simultaneously. A country is referred to as “net importer” in a specific group of product where the value of TBI is negative and as “net exporter where the value of TBI is positive.

Data collection

Data on trade patterns, information on agricultural policies and trade regulations are necessary to analyze the competitiveness of the Tunisian date trade within the Mediterranean and Iran. The study was conducted for a group of 11 countries, 10 of which belong to the Mediterranean basin (Algeria, Egypt, Spain, France, Italy, Israel, Jordan, Morocco, Tunisia and Turkey), selected based on date palm export values over the past 20 years. We also included Iran in the study, even though it is not part of the Mediterranean, due to the weight of the date-trade sector, geographical proximity to the Mediterranean, and similarity of climatic and agronomic parameters.

The agricultural trade statistics in general and of dates, in particular, were taken from the FAO. They have been complemented and contrasted with data from the United Nations Commodity Trade Statistic Database (UN CONTRADE), from official records of national statistics for each country, such as the National Institute of Statistics of

Tunisia, Israel Export Institute, and finally date export-import data missing in the sources consulted were estimated by linear interpolation using data from previous and subsequent years. The period analyzed was 20 years (1991-2010), divided in four sections, of five years each.

Results and discussion

— *Balassa's revealed comparative advantage index (RCA or BIS).* Table 1 show that Tunisia is the second most specialized country in date exports after Algeria. It presented specialization indices much higher than 200 throughout the study period, remaining more or less stable with a slight decline in the latter part of the study (BIS = 5390.84 in 2006-2010), exceeding the BIS of Egypt by 41 times on average during the study period (first producer of dates in the world), an average of 8 times that of Israel (whose improvement is ongoing) and an average of 3.3 times that of Iran (the second largest date producer in the world).

Fig. 1b shows that on analyzing the Tunisian date export (measured as the Exports/Production ratio), which gives an average of 33% over the 20 years studied and has been as high as 50% in recent years, we see that it greatly exceeds the export of Algeria, with an average of 3.5%, or Iran 7.4% and Israel 23% for the same study period. This means that Tunisia has made by far the greatest effort to export its dates to international markets, dominating with 26% of global date exports in the last decade (Rached *et al*., 2012).

—*Dependency ratio (DR).* The DR or relative advantage of imports (Vollrath, 1991) for Tunisia in the date sector ranged from 10 to 26 during the study period (Table 1). This is considered relatively low compared to Morocco (ranging from 160-1900), Jordan (300-500), France (105-201), Spain (58-80), and Italy (45-76), all considered major importers of dates.

Therefore, the fact that BIS>>200 and DR ≈ 0, shows that Tunisia is strongly specialized in exporting dates, and has a high RCA compared to Mediterranean countries and Iran. Moreover, Algeria is its main competitor in international markets with its BIS descending in the last period, almost equal to the BIS of Tunisia. This is probably due to phytosanitary problems that affect levels of production and marketing of Algerian dates. Given this situation, Tunisia should seek to improve or at least maintain its competitive position in the future, solving the problem of dates. Moth mentioned above and considered the main threat of the date palm industry in Tunisia, and seek alternatives to chemical treatments to meet international and European standards, maintaining a low infestation rate and optimum product quality.

—*Constant market share (CMS).* Table 1 shows that Tunisia ranks first in the Mediterranean, and is ahead of Iran for the time studied, with a CMS average of 41%. This score remained more or less stable throughout the observation time, with small progressive increases in one time period to another, reaching a market share of 44% in the 2006-2010 period.

Iran ranked second in market share, which is considered important; however, it was unstable given the variation between one period and another, and with significant losses after 1996, dropping from 33% in the 1991-1995 period to an average of 24%, from 1996-2010. This probably reflects economic losses due to the progression of the red palm weevil in Iran.

In the Mediterranean, Algeria began in second place in market share criterion, but has declined drastically, dropping from 30% in 1991-1995 to 4.76% in 2006-2010. It has lost more than 25% of its CMS probably due to the decline in French imports, its main destination.

The remaining countries have a negligible share, such as Egypt with an average CMS of 1.40%, over the observation period, or Italy, Jordan, Turkey, Morocco and Spain, with an average CMS <0.5%.

— *Trade balance index (TBI).* Economic development is probably the most important policy objective in less developed countries and exports are often seen as an engine for growth (Dawson, 2005). Results of TBI given in Table 1 confirm this concept and show that Tunisia is a net exporter of dates, with values greater than 0, and is very close to 100 (TBI average 99.59). Tunisia exports but does not import dates, or if it does so, in negligible quantities.

Likewise Iran, Israel and Algeria have TBI values that are equal or very close to 100, indicating a similar export structure and competitive advantage.

Egypt has an average TBI value of 65% during the study period, indicating that it is a net exporter. However, it also imports a significant amount of dates due to the large domestic demand, added to the fact that most of the varieties grown there are lacking in quality and are used for animal feed (Soliman *et al*., 2003).

— *Export competitive price index (CPIex).* The FAO (Liu, 2003) reports that the EU is the most important market for date exporters, and import mainly high-quality dates. In 1998-2000 the average unit value of imported dates ranged from between \$1.7/kg and \$2/kg in the EU, while the unit value worldwide was only \$0.6/kg, indicating that greater value is placed on this fruit by the EU than the rest of the world.

The influence that prices have on a country's competitive position is measured by the CPIex, calculated on the basis of export prices. The trend of these indices (Fig. 1c) reveals that, compared to all the countries studied, Tunisia is gaining a competitive position in date export prices. From 2001 to 2010, the CPIex was significantly reduced compared to the other countries studied, with the exception of Iran and Egypt, which have a very unstable CPIex, decreasing significantly after 2007. This could be explained by a combination of factors, which may have contributed to the reduction of the Tunisian CPIex, such as:

— Regularity of production: a steady increase in Tunisian date production, reaching 199,000 tons in 2013, of which 141,000 tons were ´Deglet Noor´.

— Export efforts: Tunisia is the first date exporter in the world in value but the seventh producer in quantity. Note that Tunisia currently exports about 60% of its total production, compared to 33% in 1999. This trend necessarily implies a special effort to avoid a decline in quality of the exported product, and a modification in export prices (APIA, 2008).

— Importance of variety, quality and presentation: Import prices can vary by up to ten times depending on the variety, origin, packaging and quality (Liu, 2003).

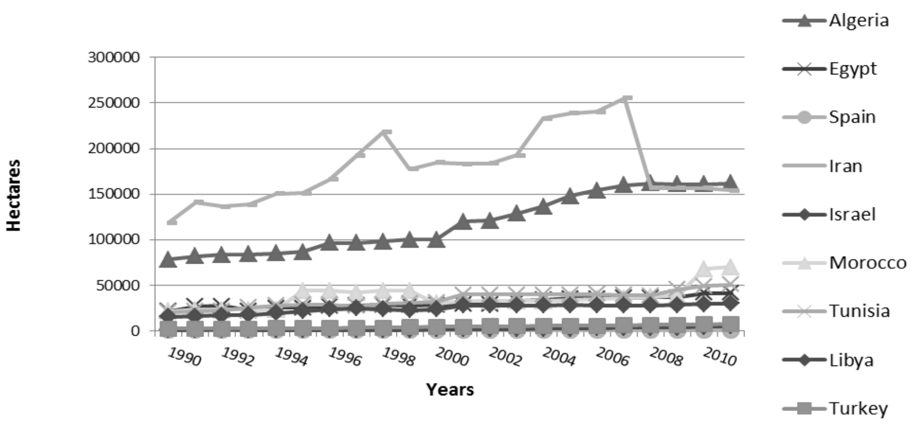
The recovery ratio calculated by APIA (2008) reveals that Tunisia sells its dates for 3.4 times more than the global average. The recovery ratio is very high for Israel, 6.3, due to the sale of ´Deglet Noor´ and cv. Medjool. France sells 4.2 times the average world price (they package and re-export ´Deglet Noor´), whereas Algeria has a coefficient of 2.7 with ´Deglet Noor´ predominating.

In conclusion, date industry is important in Tunisia, in terms of production and export, playing a key socio-economic role. Given the analysis of this sector's competitiveness within the Mediterranean basin and Iran, we conclude that Tunisia has a highly important trade position compared to the Mediterranean area. Nonetheless, recent years have seen a decline in this comparative advantage due to declining competitiveness indices.

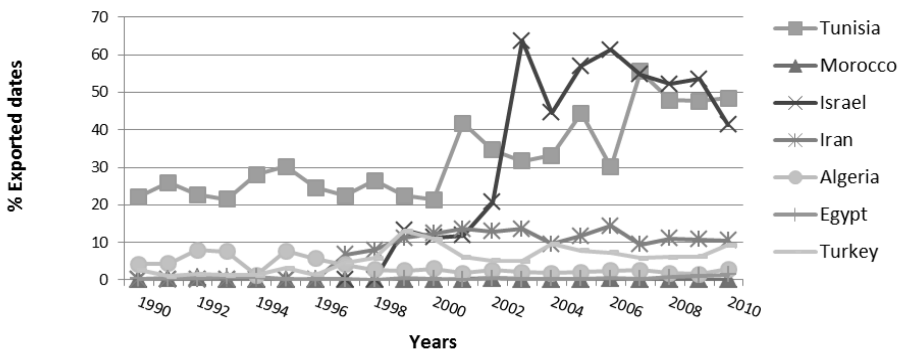
However, the market share indices (constant part of the market) are more stable, with a slight upward trend. This indicates that Tunisia is maintaining its market within the Mediterranean, and is well above the index of all competing countries throughout the period analyzed. This highlights the importance of the Tunisian date in the Mediterranean and Iran.

Moreover, the Tunisian trade balance index remains stable, and close to 100 for the 20 years analyzed, reflecting it continues to be a net exporter of dates. Conversely, imports in this sector are negligible, as in Iran, Algeria and Israel - its main competitors. Regarding the analysis of the competitive price index (CPI), indices for Tunisia were very unstable from one country to another over the period analyzed. However, we can conclude that Tunisia is gaining a competitive position in terms of date export prices, since export CPI were significantly reduced compared to the other countries studied for the period 2001-2010, except for Iran and Egypt, which have had very variable export CPIs, with significant reductions since 2007.

a)



b)



c)

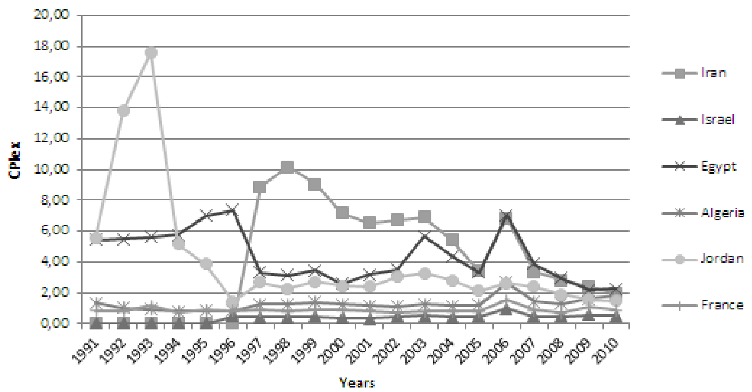


Figure 1. (a) Evolution of dates harvested area; (b) export performance of major countries; (c) export competitive price index of dates in Mediterranean countries. Source: FAO (2014).

Table 1. Analysis of no-price competitiveness of dates in the Mediterranean and Iran

Country	Tunisia	Iran	Israel	France	Egypt	Algeria	Italy	Jordan	Turkey	Morocco	Spain	Mediterranean
Specialization index (RCA or BIS)												
1991-95	6193,70	2741,62	632,83	29,51	206,91	30213,42	1,54	138,75	1,61	44,57	3,09	100
1996-00	7345,41	2188,16	305,01	36,65	170,38	35330,78	2,79	60,37	6,57	4,55	2,81	100
2001-05	6693,99	1428,91	1127,40	26,43	77,58	13713,10	2,30	137,45	7,81	6,51	2,06	100
2006-10	5390,84	1260,25	1052,64	18,22	173,30	6720,16	5,12	149,14	8,52	1,17	2,20	100
Averages	6405,99	1904,74	779,47	27,70	157,04	21494,37	2,94	121,43	6,13	14,20	2,54	100
Dependency index (DI)												
1991-95	10,69	0,00	0,00	201,13	28,66	2,24	76,25	506,68	26,67	158,22	79,68	100
1996-00	17,51	0,00	0,02	189,01	7,49	0,15	71,98	379,33	38,53	373,25	99,39	100
2001-05	26,17	0,25	0,04	125,06	5,53	0,35	46,65	343,69	54,86	1877,56	66,50	100
2006-10	15,16	0,00	0,43	105,47	14,52	0,03	42,87	304,99	77,38	1921,16	58,00	100
Averages	17,38	0,06	0,12	155,17	14,05	0,69	59,44	383,67	49,36	1082,55	75,89	100
Constant market share (CMS)												
1991-95	36,63	32,6	10,25	14,45	1,27	30,67	0,28	0,36	0,08	0,38	0,44	100
1996-00	40,12	26,95	4,27	16,33	1,07	22,77	0,53	0,23	0,35	0,04	0,49	100
2001-05	43,44	21,24	13,4	10,66	0,75	8,26	0,48	0,66	0,41	0,06	0,42	100
2006-10	43,95	25,57	13,11	6,72	2,56	4,76	1,06	0,87	0,48	0,01	0,44	100
Averages	41,04	26,59	10,26	12,04	1,41	16,62	0,59	0,53	0,33	0,12	0,45	100
Trade balance index (TBI)												
1991-95	99,76	100	100	-35,05	46,58	99,76	-94,95	-72,78	-61,24	-50,9	-83,86	32,33
1996-00	99,47	100	99,98	-36,45	68,9	99,97	-91,67	-82,4	-47,13	-97,92	-89,25	19,19
2001-05	99,18	99,96	99,99	-39,58	71,84	99,81	-89,1	-58,45	-54,76	-99,47	-88,99	15,93
2006-10	99,58	100	99,92	-40,11	74,34	99,97	-68,09	-42,98	-61,02	-99,9	-83,83	25,3
Averages	99,50	99,99	99,97	-37,80	65,42	99,88	-85,95	-64,15	-56,04	-87,05	-86,48	23,19

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CHAPTER II:

Hot water treatments combined with cold storage as a tool for *Ectomyelois ceratoniae* mortality and maintenance of Deglet Noor palm date quality.

CHAPTER II

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ABSTRACT

Insect infestation caused by *Ectomyelois ceratoniae* or carob moth is one of the main postharvest disease pests of date fruits and causes serious economic losses during storage and export. Methyl bromide is the most widely used fumigant on stored dates in several countries although it will be withdrawn in 2015 in developing countries. Heat treatment technologies, such as hot water treatment (HWT) are currently a relatively simple, non-chemical alternative that can kill quarantine pests (insects and fungi) in perishable commodities. In this article, the proper HWT treatments (dose and time) that causes *E. ceratoniae* mortality while avoiding quality losses in Deglet Noor fruit when stored for 30 d at 2 °C followed by a retail period of 4 d at 23 °C was studied. The results show that the use of HWT of 50 °C for 10 min, 55 °C for 5 min and 60 °C for 3 min lead to *E. ceratoniae* mortality, also lowering the microbial growth (<1 log cfu g^{-1} for mesophilic and < 2 log cfu g^{-1} for yeasts and molds). HWT induced a slight reduction in skin color (luminosity and hue angle), antioxidant activity (10 to 15% in FRAP and 17 to 22% in DPPH) and total phenolic compounds (9 to 14%). Overall quality was slightly reduced using 60 °C for 3 min although all treatments remained above the limit of marketability as there was no heat damage. Storage time also reduced those mentioned parameters, monosaccharides (glucose and fructose) and the concentrations of some amino acids such as alanine, aspartic acid and proline. The HWT used as an alternative to chemical treatments to control carob moth yielded optimum-quality Deglet Noor date fruits that could be exported to developed countries.

Keywords: Heat treatment; moth of pyrale; methyl bromide; microbial quality; antioxidant activity; sugars.

1. Introduction

Date palm fruit (*Phoenix dactylifera* L.) has been in the Middle East and North Africa as a staple food for thousands of years. In Tunisia, dates represent the main resource of oases and play a major role in the development of the national economy (Haouel et al., 2010). The Deglet Noor cultivar is the most popular native date variety because of its large size, texture, and particular taste and color. The world's production of dates has increased from 2.8 million in 1985 to 7.2 million tons in 2011 (FAOSTAT, 2013). Nevertheless, dates are subjected to many diseases and pests that decrease their yield and deteriorate their quality around the world. Dates are very often affected by insect infestation from coleopterans, lepidopterans and hymenopterans, as well as by pathogenic bacteria (such as *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*), and by several mold and yeast genera (Jemni et al., 2014). In North Africa, and especially in Tunisia and Algeria, one of the most economically important pest species is the carob moth (Lepidoptera: Pyralidae), *Ectomyelois ceratoniae* (Zeller), which infests 20% of the harvestable crop annually, causing great economics losses (Dhouibi, 2013). It vastly decreases the marketable quality of dates and compromises exports (Zouba et al., 2009).

Dates destined for export undergo a process of conditioning to preserve and improve their quality (Haouel et al., 2010). Chemical control using fumigation is the most economical postharvest treatment tool for managing stored-product pests; indeed, methyl bromide is the most widely-used fumigant used on stored dates in Tunisia and in several other countries (Zare et al., 2002). Nevertheless, the use of this fumigant is being phased out as it is considered harmful to human health as well as for the environment. Due to this the Montreal Protocol of the United Nations Environment Program on ozone-depleting substances (UNEP, 1995), called for its worldwide withdrawal in 2005 in developed countries, and in 2015, its withdrawal in developing countries (MBTOC, 1998) will be a requirement. Because of this, the replacement of this product has become urgent, as its use will not be allowed under any circumstance.

In recent years, several methods have been investigated as alternatives to methyl bromide fumigation in a number of commodities and pests. These include new techniques that are aimed for the avoidance of postharvest pests and diseases, as well as microbial contamination of dates, and to improve the quality of the product during the storage period. Traditional and new postharvest techniques that have been developed for date fruit include irradiation (Azelmat et al., 2006), microwave (Zouba et al., 2009; Abo-El-Saad et al., 2011), essential oils (Haouel et al., 2010), ozone (Abo-El-Saad et al., 2011, Jemni et al., 2014), vacuum and modified atmosphere packaging (Achour et al., 2003), heat treatments (Ben-Lalli et al., 2013), etc. There has been a growing interest in the application of postharvest heat treatments for controlling pests, insects and microbial agents, as a secure and non-chemical method to control postharvest diseases (Lurie, 1998; Spadoni et al., 2013; Strano et al., 2014; Zhou et al., 2014). Heat treatments can be applied through hot water dips or treatments (HWT), vapor heat or hot dry air. Heat treatment technologies are currently a relatively simple, non-chemical alternative to methyl bromide that can kill quarantine pests (insects and fungi) in perishable commodities, as well as control some postharvest diseases (U.S. EPA, 1996). Furthermore, heat has been approved as a quarantine treatment by the U.S, Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) against pests for several perishable commodities. HWT by immersion consists of submerging the commodity in a hot-water bath at a specific temperature for a specified period of time, which is based on the commodity being treated and the pests that may be present (APHIS, 1993). This type of HWT is shorter in duration and a more efficient heat transfer medium than hot air, and when properly circulated through a load of fruit, a more uniform temperature profile is established (Shellie and Mangan, 1994). However, there have been problems with heat damage and maintenance of pulp temperatures that make HWT problematic (Mulas and Schirra, 2007).

HWT have been effective in controlling *Epiphyas postvittan*a and *Pseudococcus longispinus* on Fuyu persimmons (Lester et al., 1995; Lay-Yee et al., 1997), *Ceratitis capitata* in mango fruit (Jacobi et al., 2001) and *Cydia pomonella* in sweet cherries, maintaining overall acceptable fruit quality (Feng et al., 2004). The mean lethal time (LT) values (estimated treatment x mortality) was dependent on the type of insect and fruit.

Therefore, the aim of the current work was to find the proper HWT (dose and time) that causes *Ectomyelois ceratoniae* mortality while avoiding a loss of marketable quality in Deglet Noor palm date fruit, by studying their effect during cold storage and after a retail sale period.

2. Material and methods

Chemicals

Ultrapure water was obtained from a Milli-Q system (Academic Gradient A10, Millipak™ 40, Millipore, París, France). Sodium hydroxide and methanol (HPLC grade) were purchased from Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain). Individual amino acids and sugars, hydrochloric acid (minimum 37%), sodium chloride, methanol, sodium sulfate, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), gallic acid (3,4,5-trihydroxybenzoic acid), and Folin–Ciocalteu's phenol reagent were purchased from Sigma–Aldrich Química S.A. (Madrid, Spain). Peptone water, plate count and rose Bengal were from Scharlau (Barcelona, Spain).

2.1. Experiment 1: *Ectomyelois ceratoniae* mortality under different HWT treatments

2.1.1. Plant material

Naturally-infested dates (Deglet Noor cv.) of date palm (*Phoenix dactylifera* L.) were collected, at the beginning of November, from an experimental palm orchard (33° 55′ 0″ North, 8° 8′ 0″ East), belonging to the National Institute of Agronomy of Tunisia located in Tozeur (South west, Tunisia). Naturally-infested dates are characterized by the presence of silk closing the calyx. These fruits, at the fully mature 'Tamar' stage, were carefully collected by professional entomologists from the Department of Plant Protection (National Institute of Agronomy of Tunisia).

About 50 kg of infested dates, attached to a small portion of spikelet, were placed in polystyrene boxes and transported about 500 km by car to Tunis, then by plane to Madrid (Spain), and again by car about 400 km to the Pilot Plant of the

Technical University of Cartagena. Total transport duration was about 7 d, at 8 °C. After arrival, fruits were maintained at room temperature (22 °C) and infested dates were manually detached from the spikelets and grouped according to heat dipping treatments. The fruit moisture content was found to be 25%. It is important to measure this parameter, as it influences the speed of heat transfer, with the heating being much faster when moisture content is high (Zouba et al., 2009).

These HWT treatments were performed by placing the fruits in a drilled stainless steel container and dipping it into a 50 L thermostatic water bath, with time and temperature control, continuous stirring and water recirculation. To confirm exact temperature, a digital thermometer was used to control water bath temperature and another was inserted in the center of one date fruit (Fig. 1) for the duration of the heating process. The HWT applied were based on previous experiments that were effective in controlling *E. postvittan*a and *P. longispinus* on Fuyu persimmons, with LT_{99} mean values (estimated treatment times for 99% insect mortality) ranging from 31.3 to 66.9 min, respectively, when 44 °C were used. However, that time was reduced to 8.1 min for *E. postvittana* and 13.3 min *for P. longispinus* when the temperature increased to 54 °C (Lester et al., 1995; Lay-Yee et al., 1997). In sweet cherries, HWT provided 100% *Cydia pomonella* larvae mortality using 50 °C for 10 min and 54 °C for 6 min (Feng et al., 2004). According to this information, to study larvae *E. ceratoniae* mortality in date palm, three temperatures were chosen for HWT: 50 °C, 55 °C and 60 °C with durations ranging from 0 to 10 min. The full experiment was repeated three times, each one constituting a repetition.

2.1.2. Insect mortality

To calculate *E. ceratoniae* mortality due to HWT treatments, three technical replicates per treatment were performed using 22 naturally-infested dates in each replicate. Live and dead insects (larvae) were manually counted and the total numbers of live and dead insects were used to calculate the percentage of mortality.

2.2. Experiment 2: Date palm overall quality using the HWT that caused *Ectomyelois ceratoniae* mortality

2.2.1. Plant material

Date fruits, Deglet Noor cv., were harvested in the same date than experiment 1, at the fully mature ‘Tamar’ stage from a commercial farm located in an Oasis in southern Tunisia (Tozeur). Professional pickers detached the date bunches from the head of the tree palm and these were carefully placed on the ground by hand to avoid crushing and the abscission of dates. The bunch was then cut into spikelets and placed in boxes. About 50 kg of spikelets were placed in polystyrene boxes and transported as mentioned in section 2.1.1. After arrival, visual selection was performed; dates were manually detached from the spikelets and inspected, removing damaged dates. The average weight was 12.1 ± 1.5 g, length 44.91 ± 0.52 mm and thickness 21.91 ± 0.48 mm. The fruit moisture content was 25%. The selected raw materials were then classified into four uniform groups, each group corresponding to a specific treatment. According to the results of experiment 1, three HWT were applied, 50 °C for 10 min, 55 °C for 5 min, and 60 °C for 3 min. As the control, untreated (not dipped into hot water) date palm were used. The HWT treatments were performed as explained in experiment 1. Before packaging and to avoid loss of quality, samples from HWT were water-cooled until the fruit’s internal temperature reached 15 ± 2 °C.

After, 180 g of samples (≈15 date fruits) were placed in 1L polypropylene trays (Barket, Befor Model, Chassieu, France), heat border sealed with a 35µm-thick micro-perforated oriented polypropylene film (OPP) (Danisco Flexible, Bristol, UK), and kept for 30 d at 2 °C. This micro-perforated plastic was used to avoid the effects of modified atmosphere packaging (reduced O_2 and enriched CO_2) and to focus on the HWT effects. On day 0, 15 and 30 of storage at 2 °C plus a retail period of 4 d at 25 °C, three replicates from each treatment were evaluated. This cold storage temperature is usually used to prevent possible insect feeding damage and to control insect infestation (Kader and Awad, 2009). The commonly-used temperatures in commercial distribution and export in Tunisia are between 2 °C and 4 °C at 65-75% relative humidity (GI-Fruit 2009).

2.2.2. Quality parameters

2.2.2.1. Physical measurements

Color: The surface color of the date peel was determined on three randomized sides of 5 dates from each repetition. A color-difference meter (Minolta CR 300, Ramsey, NJ, USA) was used (C standard C.I.E. illumination, 0° viewing), and the results were expressed as CIE L*a*b* color space units. L* defines the lightness and a* and b* define the red-greenness and blue-yellowness, respectively. The color was also expressed as hue angle ($h° = \text{arctangent } [(b^*)\cdot(a^*)^{-1}]$) and chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$).

Firmness: This was determined on the two flat sides of each date piece by means of a texturometer (Ibertest, Madrid, Spain) equipped with a 4.0 mm in diameter probe with a travel distance and time of 30 mm min^{-1} and with a depth of 3 mm. At each sampling day, the firmness of 5 dates from each replicate was monitored. Firmness was expressed in Newtons (N).

2.2.2.2. Chemical measurements

Pitted date fruits were flash frozen in liquid nitrogen and stored at -80 °C for a maximum of two months, ground to a fine powder in a Cryomill in liquid nitrogen for use in chemical determination.

Main free amino acids: One gram of powdered date tissue was mixed with 6 mL of ultrapure water and homogenized for 1 min with a vortex. The mixture was then centrifuged at 3,000 x *g* for 10 min at 4 °C (Heraeus Fresco 21, Thermo Scientific, Germany) and filtered (0.45µm). Free amino acid analysis was performed as reported by Özcan and Şenyuva (2006), by HPLC with UV-Vis detector (Water 2695, Photodiode array, wavelength 190 to 320 Alliance, Singapore) and mass detector (Waters Zq 4000 (m/z from 70 to 300). The chromatographic separations were performed on a Luna® C-18 column (100 Å, 5 µm, 30 x 2 mm, Phenomenex) using the isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid at 0.2 mL/min. Main individual amino acids, arginine (Arg), proline (Pro), alanine (Ala), methionine (Met), glutamic (Glu) and aspartic acid (Asp), were quantified using their respective standards and results expressed as mg kg^{-1} of fresh weight.

Composition and concentration of sugars: Sugar composition was determined from the same extract used for amino acid analysis. Glucose, fructose and sucrose contents were analyzed by ion chromatography (IC, Metrohm 871 Advance Bioscan, Herisau, Switzerland) with Pulsed Amperometric Detection (PAD) detector, using an anion exchange column (1–150 Metrosep-Carb) and isocratic conditions. Operating conditions reported by Moraga et al. (2006) for performance liquid chromatography were used, with minor modifications (mobile phase was NaOH 80 mM, at 1 mL/min flow rate). Individual sugars were quantified using their respective standards and results expressed as g 100 g^{-1} of fresh weight.

Antioxidant activity: Two methods were used to evaluated the antioxidant activity: the ferric reducing ability of plasma (FRAP) assay and the free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl, DPPH) assay. For both methods and total phenolic determination, a previous extraction was needed. One g of frozen ground date fruit was mixed with 10 mL methanol-water (1:1), then maintained for 1 h at 200 x *g* in darkness inside a polystyrene box filled with ice. The homogenates were centrifuged at 4 °C for 10 min at 15,000 × g to obtain the extracts.

The reducing activity using the FRAP assay was measured using FRAP reagent which contained 2.5 mL of a 10 mM (2,4,6-tris 2-pyridyl-s-triazine) (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$•$6H_2O$ and 25 mL of 0.3 M acetate buffer pH 3.6. Freshly prepared, FRAP reagent (198 µL) was incubated for 2 h at 37 °C, and mixed with 6 µL of date fruit extract and the reaction mixtures were later incubated in darkness for 40 min at room temperature. Absorbance at 593 nm was read with a multi-scan plate reader (Tecan Infinite M200, Männedorf, Switzerland).

The DPPH assay, which determines the free radical scavenging activity, was measured in the following way: A solution of 0.7 mM DPPH reagent in methanol prepared 2 h before the assay. An aliquot of 17 µL of the samples extracts was added to 198 µL of reagent solution. The mixture was incubated in darkness for 40 min at room temperature. After 40 min incubation at room temperature, the decrease in absorbance at 515 nm was measured, using the above-cited multi-scan plate reader. Calibration curves were made for each assay using ascorbic acid (AA) as standard.

The antioxidant activity was expressed as mg of AA equivalent (AAE) antioxidant activity per 100 g fresh weight of date tissue.

Total phenolic content (TPC): This assay was performed using the Folin–Ciocalteu reagent. Briefly, an aliquot of 17.2 µL extract of the supernatant was mixed with 30 µL of Folin–Ciocalteu reagent (1:10, v/v diluted with MilliQ water) and 193 µL sodium carbonate (20%, w/v). The mixture was incubated for 40 min at room temperature in darkness, measuring the absorption at 750 nm. Total phenols were quantified using gallic acid (GA) as standard and expressed as mg GA equivalents (GAE) per 100 g fresh weight. For each repetition, both antioxidant and phenolic assays were carried out in triplicate.

2.2.2.3. Microbiological analysis

Date fruits were pitted aseptically using sterile forceps and scalpels. Three randomized samples from each treatment containing ten grams of date fruits were aseptically placed in a sterile stomacher bag and mixed with 90 mL of sterile tryptone-hosphate water (pH 7.0) by using a Masticator (Seward Medical, London, UK). Serial dilutions were prepared in tryptone-phosphate water. Plate count agar was used for counting of mesophilic aerobic bacteria, incubated for 48 h at 30 °C. Rose Bengal Agar was used for yeast and mold, incubated at 25 °C for 3 and 7 d, respectively. Microbial counts were expressed as $\log_{10}$ cfu/g (colony forming units per g of sample).

2.2.2.4. Sensory evaluation

Sensory analyses of samples from each sampling day were performed according to Ismail et al. (2001). The panel consisted of eight assessors aged 25-40 years, from the food engineering department at the Polytechnic University of Cartagena, screened for sensory ability (Table 1).

2.2.2.5. Statistical analysis

To determine the effect of HWT and storage time on each dependent variable, a two-way analysis of variance (ANOVA, $p < 0.05$) was carried out (Statgraphic Plus, version 5.1, 2001, Manugistic Inc, Rockville, MD, USA). Mean values were compared using Tukey's test when significant differences among treatments and interactions between factors was found.

3. Results and discussion

3.1. Experiment 1: *Ectomyelois ceratoniae* mortality under different HWT treatments

Fig. 2 shows the thermal susceptibility of *E. ceratoniae* larvae when HWT were used. To obtain 100% *E. ceratoniae* mortality, a lower temperature meant a longer time of application; in contrast, a higher temperature meant a shorter time. One-hundred percent mortality was obtained when using 50 °C for 10 min, 55 °C for 5 min or 60 °C for 3 min. As reported in material and methods, the LT_{99} for *E. postvittana* and *P. longispinus* insects on Fuyu persimmons ranged from 31.3 to 66.9 min, respectively, when 44 °C was used. However, only 8.1 min was needed for *E. postvittana* and 13.3 min for *P. longispinus* when the temperature increased to 54 °C (Lester et al., 1995; Lay-Yee et al., 1997). Feng et al. (2004) reported similar findings for *C. pomonella* larvae on sweet cherries, reaching 100% mortality at 50 °C for 9 min or at 54 °C for 5 min. Along the same lines, Wang et al. (2009) obtained 99.8% mortality of *Amylois transitella* by applying 48 °C for30 min, and when 50 °C was used, the time needed was only 10 min.

As for *E. ceratoniae* larvae mortality, other treatments such as microwaves (1 Kw) for 55-90 s increased internal and external temperatures of date fruits to more than 52 °C, which disinfested 100% of the larvae and eggs (Zouba et al., 2009). Dhouibi (2013) also showed 100% mortality of *E. ceratoniae* larvae using only CO_2 and freezing organic dates, and CO_2 plus phosphine for conventional treatments of dates. In contrast, other novel treatments such as chlorine, UV-C radiation, ozonated water and alkaline and neutral electrolyzed water only obtained between 8 to 15% of mortality (Jemni et al., 2014).

3.2. Experiment 2: Date fruit overall quality using the HWT that caused *Ectomyelois ceratoniae* mortality

Table 2 shows ANOVA results of simple factors as HWT treatments, time of storage and their interaction on date fruits.

3.2.1. Physical parameters

Color: HWT induced a significant reduction in L* and h° values (Table 3). Date fruit color changed from a light brown to a slight dark brown. A similar effect has been found when high temperatures are used in palm dates treated with a combination of HWT (60 °C, few seconds) followed by hot air treatment (Hazbavi et al. 2014), in banana (50 °C 10 min) (Ummarat et al. 2011) or leeks (treated with various combinations of temperatures/times) (Tsouvaltzis et al. 2006). The use of high temperatures (50 to 55 °C) usually increases color darkening in date palm fruits (Kader and Awad, 2009), probably due to the oxidative browning of phenolic compounds and sugar browning, the main factors of darkening using elevated temperatures on palm date (Vandercook et al., 1979). Time of storage, and in particular, retail period also diminished L* and h° data (Table 4). Hazbavi et al. (2014) also reported this storage time effect, and as we have found, the reduction of lightness was higher due to storage time than by the use of HWT. The reduction of h° values probably corresponded to an increase in carotenoid biosynthesis as the fruit ripened, which is associated with the climacteric increase in respiration, initiated by the action of ethylene (Saltveit, 1999). These results are in accordance with Porat et al. (2000) and Kim et al. (2007) who reported that storage time affects fruit color, decreasing h° in HWT-treated grapefruit and mangoes, respectively.

Firmness: This is one of the main quality parameters in sensory acceptance of date fruits by the consumers. Fruit firmness was affected by the interaction of HWT and storage time (Fig. 3). Firmness values tended to decrease without significant differences in most of treated samples. Only in the initial evaluation (day 0), was a significant loss of fruit firmness detected in the 50 °C for 10 min HWT when compared to control (14 N versus 10 N), probably due to the long duration of the treatment. High water temperature could cause disturbances in cell structure that causes membrane damage in fruit samples, decreasing fruit firmness (Wills et al., 1989). Storage time did not promote significant changes in firmness, although Rohrbach et al. (2003) confirmed that fruit structure declines fast with a long storage period in pineapple.

3.2.2. Chemical measurements

Main free amino acid: Amino acids are abundant in palm dates, and twenty-three different amino acids have been found in date fruit, some of which are not present in the most popular fruits such as oranges, apples and bananas (Al-Shahib and Mardhall, 2003). Reynes et al. (1994) indicated that total amino acid levels in palm dates are greatly variable, mainly depending on the type of cultivar, with glutamic and aspartic acids being the ones found in greater concentrations, which can be superior to the content of essential amino acids, as we also found. In our experiment, statistical analysis showed that the contents of amino acids were not at all affected by HWT (Table 2). In our opinion, this fact means that the temperature used was not significantly high to promote heat degradation/denaturation or even the Maillard reaction, a chemical reaction between amino acids and reducing sugars that usually reduces the content of free amino acids (Chichester, 1986). According to the results of the amino acid changes by storage time, the concentrations of alanine, aspartic acid and proline increased slightly during cold storage when compared to the initial evaluation (Table 6). Similar results were observed by Jiang et al. (2011), who showed that low temperatures, in particular, close to the freezing point, can increase amino acid content in litchi fruit. When samples were kept under retail period conditions of 23 °C, the content of those amino acids started to decrease. In contrast to this trend, arginine, glutamic acid and methionine remained stable throughout the experiment. Kazami and al. (1991) also found that broccoli heads dipped in 45 °C water for 14 min did exhibit changes in soluble protein or free amino acid content during storage.

Sugar concentration: The concentration of sugars in palm date change with ripening stage, firmness and water content. The individual concentration levels of glucose, fructose and sucrose, as well as total sugars, are shown on Table 5. Sucrose was the predominant sugar in all samples, followed by fructose and glucose, with approximately similar proportions, which is characteristic of the Deglet Noor cultivar and one of the main reasons behind the very pleasant taste of the fruit (Mrabet et al., 2008). The interaction between storage time and HWT had a significant effect on sucrose, but only on day 0 was a significant loss of sucrose content observed on samples treated with 60 °C for 3 min compared to control (23.98 versus 35.09 g 100 g^{-1}). In the rest of the treatments, no significant changes were observed, although a slight but non-significant decrease was observed during the storage period. Glucose and fructose had the same behavior throughout the experiment. HWT did not affect glucose and fructose concentrations (Table 5). In contrast, Cruz et al. (2012) reported that HWT applied to mangoes at 46 °C for 90 min increased glucose and fructose content. Mirdehghan et al. (2006) also reported a significant increase of glucose and fructose content in pomegranate arils treated with HWT and added that the increase of sugar concentrations due to heat treatments could be attributed to the increase in glucosidase, galactosidase, and arabinose activities, which would release sugars from cell wall polymers. During cold storage, the concentrations of both monosaccharides were maintained without significant changes, but increased significantly at the end of storage, during the retail period, reaching 13.2 g 100 g^{-1} for glucose and 12.0 g 100 g^{-1} for fructose. These results, along with the decrease of sucrose content mentioned above, are in agreement with the findings of Ismail et al. (2008), who showed an increase of glucose and fructose content, obtained alongside a slight decrease of sucrose content on two cultivars of palm date during 2 and 12 months of storage. This is probably due to invertase activity, which, although slowed down by low temperatures, can bring about the slow conversion of sucrose to glucose and fructose (Zare et al., 2002). Also, cellular respiration could use monosaccharides as the main respiratory substrate (Jemni et al., 2014), particularly at high temperatures, such as found in the retail sale period. Therefore, the differences in total sugar content found, as well as the decreasing tendency of sucrose content could be justified by the hydrolysis of sucrose and/or respiration, or a combination of both (Ismail et al., 2008). Total sugars was only affected by HWT treatments, tending to decrease in all treated samples compared to control, with significant differences in samples treated with 55 °C and 60 °C water (Table 5).

Antioxidant activity: Palm date fruit constitutes a natural source of antioxidants that may prevent many diseases and could potentially be used in food and nutraceutical formulations (Saafi et al., 2009). In our experiment, FRAP values were strongly affected by HWT (Table 3). Immediately after hot water dipping, FRAP values significantly decreased between 10 to 15%, with differences between control (74 mg AAE 100 g^{-1}) and HWT treatments (66 to 63 mg AAE 100 g^{-1}). The decrease was more pronounced when the highest temperature (60 °C) was used although without significant differences among HWT. Natural antioxidants present in the date fruit (mainly ascorbic acid) could undergo thermal degradation. Similar results were found by Shen et al. (2013), who confirmed that higher temperatures might have a negative effect on antioxidant activity in treated HWT mandarins. However, Schirra et al. (2011) reported that antioxidant activity in oranges (*Fortunella japonica* Lour. Swingle, cv. Ovale) was unaffected by HWT although a slight reduction was found. FRAP values were also affected by storage time, decreasing significantly after 30 d of cold storage and retail period (70 to 64 mg AAE 100 g^{-1}) (Table 4). In the same line with our results, Al-Najada and Mohamed (2014) reported that antioxidant activity measured by FRAP and DPPH assays, decreased enormously on two cultivars of Saudi palm dates, Khalas and Shishi, when stored for 12 months at 4 °C. A similar response was found recently on Bahree dates stored for 50 d at 1 °C (Mohamed et al. 2014), with a significant loss on the antioxidant activity as measured by DPPH and FRAP methods during cold storage. Results on DPPH showed the same trend as FRAP, with both considerably affected by HWT. The initial antioxidant activity for untreated date fruit was 62.7 mg AAE 100 g^{-1} with a decrease of 17 to 22% depending on the HWT treatment (Table 3). As with FRAP, storage time had decreased the antioxidant activity from 57 mg AAE 100 g^{-1} during the first night to 50 mg AAE 100 g^{-1}, obtained at the end of the cold storage and retail period (Table 4).

Comparing both antioxidant activity assays, FRAP results were 1.2 to 1.3-fold higher than the value obtained by the DPPH assay as is usually found in fruits (Aguayo et al. 2010). The antioxidant activity measured here was almost double than those reported by Jemni et al. (2014) even using the same Deglet Noor cultivar. The differences in antioxidant activities are due to differences in cultivar, growing conditions, maturity, season, geographic origin, fertilizer, soil type, storage conditions, and amount of sunlight received (Al-Farsi et al. 2007). According to our results, date fruits are a good source of antioxidants as compared to other fruits, as the antioxidant activity was about 75% of fresh apple (Aguayo et al., 2010). Presumably, that less antioxidant activity was due to a high proportion of sugar in date fruits (Hsu et al., 2006).

Total Phenolic Content: The average phenolic content was 84 mg GAE 100 g^{-1}. Chaira et al. (2009) reported values for different Tunisian cultivars ranging from 3.57 ± 1.11 to 31.82 ± 1.29 mg GAE 100 g^{-1} fw. However, Besbes et al. (2004) showed higher values in Deglet Noor fruit (493.5 mg GAE 100 g^{-1} fw). This large difference depended on date cultivars and storage duration. In our experiment, HWT significantly reduced the TPC from 90 mg GAE 100 g^{-1} (control) to 82 to 78 mg GAE 100 g^{-1} (HWT samples). These losses ranged from 9 to 14% depending on the HWT, with 60 °C for 3 min being the one which provoked a higher reduction (Table 3). According to our results, Siddiq et al. (2013) also found that changes on TPC on fresh cut onions depended on dip temperature and that a 70 °C treatment resulted in a significant loss on TPC, whereas a significant increase was observed with 50º and 60 °C treatments. In contrast, Bassal and El-Hamahmy (2011) found that HWT enhanced the concentration of free phenols in Navel and Valencia late orange fruit during storage, which was associated with reduced chilling injury symptoms. Storage time did not significantly change the TPC except for a slight but non-significant increase with longer storage time (data not shown). Several authors have reported a significant increase of TPC with storage time in palm date fruit (Biglari et al., 2009; Al-Najada and Mohamed, 2014).

3.2.3. Microbiological analysis

In the present study, the initial microbial counts for untreated samples were 2.88 ± 0.02 log cfu g^{-1} for mesophilic, 3.48 ± 0.22 log cfu g^{-1} for yeast and 2.52 ± 0.06 log cfu g^{-1} for molds (Table 7). Control samples maintained this microbial count with a slight increased during storage time. However, immediately after HWT applied, the microbial counts were significantly lowered below detection limits (<1 log cfu g^{-1} for mesophilic and <2 log cfu g^{-1} for yeasts and molds) with this level maintained throughout the storage period. In our opinion, this sanitizer effect was obtained through the cleansing effect of hot water. The significant effect of HWT on the reduction of microbiological counts was in agreement with other authors, who have confirmed that HWT, with or without fungicides, were capable of significantly reducing microbial growth on whole and fresh-cut product (Karabulut et al., 2002; Zong et al., 2010; Schirra et al. 2011; Spadoni et al., 2013; Ansorena et al., 2014; Strano et al., 2014; Zhou et al., 2014). The treatments have also improved storage quality (Porat et al., 2000).

3.2.4. Sensorial analysis

Date fruits showed no significant changes in color and texture (data no shown) mainly because heat did not cause any superficial damage. Only storage time had an effect on these sensorial parameters, with color and texture scores decreasing when storage time was extended, with significant differences found between evaluation at day 0 and the final retail period storage time, 4.05 versus 3.57 for color and 3.83 versus 3.35 for texture, but always above the limit of marketability. In this sense, Spadoni et al. (2013) reported that no significant effects were induced by HWT on sensory parameters of peach and only cold storage affected these parameters. Minor changes in taste were found, where the interaction time x temperature had a significant effect, and only samples treated with 60 °C for 3 min showed remarkable differences in their taste, 4.60 (day 0) versus 3.20 (after retail period), without off-flavor detection. Overall acceptability of palm date was affected by HWT (Table 3), where fruits treated with 60 °C for 3 min had a reduced overall acceptability, decreasing from 4.15 (untreated samples) to 3.65. Storage time also affected overall acceptability, decreasing when time of storage was extended, with a significant loss at the end of the retail period (4.35 at day 0 versus 3.69) (Table 4). In general, all sensorial parameters suffered a reduction at the end of the retail period but were always above the limit of marketability (data not shown). Similar results were found by Ismael et al. (2001; 2008) and Kulkarni et al. (2008) who indicated that storage conditions (duration, temperature and relative humidity) were the main factors for maintaining sensory quality of dates.

4. Conclusions

The use of the HWT of 50 °C for 10 min, 55 °C for 5 min and 60 °C for 3 min, in laboratory conditions, lead to 100% *E. ceratoniae* larvae mortality in date fruit and it can be used as an alternative to chemical treatments. The treatments did not cause any heat damage and kept the fruits at optimal quality, although with a reduction in color parameters and bioactive compounds when dates were stored for 30 d at 2 °C plus 4 d at 23 °C. For industrial implementation, parameters such as water quality, date fruit moisture and reduced thermal differences between hot water and fruit has to be investigated.

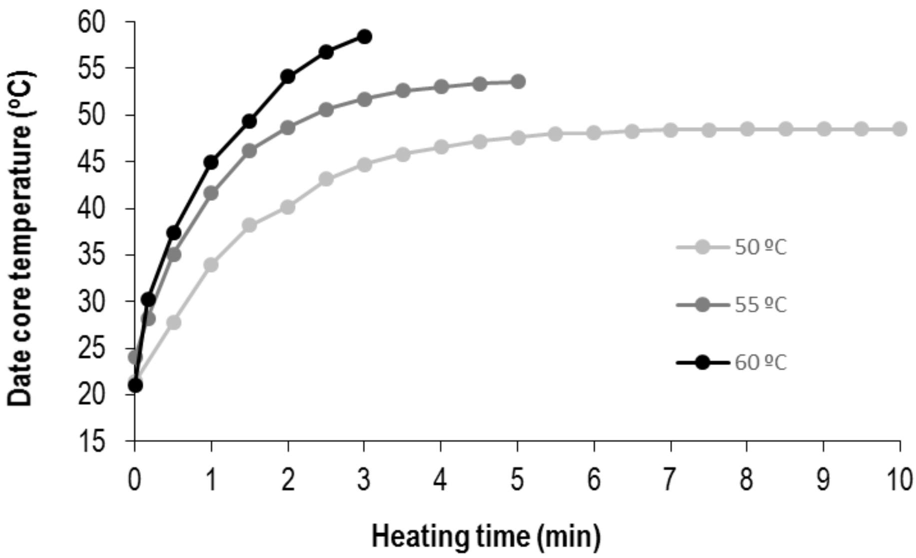

Figure 1. Temperature mapping of the date core under hot water treatments

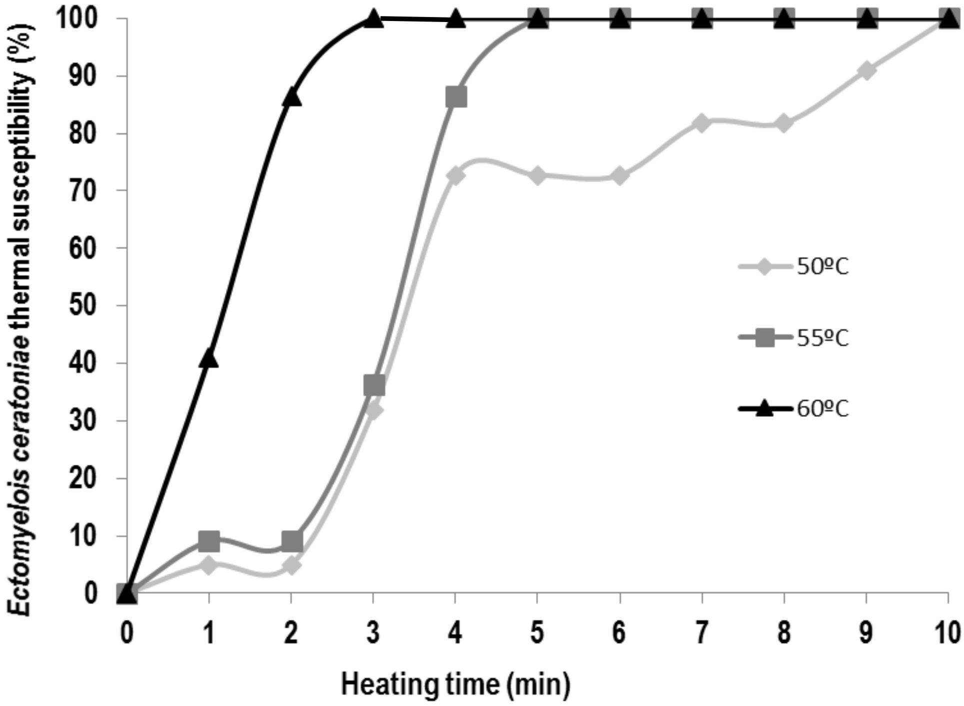

Figure 2. *Ectomyelois ceratonieae* larvae thermal susceptibility (%) in date fruit treated with three different hot water treatments. Data are the minimum thermal susceptibility obtained in 3 replicates using 22 naturally infested dates in each replicate.

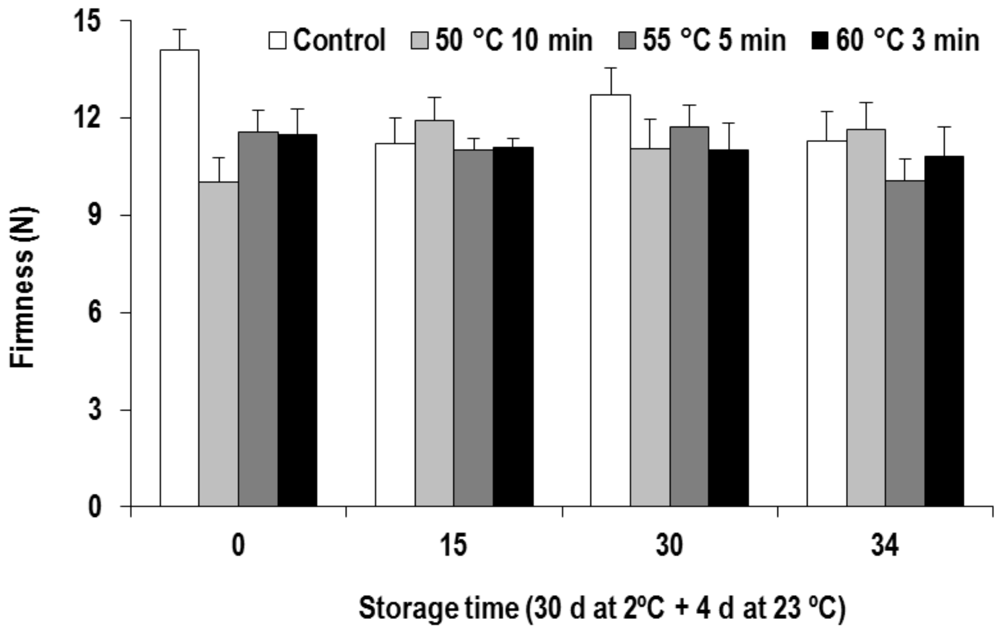

Figure 3: Effect of hot water treatments (HWT) and storage time (30 d at 2 °C plus 4 d at 23 °C) on firmness (N). Data are the means of 3 replicates ± SE. Analysis of variance showed HWT x storage time as significant factors. Tukey's value ($p \leq 0.05$) = 3.70.

Table 1. Guide of sensory evaluation for date fruits.

	1	2	3	4	5
Color	Very dark/Very yellow	Dark brown/ Yellowish brown	No uniform brown	Caramelized brown	Translucent brown uniform and bright color
Texture	Very soft, deformed and glued/very firm and wrinkled	Quite soft, skin separated from pulp and sticky	Soft but keeps its shape	Uniform firmness	Firmness uniform with its original shape when gently pressed with your fingers, not too dry, not too sticky
Flavor	High acidity/High fermentation/Bitterness	Slightly acid/fermented, strange taste	Sweet just enough without foreign flavors	Sufficiently sweet	High perception of sweetness
Overall quality	Extremely poor	Poor	Acceptable and limit of usability	Good	Excellent

Table 2. Statistical analysis of quality parameters according to the simple factors as hot water treatments (HWT: control, 50 °C 10 min, 55 °C 5 min, 60 °C 3 min), storage time (ST: 30 d at 2 °C plus 4 d at 23 °C) and their interaction for Deglet Noor date fruits through analysis of variance.

Quality parameters	HWT	ST	HWT x ST
L*	***	***	NS
h°	***	**	NS
Firmness	**	**	*
Alanine	NS	***	NS
Arginine	NS	NS	NS
Aspartic acid	NS	*	NS
Glutamic acid	NS	NS	NS
Methionine	NS	NS	NS
Proline	NS	***	NS
Glucose	NS	*	NS
Fructose	NS	***	NS
Sucrose	***	NS	*
Total sugars	*	NS	NS
FRAP	***	***	NS
DPPH	***	***	NS
Total phenolic content	***	NS	NS
Overall quality	*	***	NS

NS: not significant difference at $P > 0.05$ and *, **, *** significant difference at $P < 0.05$; 0.01 and 0.001, respectively.

Table 3. Effect of hot water treatments on physico-chemical parameters and overall quality of Deglet Noor date fruits.

Treatments	**L***	**h°**	**FRAP (mg AAE 100 g^{-1})**	**DPPH (mg AAE 100 g^{-1})**	**TPC (mg GAE 100 g^{-1})**	**OQ (1 to 5)**
Control	36.26^{z} a	62.79 a	73.83 a	62.67 a	90.09 a	3.85 ab
50 °C 10 min	34.82 b	60.71 b	65.95 b	51.92 b	82.17 b	3.78 ab
55 °C 5 min	35.18 b	61.91 ab	65.75 b	48.80 b	80.18 b	4.15 a
60 °C 3 min	34.82 b	60.11 b	62.51 b	48.54 b	78.26 b	3.65 b

^{z} Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to Tukey's test. TPC: Total phenolic content. OQ: Overall quality.

Table 4. Effect of storage time (30 d at 2 °C plus 4 d at 23 °C) on physico-chemical parameters and overall quality of Deglet Noor date fruits.

Days of storage	**L***	**h°**	**FRAP (mg AAE 100 g^{-1})**	**DPPH (mg AAE 100 g^{-1})**	**OQ (1 to 5)**
0 d	36.85^{z} a	62.97 a	69.68 a	57.05 a	4.35 a
15 d	35.28 b	62.13 ab	70.19 a	54.31 a	3.93 b
30 d	34.97 b	60.63 bc	63.78 b	50.63 b	3.53 b
30 d + 4 d	33.98 c	59.80 c	64.39 b	49.94 b	3.63 b

^{z} Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to Tukey's test. OQ: Overall quality.

Table 5. Effect of hot water treatments (HWT) and storage time (30 d at 2 °C plus 4 d at 23 °C) on sugar content (g 100 g^{-1}) of Deglet Noor date fruits.

Storage time	HWT	Sucrose	Glucose	Fructose	Total sugar
0 d	**Control**	35.09[z]	12.27	10.19	57.55
	50 °C 10 min	29.82	11.13	9.09	50.05
	55 °C 5 min	32.51	10.95	8.51	51.96
	60 °C 3 min	23.98	13.33	10.54	47.85
15 d	**Control**	32.33	10.43	8.76	51.52
	50 °C 10 min	30.14	12.15	10.19	52.49
	55 °C 5 min	30.78	11.23	9.34	51.35
	60 °C 3 min	27.39	12.02	9.22	48.62
30 d	**Control**	30.93	11.29	9.21	51.43
	50 °C 10 min	24.11	13.10	11.62	48.82
	55 °C 5 min	30.17	12.19	10.42	52.78
	60 °C 3 min	31.23	11.29	10.16	52.68
30 d + 4 d	**Control**	32.19	12.94	11.96	57.09
	50 °C 10 min	26.28	12.80	11.76	50.85
	55 °C 5 min	26.52	14.17	12.91	53.60
	60 °C 3 min	30.62	12.72	11.32	54.66
Storage time		NS	(1.81)	(1.13)	NS
HWT		(2.79)	NS	NS	(3.37)
Storage time x HWT		(8.54)	NS	NS	NS

[z]Data are the means of 3 replicates. NS: not significant difference. Tukey's values are in brackets at $p \leq 0.05$

Table 6. Effect of storage time (30 d at 2 °C plus 4 d at 23 °C) on free amino acids (mg kg^{-1}) of Deglet Noor dates.

Days of storage	Alanine	Arginine	Aspartic acid	Glutamic acid	Methionine	Proline
0 d	2.16 c	3.65 NS	6.91 b	8.08 NS	10.03 NS	6.66 c
15 d	2.47 bc	3.80	7.32 a	7.92	10.03	8.30 b
30 d	3.20 a	3.81	7.40 a	8.10	10.07	10.60 a
30 d + 4 d	2.78 ab	3.93	7.20 ab	7.85	9.97	9.64 a

[z]Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to Tukey's test. NS: non-significant difference.

Table 7. Microbiological counts (log cfu g^{-1}) of unheated samples (control) of Deglet Noor date fruits when stored for 30 d at 2 °C plus 4 d at 23 °C.

Days of storage	Mesophilic	Yeasts	Molds
0 d	2.52 ± 0.06[z]	3.48 ± 0.22	3.88 ± 0.11
15 d	2.78 ± 0.09	3.70 ± 0.04	3.99 ± 0.05
30 d	2.74 ± 0.11	3.74 ± 0.08	4.01 ± 0.50
30 d + 4 d	2.88 ± 0.02	3.88 ± 0.52	4.13 ± 0.08

[z]Data are the means of 3 replicates ± ES. Microbial counts for all HWT and each sampling day was <1 log cfu g^{-1} for mesophilic and < 2 log cfu g^{-1} for yeasts and molds.

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CHAPTER III:

Effect of hot air on Deglet Noor palm quality parameters and on *Ectomyelois ceratoniae*.

CHAPTER III

Ben Amor, R., Miguel-Gómez, M.D., Martínez-Sánchez, A., Aguayo, E., 2016. Effect of hot air on Deglet Noor palm quality parameters and on *Ectomyelois ceratoniae*. Journal of Stored Products, DOI: 10.1016/j.jspr.2016.03.001.

ABSTRACT

Insect infestation caused by *Ectomyelois ceratoniae* or carob moth is one of the main postharvest problems that lead to a decrease of marketable quality of dates. The control of carob moth is a mandatory process for exported fruits, and the main chemical method used to prevent pest diseases of palm date is methyl bromide. However, its use is being restricted due to direct, harmful effects to the environment, and indirect effects on humans. Hot air treatments (HATs) could be physical alternatives to methyl bromide and other chemicals. Three HATs (55 °C for 30 min, 60 °C for 15 min, 60 °C for 20 min) were studied on Deglet Noor date fruits when stored for 45 d at 2 °C followed by a retail period of 4 d at 23 °C. The results showed that the use of HATs led to 100% *E. ceratoniae* larvae mortality in naturally-infested date. These HATs did not cause any damage on fruit quality and reduced mesophilic bacterial counts (< 1 log CFU g^{-1}). These HATs, especially the combination of highest temperature and longer time of application (60 °C for 20 min), decreased the antioxidant activity (25% in DPPH and 14% in FRAP assay). Storage time also had a significant impact, reducing color (h°), antioxidant activity, and total phenolic content of date fruits (39%). When this storage was accompanied by a retail period at 23 °C, the firmness and sensorial parameters of date fruits was significantly reduced. Nevertheless, all date fruits from HATs and control treatments maintained their marketability quality for 45 d at 2 °C followed by a retail period of 4 d at 23 °C. Results show that HATs are a physical, non-chemical, treatment for *E. ceratoniae* larvae mortality that maintained date fruits' postharvest quality.

Keywords: Heat treatment; carob moth; non-chemical treatment; amino acids; sugar content; microbial counts.

1. Introduction

The date palm (*Phoenix dactylifera* L.) is considered a symbol of life in the desert because it tolerates high temperatures, drought and salinity (Haouel et al., 2010). In 2011, 7 million tons of dates were grown worldwide, of which 2.5% corresponded to Tunisia, where the date sector constitutes around 4% of its agricultural production and 12% of its agricultural exports (GI Fruit, 2009). Tunisia is considered a leader in the production and export of Deglet Noor dates, which have specific organoleptic characteristics, with over 85% of exports (GI Fruit, 2009). This cultivar is the most marketed in Europe, with 90% of the dates imported being of this variety and about 80% of these imports coming from Tunisia (FAOSTAT, 2013). However, the carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), is the most important insect pest both in the field and in storage, causing great economic losses and yearly infestation on about 20% of dates in Tunisia (Zouba et al., 2009; Jemni et al., 2014). It greatly depreciates the commercial quality of dates and increases the risks that negatively affect exports, which constitute an integral part of the agriculture-based economy of Tunisia. Postharvest methyl bromide fumigation effectively controls *E. ceratoniae*, however, the Montreal Protocol of the United Nations Environment Program (UNEP, 1995) has recommended the phasing out of methyl bromide by 2005 in developed countries and by 2015 in developing countries (MBTOC, 1998). Therefore, alternative treatments are needed.

In the past few years, several methods have been investigated for replacing methyl bromide fumigation as a treatment for postharvest commodities. For avoiding postharvest pest diseases and microbial attacks of dates and to maintain the quality during storage, traditional and new postharvest tools have been developed for date fruits such as irradiation (Azelmat et al., 2006), microwave (Zouba et al., 2009; Abo-El-Saad et al., 2011), ozone (O_3) (Abo-El-Saad et al., 2011), heat treatments (Ben-Lalli et al., 2013), etc. Heat treatments are an interesting postharvest technology for the control of pests and insects, as it can replace the use of chemicals (Lurie, 1998). Heat treatments can be provided through hot water dips, vapor heat or hot dry air, but an optimal combination of time and temperature is necessary to provide the desired control, without significant losses in the quality of the product (Finkelman et al., 2006; Ben-Amor et al., 2016).

Previous works performed in date fruits using hot air treatments (HATs) have shown that an exposure at 50 °C for 25 to 60 min has been effective in adults of *Carpophilus hemipterus* L. (Al-Azawi et al., 1984) and applying 50 to 55 °C for 2 h has been effective with the larval stage (Finkelman et al., 2006) to achieve total mortality. Others works have described different temperatures and different lengths of time for application depending on the type and stage of the insect (Barreveld, 1993; Navarro, 2006). Thus, a HAT of 60 °C for 33 min has been efficient with all life stages of the *Cadra cautella* insect in Iraq date palm (Al-Azawi et al., 1983). Several cultivars of dates palm do not tolerate high temperatures, for example, the Madjool cultivar tolerates moderate temperatures between 45 °C and 55 °C (Finkelman et al., 2006). Although

HATs could be a physical alternative to chemicals, studies on dates are still scarce, with only a few varieties of dates and specific insects being studied (Barreveld, 1993).

Therefore, the aim of the current work was to focus on the application of three HAT to later assess their effects on the physico-chemical, sensory and microbiological parameters of Deglet Noor Tunisian date fruits, as a possible treatment to control carob moth, *Ectomyelois ceratoniae* during the cold storage retail sale period.

2. Material and methods

2.1. Chemical and plant material

To develop this study, the following chemical products were used. Ultrapure water was obtained from a Milli-Q system (Academic Gradient A10, Millipak™ 40, Millipore, París, France). Sodium hydroxide and methanol (HPLC grade) were purchased from Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain). Individual amino acids and sugars, hydrochloric acid (minimum 37%), sodium chloride, methanol, sodium sulfate, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine), gallic acid (3,4,5-trihydroxybenzoic acid), and Folin–Ciocalteu’s phenol reagent were purchased from Sigma–Aldrich Química S.A. (Madrid, Spain). Peptone water, plate count and rose bengal were purchased from Scharlau (Barcelona, Spain).

For plant material, two groups of date fruits were harvested. The first group, naturally-infested dates (Deglet Noor cv.) of date palm was used for HAT mortality study. Those naturally-infested dates by *E. ceratoniae* insect at larval state and were characterized by the presence of silk closing the calyx. The second group of date fruits was used for the HAT quality study, and came from a commercial farm. Naturally-infested and commercial date fruits were harvested in Tozeur (South west, Tunisia), in November, at the fully mature ‘Tamar’ (dark-brown and dry). Professional pickers detached the date bunches from the head of the palm tree and these were carefully placed on the ground by hand to avoid crushing and the abscission of dates. The bunch was then cut into spikelets and placed in boxes.

About 50 kg of spikelets from each group were placed in polystyrene boxes and transported about 500 km by car to Tunis, then by plane to Madrid (Spain), and again by car about 400 km to the Pilot Plant of the Technical University of Cartagena. Total transport duration was about 7 d at 8 °C. The average weight was 12.1 ± 1.5 g, length 44.91 ± 0.52 mm and thickness 21.91 ± 0.48 mm. The fruit moisture content was found to be 25%. After arrival, fruits were maintained at room temperature (22 °C) and a visual selection was performed, removing damaged dates and carefully extracting the fruit from their branch before treatment.

2.2. HAT treatments

HATs were performed inside a hot air chamber (2.5 m/s), by randomly placing the naturally-infested date fruits for the insect mortality study or the non-infested fruits for the quality study, in a drilled stainless container with hot air flow, control of time, temperature and relative humidity (RH) (COPLAN, Material Science Teaching, Madrid, Spain). For each insect mortality or quality study, three HATs were applied: 55 °C for 30 min, 60 °C for 15 min, and 60 °C for 20 min, untreated air date palm was used as control. After HATs, samples were air-cooled until the fruit's internal temperature reached 15 ± 2 °C.

To confirm exact temperature, a digital thermometer was used to control air chamber temperature and another was inserted in the center of one date fruit during the heating process. RH range was between 14-20%. The HATs applied were selected according to previous experiments on date fruits (Al-Azawi et al., 1983; Barreveld, 1993; Finkelman et al., 2006).

2.3. Insect mortality study

For the *E. ceratoniae* mortality study of naturally-infested dates, three replicates per treatment were performed, using 22 infested dates in each replicate. HATs were evaluated for each specific temperature whilst control date fruits were also placed inside the hot air chamber but at room temperature. Live and dead insects (larvae) were manually counted immediately after the exposure period of each HAT. The total numbers of live and dead insects were used to calculate the percentage of mortality.

2.4. Quality study

Samples weighing 180 g (≈15 date fruits) were placed in 1 L polypropylene trays (Barket, Befor Model, Chassieu, France), borders were heat-sealed with a 35 µm-thick micro-perforated oriented polypropylene film (OPP) (Danisco Flexible, Bristol, UK), and kept for 45 d at 2 °C. This micro-perforated plastic was used to avoid the effects of modified atmosphere packaging (reduced O_2 and enriched CO_2) and to focus on the HAT effects. On days 0, 15, 30 and 45 of storage at 2 °C plus a retail period of 4 d at 23 °C, three repetitions (trays) from each HAT were evaluated. This cold storage temperature is usually used to prevent possible insect feeding damage and to control insect infestation (Kader and Awad, 2009). The commonly-used temperatures in commercial distribution and export in Tunisia are between 2 °C and 4 °C at 65-75% relative humidity (GI-Fruit, 2009).

2.4.1. Physical measurements

Color: The surface color of the date peel was determined on three randomized sides of 5 dates from each repetition. A color-difference meter (Minolta CR 300, Ramsey, NJ, USA) was used (C standard C.I.E. illumination, 0° viewing), and the results were expressed as CIE L*a*b* color space units. L* defines the lightness and a* and b* define the red-greenness and blue-yellowness, respectively. The color was also expressed as hue angle (h° = arctangent $[(b^*)\cdot(a^*)^{-1}]$) and chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$).

Firmness: This was determined on the two flat sides of each date piece by means of a texturometer (Ibertest, Madrid, Spain) equipped with a 4.0 mm diameter probe with a travel distance and time of 30 mm min^{-1} and with a depth of 3 mm. At each sampling, the firmness of 5 dates from each replicate was monitored. Firmness was expressed in Newtons (N).

2.4.2. Chemical measurements

From each repetition, five pitted date fruits were flash frozen in liquid nitrogen and stored at -80 °C for a maximum of two months, ground to a fine powder in a Cryomill in liquid nitrogen for use in chemical determination.

Main free amino acids: One gram of powdered date tissue was mixed with 6 mL of ultrapure water and homogenized for 1 min with a vortex. The mixture was then centrifuged at 3,000 x *g* for 10 min at 4 °C (Heraeus Fresco 21, Thermo Scientific, Germany) and filtered (0.45µm). Free amino acid analysis was performed as reported by Özcan and Şenyuva (2006), by HPLC with UV-Vis detector (Waters 2695, Photodiode array, wavelength 190 to 320 Alliance, Singapore) and mass detector (Waters Zq 4000 (m/z from 70 to 300). The chromatographic separations were performed on a Luna® C-18 column (100 Å, 5 µm, 30 x 2 mm, Phenomenex) using the isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid at 0.2 mL min^{-1}. The main individual amino acids, arginine, proline, alanine, methionine, glutamic and aspartic acid were quantified using their respective standards and results expressed as mg kg^{-1} of fresh weight.

Composition and concentration of sugars: Sugar composition was determined from the same extract used for amino acid analysis. Glucose, fructose and sucrose contents were analyzed by ion chromatography (IC, Metrohm 871 Advance Bioscan, Herisau, Switzerland) with a Pulsed Amperometric Detection (PAD) detector, using an anion exchange column (1–150 Metrosep-Carb) and isocratic conditions. Operating conditions reported by Moraga et al. (2006) for performance liquid chromatography were used, with minor modifications (mobile phase was NaOH 80 mM, at 1 mL min^{-1} flow rate). Individual sugars were quantified using their respective standards and results expressed as g 100 g^{-1} of fresh weight.

Antioxidant activity: Two methods were used to evaluated the antioxidant activity: the free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl, DPPH) assay according to Aguayo et al. (2015) and the ferric reducing ability of plasma (FRAP) assay according to Benzie and Strain (1996) with some modifications. For both methods and for total phenolic determination, a previous extraction was needed. One g of frozen ground date fruit was mixed with 10 mL methanol-water (1:1), maintained for 1 h at 200 x *g* in darkness inside a polystyrene box filled with ice. The homogenates were centrifuged at 4 °C for 10 min at 15,000 × *g* to obtain the extracts.

The DPPH assay, which determines the free radical scavenging activity, was measured in the following way: A solution of 0.7 mM DPPH reagent in methanol was prepared 2 h before the assay. An aliquot of 17 µL of the sample extracts was added to 198 µL of reagent solution. The mixture was incubated in darkness for 40 min at room temperature. After 40 min incubation at room temperature, the decrease in absorbance at 515 nm was measured, using the above-cited multi-scan plate reader.

The reducing activity using the FRAP assay was measured using FRAP reagent which contained 2.5 mL of a 10 mM (2,4,6-tris 2-pyridyl-s-triazine) (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$•$6H_2O$ and 25 mL of 0.3 M acetate buffer pH 3.6 (Benzie and Strain, 1996). Freshly prepared FRAP reagent (198 µL) was incubated for 2 h at 37 °C, and mixed with 6 µL of date fruit extract, and the reaction mixtures were later incubated in darkness for 40 min at room temperature. Absorbance at 593 nm was read with a multi-scan plate reader (Tecan Infinite M200, Männedorf, Switzerland).

Calibration curves were made for each assay using ascorbic acid (AA) and the antioxidant activity was expressed as mg of AA equivalent (AAE) antioxidant activity per 100 g fresh weight of date tissue.

Total phenolic content (TPC): This assay was performed using the Folin–Ciocalteu reagent. Briefly, 17.2 µL of the extract was mixed with 30 µL of Folin–Ciocalteu reagent (1:10, v/v diluted with MilliQ water) and 193 µL sodium carbonate (20%, w/v). The mixture was incubated for 40 min at room temperature in darkness, and the absorption measured at 750 nm. Total phenols were quantified using gallic acid (GA) as the standard and expressed as mg GA equivalents (GAE) per 100 g fresh weight. For each repetition, both antioxidant and phenolic assays were carried out in triplicate.

2.4.3. Sensory evaluation

Sensory analyses of samples from each sampling day were performed according to Ismail et al. (2001). The panel consisted of eight individuals aged 25-40 years, from the food engineering department at the Polytechnic University of Cartagena, who had been previously screened for sensory ability (Table 1). Since high temperature and high humidity at a stage before the start of ripening may predispose the dates to skin separation (Navarro, 2006), date fruits were also visually inspected for this parameter.

2.4.4. *Microbiological analysis*

Date fruits were pitted aseptically using sterile forceps and scalpels. Three randomized samples from each treatment containing ten grams of date fruits were aseptically placed in a sterile stomacher bag and mixed with 90 mL of sterile tryptone-phosphate water (pH 7.0) by using a Masticator (Seward Medical, London, UK). Serial dilutions were prepared in tryptone-phosphate water. Plate count agar was used for counting of mesophilic aerobic bacteria, with the plates incubated for 48 h at 30 °C. Rose Bengal Agar was used for yeast and mold, and the plates were incubated at 25 °C for 3 and 7 d, respectively. Microbial counts were expressed as $\log_{10}$ CFU/g (colony forming units per g of sample).

2.5. *Statistical analysis*

The data were subjected to a two-way ANOVA for HAT treatments and storage time as the main effects. The means were separated by using the Least Significant Difference (LSD) at $P = 0.05$. Correlation coefficients were calculated for the different antioxidant activity evaluated by FRAP and DPPH with total phenolic content and amino acids. These coefficients were tested for significance at $P = 0.01$ with a two-tailed t-test at n-2 df. All analyses were performed by using SPSS software (SPSS 22 for Windows, SPSS Inc. Chicago IL.).

3. Results

3.1. *Ectomyelois ceratoniae mortality under different HATs*

All the studied HATs resulted in 100 % mortality of *E. ceratoniae* insect (larvae) without significant differences between temperature and length of time applied. In contrast, control samples contained all the larvae in a live state during and after treatment application.

3.2. Quality parameters under different HATs

Table 2 shows the ANOVA results of simple factors such as HATs, storage time and their interaction according to each date fruit parameter studied fruits.

3.2.1. Physical measurements

Color: During storage, the color of date fruits changed from light brown to dark brown as shown by the decrease of L* and h° values (Fig. 1, Tables 2 and 3). Significant decreases in L* were found between dates from day 0 and at the end of the retail period on control, 55 °C 30 min and 60 °C 15 min treatments. On the other hand, the longest HAT (50 °C for 30 min) led to a decreased h° level with respect to control dates (Table 4). Moreover, all the treatments had reduced h° values after storage and retail period (Table 3). Changes in Chroma ranged from 15 to 16 and were not significant for any factor studied.

Firmness: Firmness was reduced due to storage time (Tables 2 and 3) without significant differences when HATs were used (data not shown). Dates maintained their firmness values all through the storage time with a significant reduction at the end of retail period (10.6 N) with respect to day 0 (12.7 N).

3.2.2 Chemical measurements

Main free amino acids: In our experiment, some of the main free amino acids (glutamic acid, aspartic acid and methionine) were not affected by HATs or storage time (Table 2). The concentration of these amino acids were stable during storage after the different HATs, ranging from 7.9 to 8.6 mg kg^{-1} for glutamic acid, 7.8 to 10.9 mg kg^{-1} for aspartic acid and 9.7 to 10.3 mg kg^{-1} for methionine. On the contrary, the concentrations of alanine, proline and arginine were significantly ($P < 0.05$) affected by the interaction of HATs and storage time (Table 2). Date fruits had a slightly reduced concentration of proline and arginine in the retail period at 23 °C, reaching an average of 19.3 to 17.7 mg kg^{-1} for proline and 3.9 to 3.7 mg kg^{-1} for arginine. In both cases, the 60 °C for 15 min HAT was the treatment that better maintained the concentration of amino acids in dates. Alanine concentration was also reduced during the retail period (7.7 to 5.8 mg kg^{-1}), with the lowest level obtained when 60 °C for 20 min HAT was used.

Sugar content: The individual concentrations of glucose, fructose and sucrose, as well as total sugars, are shown on Fig. 2. Sucrose was the main sugar in all samples, followed by fructose and glucose, with approximately similar proportions. Sugar content was affected by the interaction of HATs and storage time (Table 2). At day 0, the HATs applied reduced the sucrose, glucose and fructose concentration with respect to control samples, with minor reductions observed in date samples treated with the 60 °C for 20 min HAT. During storage, sucrose concentrations generally increased (Fig. 2), mainly in samples treated with 55 °C for 30 min (22.4 g 100 g^{-1} *vs*. 44.1 at the beginning of storage time and at end of retail time, respectively). A similar tendency was observed in the reducing sugars (glucose and fructose) (Fig. 2). At the end of the retail period, the highest total sugar concentration was found in samples treated with 55 °C for 30 min and 60 °C for 20 min.

Antioxidant Activity: Both assays used to evaluate the antioxidant activity of date fruit extracts, DPPH and FRAP, were significantly affected by the simple factors storage time and HAT but not by their interaction (Tables 2 to 4). The initial antioxidant capacity (DPPH assay) was 40.7 mg AAE 100 g^{-1} and was significantly reduced in the retail period, up to 25%. The storage time also affected antioxidant capacity as determined by the FRAP assay, showing a similar trend to DPPH but with lower reductions.

The results of the HATs showed that control dates had 41.2 mg AAE 100 g^{-1} (DPPH assay), which was significantly reduced when the highest temperature HAT was applied. More specifically, the 60 °C for 20 min treatment decreased DPPH by 25% (Table 4). As with storage time, FRAP followed the same trend as DPPH but with lower reductions. The decrease for the 60 °C for 20 min treatment was 14%.

Total Phenolic Content (TPC): The initial phenolic content was between 94 to 84 mg GAE 100 g^{-1}. The TPC decreased with storage time (Tables 2 and 3), mainly when the storage temperature was high as in the retail period (about39 % lost *vs.* the initial content). No significant differences between the control and HATs were found.

The total phenolic compound content showed a high correlation in these two antioxidant assays, where the number of data points analyzed was 60. The correlation coefficients between DPPH and TPC were positive and significant (0.569, $P \leq 0.001$), between FRAP and TPC were positive and significant (0.540, $P \leq 0.001$) and between DPPH and FRAP were positive and significant (0.631, $P \leq 0.001$). In addition, the correlation coefficient between alanine and FRAP were positive and significant (0.279, $P \leq 0.05$) and the correlation coefficient between arginine and FRAP were positive and significant (0.256, $P \leq 0.05$).

3.2.3. Sensory analysis

Sensory parameters such as color, flavor, texture and overall quality were evaluated. After the application of the HATs, statistical differences in sensorial parameters were observed (Table 2). At day 0, dates treated with 60 °C for 20 min had the best color (4.9-caramelized brown) and texture values (3.8) than the rest of the treatments, with an average of 4.0 for color and 3.0 for texture. However, these texture changes were subjective since firmness was only influenced by storage time.

Date color was maintained until 45 d of storage but at the end of the retail period there was a significant change, changing from “caramelized brown” (4.2) to “no uniform brown” (3.4). Flavor showed a similar tendency; at day 0, dates from the 60 °C for 20 min treatment reached the best flavor (4.8) but all the treatments had significantly-decreased flavor scores at the end of storage, from an initial and averaged “sufficiently sweet” (4.6) to “sweet just enough without foreign flavors” (3.2). These reductions in the sensorial parameters scores affected the overall quality score, but all samples were above the limit of marketability (> 3) at the end of retail period (Table 3). The high temperature used in HATs did not affect skin separation (data not shown).

3.2.4. Microbiological analysis

Total mesophilic bacterial count, yeasts and molds were assessed (Table 5). The results showed that microbiological counts from the control treatment at day 0 were relatively high because fruits were harvested from the field without any sanitizing treatment. The control samples had a stable microbial count which slightly increased during storage time. However, immediately after the HATs were applied, the mesophilic bacterial counts were significantly reduced to under the detection limits (< 1 log cfu g^{-1}). On the other hand, the microbial count for molds was not reduced by the HATs and was stable during the storage time. At the end of retail period, low levels of yeast appeared in control samples.

4. Discussion

All HATs applied in this research were effective in achieving total mortality of *E. ceratoniae*, which is in agreement with Kader and Awad (2009), who obtained an effective date palm insect disinfestation after the application of hot air at 50 to 55 °C for 2 to 4 hours. Previously, heat treatments had been described as having the potential to control a number of pests and diseases in different crops (Lurie, 1998). Thus, different authors have described a range of 92 to 100% insect mortality, depending on date fruit cultivar, when applying a temperature of 50-60 °C for 33-60 min (Al-Azawi et al, 1983; Finkelman et al, 2006; Navarro, 2006). Treatments using hot water dips at 50 °C for 10 min, 55 °C for 5 min and 60 °C for 3 min have led to *E. ceratoniae* mortality (Ben-Amor et al., 2016), microwaves (1 Kw) for 55-90 s have increased internal and external temperatures of date fruits to more than 52 °C, which disinfested 100% of the larvae and eggs (Zouba et al., 2009). However, other treatments using sanitizers such as chlorine, UV-C radiation, ozonated water and alkaline and neutral electrolyzed water only obtained mortality percentages between 8 to 15% (Jemni et al., 2014).

According the quality parameters, for the color, the decreases in L* and h° caused by the storage time and HATs in this experiment were probably due to the oxidative browning of phenolic compounds and sugar browning, the main causes of darkening using elevated temperatures combined with longer-term heat treatments (Vandercook et al., 1979). On the other hand, the reduction of h° values linked to the end of storage and retail period probably corresponded to an increase of carotenoid biosynthesis as the fruit ripened, which is associated with the climacteric increase in respiration, initiated by the action of ethylene (Saltveit, 1999).

Likewise, storage time such as the retail period led to a decrease in the firmness of date fruits in this experiment, which was probably due to the 'high' temperature used during the retail period. The optimal temperature for Tamar dates is 0 °C for 6 – 12 months, with the temperature being the most important tool for maintaining quality of dates (Kader and Awad, 2009). Previously, different authors had observed that increased storage time gradually decreased date palm firmness due to the decay of fruit structure (Lazan et al., 1993; Rohrbach et al., 2003) caused by enzymatic activity and other factors such as moisture content and respiration (Ismail et al., 2008).

Dates have very important nutritional aspects, as they have an important content of diverse essential and non-essential amino acids, which are considerably variable depending on the cultivar (El Hadrami and Al-Khayri, 2012; Sadiq et al., 2013). The content of some of amino acid present in dates decreased due to the interaction of HATs and storage time, with this loss of amino acids by heat treatment previously described by other authors. For example, broccoli heads dipped in 45 °C water for 14 min also exhibited changes in soluble proteins or free amino acid content during storage (Kazami and al., 1991). Greater reductions in amino acid content with longer storage time and higher temperature in apricot jam have also been reported (Touati et al., 2014).

The taste of the Deglet Noor cultivar fruits was associated to their sugar profile, with the main sugar being sucrose, followed by fructose and glucose (with the last two having similar proportions) (Mrabet et al., 2008). Sugar concentration in dates depends on ripening stage, firmness and water content (El Hadrami and Al-Khayri, 2012). The observed increase in sugar concentrations during the storage found in our experiment could be due to the accumulation of sugars at the end of maturity, which makes them more palatable (Ahmed at el., 1995) and/or to the increase in glucosidase, galactosidase and arabinose activities, which could release sugars from cell wall polymers as in pomegranate arils treated with a hot water treatment (Mirdehghan et al., 2006). The longer-time HATs could have activated these enzymes.

Additionally, date fruit constitutes a natural source of antioxidants that may prevent many diseases and could potentially be used in food and nutraceutical formulations (Saafi, et al., 2009). However, differences in cultivar, growing conditions, maturity, season, geographic origin, fertilizer, soil type and storage conditions could affect the antioxidant activity of dates (Al-Farsi et al., 2007). In our case, the use of 60 °C in the HATs significantly decreased the antioxidant activity of dates. This was probably due to the degradation of thermolabile antioxidants from date palm by the heat treatments. Many studies have reported losses in antioxidant activity of plant samples due to heat treatment. For example, Demarchi et al. (2013) reported that antioxidant activity retention decreased with higher air temperatures in apples. Moreover, *Vitex negundo L*. (Family: Verbenaceae) tea leaves treated with high-temperature hot air had significantly reduced antioxidant activity values (Chong and Lim, 2012). Likewise, several studies have indicated the possibility that the concentrations of antioxidant compounds in fruits were affected by storage (Kalt, 2005; Kevers et al., 2007). Al-Najada and Mohamed (2014) have reported on an important decrease in antioxidant activity as measured by FRAP and DPPH assays on two cultivars of Saudi palm dates when stored for 12 months at 4 °C. A similar response was found on Bahree dates stored for 50 d at 1 °C (Mohamed et al. 2014). On the contrary, and in disagreement with our results, Ritenoor et al. (1995) observed an increase in the concentration of antioxidant compounds during cold storage of dates that could be due to ethylene action.

In our study, at the end of storage, different reduction percentages of antioxidant activity were observed by the DPPH and FRAP assays. The differences in the antioxidant activity measurements between assays could be expected, as each assay has a different mechanism of action (Biglari et al., 2008). The DPPH assay is a method based on the reduction of the 2,2-diphenyl-1-picrylhydrazyl radical while the FRAP assay measures the reducing ability of ferric ions. The antioxidant properties of arginine and its decrease in concentration with storage time have been previously described by Aluko (2015).

Moreover, the storage time significantly reduced the TPC, with similar losses due to storage reported in many fruits; some examples have been described for pistachios nuts (Tsantili et al. 2011) and pomegranate (Sayyari et al., 2011). The decline in TPC in date fruit may be related to the breakdown of phenolic compounds as a result of enzymatic activity occurring during storage, as previously described in pomegranate fruit (Fawole and Opara, 2013). On the contrary, several authors have reported an increase of TPC with storage time in dates (Biglari et al., 2009; Al-Najada and Mohamed, 2014).

The sensory analysis results clearly show that the low storage temperature (2 °C) used in this study preserved the quality of dates, but during the retail period at 23 °C, color, flavor, and overall quality slightly deteriorated in all the samples. Previous authors have reported that the storage conditions (duration and temperature) were the main factors that affected the maintenance of sensory quality of dates (Ismail et al., 2008; Kulkarni et al., 2008), with low temperature being the most important factor for maintaining color, flavor and textural quality (Kader and Awad, 2009). Moreover, Baloch et al. (2006) reported that in the Tamar stage (full maturity) of palm date fruit, the browning of the fruit continuously increased under any storage condition, degrading the sensory quality of the fruit.

Lastly, the significant effect of HATs on the reduction of mesophilic bacteria and yeast counts obtained in this research was in agreement with a previous study where the sanitizing effect of HATs (48 °C for 3 h) was described in Chinese berries, effectively reducing fruit decay, probably by directly inhibiting bacterial growth and indirectly inducing disease resistance (Wang et al., 2010). However, in our study, mold counts were not reduced with HATs, probably due to the short treatment time. As Lurie (1998) has reported, exposure to high temperature using forced or static air also decreased fungal infections, but only when long term heating, from 12 to 96 h at temperatures ranging from 38 to 46 °C was applied.

We can conclude that HATs, particularly the use of 60 °C for 15 min, are a suitable physical treatment for *E. ceratoniae* larvae, inducing mortality while maintaining postharvest quality.

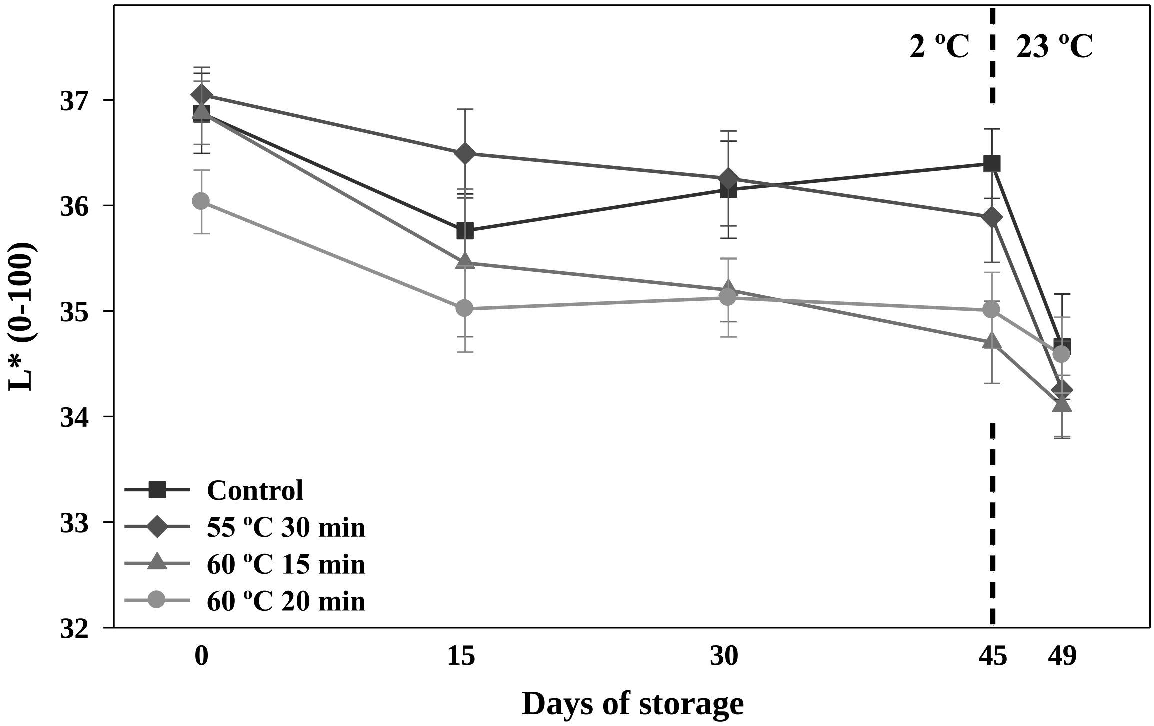

Fig. 1. Effect of hot air treatments (HATs) and storage time (45 d at 2 ^{o}C plus 4 d at 23 °C) on luminosity (mean ± SD) of Deglet Noor date fruits (see Table 2 for statistical analysis description).

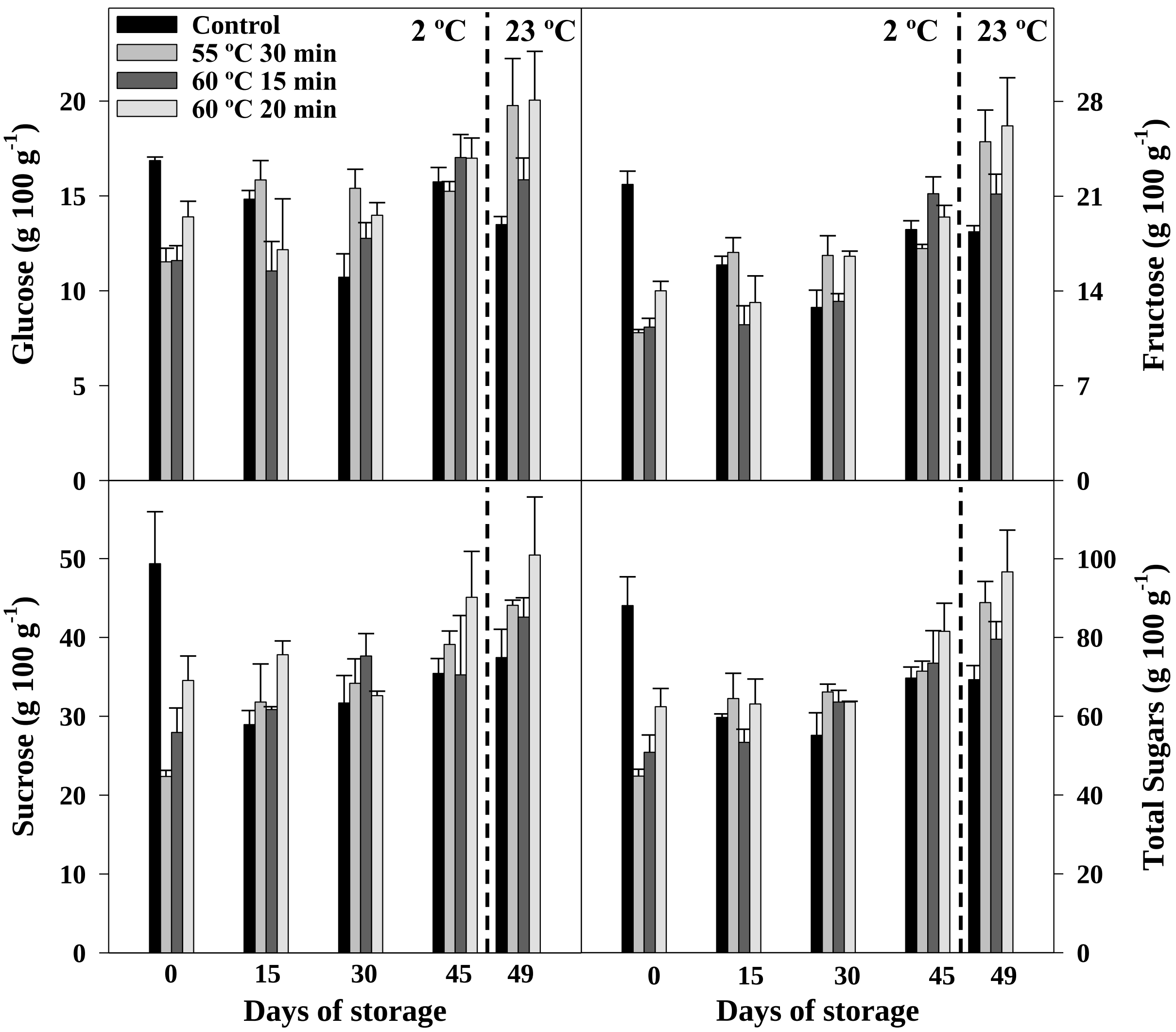

Fig. 2. Effect of hot air treatments (HATs) and storage time (45 d at 2 °C plus 4 d at 23 °C) on sugar content (mean ± SD) of Deglet Noor date fruits (see Table 2 for statistical analysis description).

Table 1. Guide of sensory evaluation for date fruits.

	1	**2**	**3**	**4**	**5**
Color	Very dark/Very yellow	Dark brown/ Yellowish brown	No uniform brown	Caramelized brown	Translucent brown uniform and bright color
Texture	Very soft, deformed and glued/very firm and wrinkled	Quite soft, skin separated from pulp and sticky	Soft but keeps its shape	Uniform firmness	Firmness uniform with its original shape when gently pressed with your fingers, not too dry, not too sticky
Flavor	High acidity/High fermentation/Bitterness	Slightly acid/fermented, strange taste	Sweet just enough without foreign flavors	Sufficiently sweet	High perception of sweetness
Overall quality	Extremely poor	Poor	Acceptable and limit of usability	Good	Excellent

Table 2. ANOVA results for quality parameters according to main effects, hot air treatments (HAT: control, 55 °C for 30 min, 60 °C for 15 min, 60 °C for 20 min) and storage time (ST: 45 d at 2 °C plus 4 d at 23 °C), and their interaction for Deglet Noor date fruits.

Quality parameters	HAT (df, 3)			ST (df, 4)			HAT x ST (df, 12)		
	df_{error}	F	*P*	df_{error}	F	*P*	df_{error}	F	*P*
L*	580	6.8	<0.001	580	12.1	<0.001	580	3.3	<0.001
h°	596	3.0	<0.05	595	6.1	<0.001	580	1.5	>0.05
Chroma	595	7.1	>0.05	595	9.7	>0.05	579	2.9	>0.05
Firmness	236	0.7	>0.05	235	4.0	<0.001	220	1.1	>0.05
Alanine	40	3.1	<0.05	40	4.8	<0.001	40	2.4	<0.05
Aspartic acid	53	3.1	>0.05	52	1.9	>0.05	37	1.0	>0.05
Glutamic acid	56	0.8	>0.05	55	3.3	>0.05	40	0.7	>0.05
Methionine	56	1.1	>0.05	55	0.3	>0.05	40	1.4	>0.05
Proline	40	0.7	>0.05	40	1.7	>0.05	40	2.4	<0.05
Arginine	40	2.5	>0.05	40	7.5	<0.001	40	3.9	<0.001
Glucose	40	2.4	>0.05	40	8.5	<0.001	40	3.3	<0.001
Fructose	40	2.5	>0.05	40	29.6	<0.001	40	6.4	<0.001
Sucrose	40	2.3	>0.05	40	6.0	<0.001	40	2.9	<0.01
Total sugars	40	2.4	>0.05	40	13.8	<0.001	40	4.2	<0.001
DPPH	56	12.1	<0.001	55	7.8	<0.001	40	1.6	>0.05
FRAP	56	11.2	<0.001	55	5.7	<0.001	40	1.7	>0.05
Total phenolic content	40	0.4	>0.05	40	43.0	<0.001	40	2.0	<0.05
Flavor	80	1.2	>0.05	80	29.3	<0.001	80	5.6	<0.001
Color	80	7.1	<0.001	80	10.3	<0.001	80	4.1	<0.001
Texture	80	5.6	<0.001	80	1.8	>0.05	80	2.5	<0.01
Overall quality	80	0.8	>0.05	80	6.6	<0.001	80	1.8	>0.05

Table 3. Effect of storage time (45 d at 2 °C plus 4 d at 23 °C) on color, firmness, antioxidant activity (DPPH and FRAP), total phenolic content (TPC) and overall quality of Deglet Noor date fruits.

Days of storage	**h°**	**Firmness (N)**	**DPPH (mg AAE 100 g^{-1})**	**FRAP (mg AAE 100 g^{-1})**	**TPC (mg GAE 100 g^{-1})**	**Overall quality (1 to 5)**
0	62.8 ± 5.6 a	12.7 ± 3.6 a	40.7 ± 5.3 a	65.6 ± 5.5 a	88.7 ± 7.1 a	4.0 ± 0.5 a
15	62.8 ± 5.4 a	11.7 ± 2.9 ab	40.1 ± 7.3 a	60.0 ± 5.9 b	70.4 ± 9.1 b	3.9 ± 0.4 a
30	62.8 ± 5.7 a	11.5 ± 3.0 ab	35.8 ± 4.1 b	57.3 ± 4.4 b	65.6 ± 4.3 bc	3.8 ± 0.3 a
45	60.4 ± 6.0 b	11.1 ± 3.2 ab	34.1 ± 2.3 c	57.9 ± 5.6 b	65.2 ± 3.8 c	3.8 ± 0.6 a
45 + 4	60.4 ± 5.9 b	10.6 ± 3.2 b	30.5 ± 5.9 c	58.0 ± 2.6 b	54.1 ± 7.5 d	3.3 ± 0.4 b

Means within the same column with different letters are significantly different ($P \leq 0.05$) according to LSD test (see Table 2 for statistical analysis description). Values are means ± SD.

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Table 4. Effect of hot air treatments on color and antioxidant activity (DPPH and FRAP) of Deglet Noor date fruits.

Treatments	h°	DPPH (mg AAE 100 g^{-1})	FRAP (mg AAE 100 g^{-1})
Control	62.3 ± 6.1 a	41.2 ± 5.0 a	63.7 ± 4.8 a
55°C 30 min	60.7 ± 6.0 b	38.5 ± 5.7 ab	62.2 ± 5.3 a
60°C 15 min	62.6 ± 5.3 ab	34.4 ± 4.6 b	58.3 ± 4.6 b
60°C 20 min	61.8 ± 5.8 ab	30.8 ± 4.7 c	54.9 ± 3.5 c

Means within the same column with different letters are significantly different (*P* ≤ 0.05) according to LSD test (see Table 2 for statistical analysis description). Values are means ± SD.

Table 5. Effect of hot air treatments and time of storage on microbiological counts (log CFU g^{-1}) of Deglet Noor date fruits at day 0 and after being stored for 45 d at 2 °C plus 4 d at 23 °C.

Storage time	Treatments	Mesophilic bacteria	Yeasts	Molds
Day 0	**Control**	3.0 ± 0.2	<2	4.1 ± 0.5
	55 °C 30 min	<1	< 2	4.1 ± 0.3
	60 °C 15 min	<1	< 2	3.5 ± 0.4
	60 °C 20 min	<1	< 2	3.9 ± 0.1
Day 49	**Control**	2.7 ± 0.3	2.5 ± 0.5	4.2 ± 0.2
	55 °C 30 min	< 1	< 2	4.3 ± 0.2
	60 °C 15 min	< 1	< 2	3.9 ± 0.1
	60 °C 20 min	< 1	< 2	3.6 ± 0.2

Values are means (n = 3) ± SD.

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CHAPTER IV:

Freezing treatments for *Ectomyelois ceratoniae* mortality and maintenance of Deglet Noor palm date quality.

CHAPTER IV

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ABSTRACT

Insect infestation caused by *Ectomyelois ceratoniae* or carob moth is one of the main postharvest problems that can lead to a decrease of the marketable quality of dates. The control of carob moth is a mandatory process for exported fruits, and the main chemical method used to prevent pest diseases of palm date is treatment with methyl bromide. However, its use is being restricted due to direct harmful effects to the environment and indirect effects on humans. Freezing treatments could be physical alternatives to methyl bromide and other chemicals. Three freezing treatments at -18 °C (50 hours, 77 hours, and 125 hours) were studied for *E. ceratoniae* mortality in Deglet Noor date fruits. The results showed that the use of freezing at -18 °C led to 100% mortality of all the stages of *E. ceratoniae* found in naturally-infested dates. Fruit quality was examined under a selected sanitizing freezing treatment (50 hours at -18 °C). This freezing treatment induced an increase of monosaccharides and a reduction in antioxidant activity (40 to 45%, measured with FRAP and DPPH assays). However, other parameters such as color, amino acids, total phenolic content, microbial and sensorial quality were not affected by that treatment. All samples remained above the limit of marketability as there was no chilling injury. This treatment can be recommended as an alternative to chemical treatments to control carob moth while

yielding optimum-quality Deglet Noor date fruits that could be exported to developed countries.

Keywords: Cold treatment; carob moth; methyl bromide; overall quality; antioxidant activity; sugars.

1. Introduction

Date palm (Phoenix *dactylifera* L.) is the main fruit crop in arid and semi-arid regions of western Asia and North Africa between 24 °N and 34 °N (Al-Yahyai and Al-Kharusi, 2012). Tunisia and Algeria are the traditional Deglet Noor cultivar suppliers for Europe (Guizani et al., 2010). This native cultivar is the most popular because of its large size, texture, and distinctive taste and color, which gives it a high commercial value (Benmeddour et al., 2012), and account about 80% of the production. In contrast, common cultivars are less appreciated and account for approximately 20% of the production of Tunisia dates (FAOSTAT, 2015).

In Tunisia, the carob moth, *Ectomyelois ceratoniae* Zeller, is a major insect pest of dates, pomegranate and several other host plants (Dhouibi, 1989). This pest causes great economics losses and the yearly infestation rate can reach 20% of harvestable dates in Tunisia (Jemni et al., 2014). It decreases the marketable quality of dates and risks compromising exports, especially those of Deglet Noor cv. (Zouba et al., 2009).

Methyl bromide, which is being used in many countries, is very effective for controlling insects in stored dates (Al-Kahtani et al., 1998). However, it is an ozone depleting substance, and according to the Montreal Protocol, developed countries were expected to phase methyl bromide out by 2005, while developing countries were expected to phase it out by 2015 (El-Mohandes, 2012). Hence alternative physical treatments are needed urgently.

The use of cold temperatures for the managing of stored products can be an important component of insect pest management programs (Navarro et al., 2002; 2012). Since cold temperatures directly affect the spread and impact of invasive pests (Bale and Hayward, 2010), depending of the geographic zone, insects can be regularly exposed to potentially-lethal freezing conditions (Sømme et al., 1996). Most insects require only a short exposure to very low temperatures (-10 °C or below) to ensure control (Chauvin and Vannier, 1991; Fields, 1992). At sub-zero temperatures, insects risk freezing of their body fluids, as well as a host of other low temperature-related injuries (Denlinger and Lee, 2010). As for fruit quality under freezing conditions, Barbosa-Cánovas et al. (2005) indicated that this method is one of the few that allows for the preservation of food attributes such as taste and texture whilst maintaining the nutritional value, with this retention of quality better achieved when foods were kept at -18 °C or even lower temperatures. At these temperatures, micro-organisms cannot grow and any food-deterioration processes take place at very slow rates.

Therefore, the aim of the current work was to find the minimum length of time at -18 °C needed that would induce *Ectomyelois ceratoniae* mortality and to know the effects of this treatment on fruit quality.

2. Material and methods

Chemicals

Ultrapure water was obtained from a Milli-Q system (Academic Gradient A10, Millipak™ 40, Millipore, París, France). Sodium hydroxide and methanol (HPLC grade) were purchased from Panreac Química S.A. (Castellar del Vallés, Barcelona, Spain).

Individual amino acids and sugars, hydrochloric acid (minimum 37%), sodium chloride, methanol, sodium sulfate, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), gallic acid (3,4,5-trihydroxybenzoic acid), and Folin–Ciocalteu's phenol reagent were purchased from Sigma–Aldrich Química S.A. (Madrid, Spain). Peptone water, plate count and rose bengal were from Scharlau (Barcelona, Spain).

2.1. Experiment 1: *Ectomyelois ceratoniae* mortality under different freezing treatments

2.1.1. Plant and entomological material

Deglet Noor cv. dates that were naturally infested with *E. ceratoniae* of date palm (*Phoenix dactylifera* L.) were collected at the beginning of November from an experimental palm orchard (33° 55′ 0″ North, 8° 8′ 0″ East), belonging to the National Institute of Agronomy of Tunisia located in Tozeur (South west, Tunisia). Naturally-infested dates are characterized by the presence of silk closing the calyx. These fruits were at the fully mature 'Tamar' stage, and were carefully collected by professional entomologists from the Department of Plant Protection (National Institute of Agronomy of Tunisia), and used to study the effect of freezing treatment on *E. ceratoniae* mortality.

For insect mortality studies, three different freezing treatments were applied, using three different lengths time at -18 °C, with 50, 77 and 125 h being the actual duration of each freezing treatment at that temperature. Untreated date palm fruits were used as the control, and these were placed in a chamber at room temperature. All of these treatments were carried out on three bunches of 100 naturally-infested dates, for a total of 300 dates per treatment. Those bunches were placed in plastic bags in different positions inside a freezing chamber, which was designed for the treatment of exported dates.

The development stages of *E. ceratoniae* present inside the fruit were mainly: larvae (young larvae: L_1, L_2 and L_3, and old larvae L_4 and L_5), pupae and other pests and feces and webbings of larvae. The finding of infestation by different insect stages is representative of a normal date fruit sample. Date infestation rates varied between 53 and 78% (Table 1).

2.1.2. Equipment

The freezing treatments were conducted in a chamber with a 140 m^3 freezer system. . Temperature (°C) and relative humidity (%RH) inside the freezing chamber were measured using hygro buttons. Temperature (° C) was recorded by placing nine thermo buttons in different places inside the chamber.

2.1.3. Insect mortality

To calculate *E. ceratoniae* mortality, live and dead insects were manually counted and examined with a binocular microscope. The total numbers of live and dead insects were used to calculate the percentage of mortality.

2.2. Experiment 2: Overall date fruit quality after treating with the temperature that caused 100% mortality of *Ectomyelois ceratoniae.*

Once the shortest freezing treatment that resulted in 100% *Ectomyelois ceratoniae* mortality was known, non-infested date fruits were subjected to the same treatment to evaluate its effect on fruit quality.

2.2.1. Plant material

Non-infested date fruits, Deglet Noor *cv.*, were harvested at the fully mature 'Tamar' stage from a commercial farm located in an Oasis in southern Tunisia (Tozeur). Professional pickers detached the date bunches from the head of the palm tree and these were carefully placed on the ground by hand to avoid crushing and the abscission of dates. These bunches were then cut into spikelets and placed in boxes. About 50 kg of spikelets were placed in polystyrene boxes and transported about 500 km by car to Tunis, then by plane to Madrid (Spain), and again by car about 400 km to the Pilot Plant of the Technical University of Cartagena. Total transport duration was about 7 d at 8 °C. After arrival, a visual inspection and selection was performed to remove damaged dates. The average weight was 12.1 ± 1.5 g, length 44.91 ± 0.52 mm and thickness 21.91 ± 0.48 mm. The fruit moisture content was 25%. Samples of 180 g of date fruits (≈15 fruits) were placed in 1 L polypropylene trays (Barket, Befor Model, Chassieu, France), and the borders were heat sealed with a 35µm-thick micro-perforated oriented polypropylene film (OPP) (Danisco Flexible, Bristol, UK). Samples (trays) were classified into two uniform groups: Control date fruits were stored for 72 h in a chamber at room temperature (20 °C ± 2 °C) and frozen treatment kept at -18 °C for 50 hours (72 h for the total freezing and thawing processes, figure 1). Three replicates (trays) from each treatment were used, and the evaluation of quality was performed at day 0 (initial) and after 3 days at room temperature or at the end of the freezing storage process (Figure 1). After that time, frozen date fruits were removed from the freezer and kept at room temperature until they were completely thawed before quality analysis.

2.2.2. Quality parameters

2.2.2.1. Physical measurements

Color: The date peel’s surface color was determined on three random sides of 5 dates from each repetition. A color-difference meter (Minolta CR 300, Ramsey, NJ, USA) was used (C standard C.I.E. illumination, 0° viewing), and the results were expressed as CIE L*a*b* color space units. L* defines the lightness and a* and b* define the red-greenness and blue-yellowness, respectively. The color was also expressed as hue angle (h° = arctangent $[(b^*)\cdot(a^*)^{-1}]$) and chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$).

Firmness: Firmness was determined on the two flat sides of each date piece by means of a texturometer (Ibertest, Madrid, Spain) equipped with a 4.0 mm in diameter probe with a travel distance and time of 30 mm min^{-1} and to a depth of 3 mm. At each sampling day, the firmness of 5 dates from each replicate was measured. Firmness was expressed in Newtons (N).

Total Soluble Solids: Total soluble solids (TSS) were determined by measuring the refraction index with a handheld refractometer (Atago N1, Tokyo, Japan). The refractive index was recorded and expressed as percentages. Measurements were performed at 20 °C.

2.2.2.2. Chemical measurements

Pitted date fruits were flash-frozen in liquid nitrogen and stored at -80 °C for a maximum of two months, ground to a fine powder in a Cryomill in liquid nitrogen for use in the determination of chemical qualities.

Main free amino acids: One gram of date tissue powder was mixed with 6 mL of ultrapure water and homogenized for 1 min with a vortex. The mixture was then centrifuged at 3,000 x *g* for 10 min at 4 °C (Heraeus Fresco 21, Thermo Scientific, Germany) and filtered (0.45µm). Free amino acid analysis was performed as reported by Özcan and Şenyuva (2006), by HPLC with a UV-Vis detector (Water 2695, Photodiode array, wavelength 190 to 320 Alliance, Singapore) and mass detector (Waters Zq 4000 (m/z from 70 to 300). The chromatographic separations were performed with a Luna® C-18 column (100 Å, 5 µm, 30 x 2 mm, Phenomenex) using the isocratic mixture of 0.01 mM acetic acid in a 0.2% aqueous solution of formic acid at 0.2 mL/min. The main individual amino acids arginine, proline, alanine, methionine, glutamic and aspartic acid, were quantified using their respective standards and results were expressed as mg kg^{-1} of fresh weight.

Composition and concentration of sugars: Sugar composition was determined from the same extract used for amino acid analysis. Glucose, fructose and sucrose contents were measured by ion chromatography (IC, Metrohm 871 Advance Bioscan, Herisau, Switzerland) with a Pulsed Amperometric Detection (PAD) detector, using an anion exchange column (1–150 Metrosep-Carb) and isocratic conditions. The operating conditions reported by Moraga et al. (2006) for performance liquid chromatography were used, with minor modifications (mobile phase was NaOH 80 mM, at 60 mL/h flow rate). Individual sugars were quantified using their respective standards and the results were expressed as g kg^{-1} of fresh weight.

Antioxidant activity: Two methods were used to evaluate antioxidant activity: the ferric reducing ability of plasma (FRAP) assay according to Benzie and Strain (1996) and the free radical scavenging activity (2,2-diphenyl-1-picrylhydrazyl, DPPH) assay according to Aguayo et al. (2015) with some modifications. The extracts were prepared as follows: one g of frozen, ground date fruit was mixed with 10 mL methanol-water (1:1), and then maintained for 1 h at 200 x *g* in darkness inside a polystyrene box filled with ice. The homogenates were centrifuged at 4 °C for 10 min at 15,000 × g to obtain the extracts.

The reducing activity using the FRAP assay was measured using FRAP reagent which contained 2.5 mL of a 10 mM (2,4,6-tris 2-pyridyl-s-triazine) (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer pH

3.6. Freshly prepared, FRAP reagent (198 µL) was incubated for 2 h at 37 °C, and then mixed with 6 µL of date fruit extract. The reaction mixtures were later incubated in darkness for 40 min at room temperature. Absorbance at 593 nm was read with a multi-scan plate reader (Tecan Infinite M200, Männedorf, Switzerland).

The DPPH assay, which determines the free radical scavenging activity, was measured in the following way: A solution of 0.7 mM DPPH reagent in methanol was prepared 2 h before the assay. An aliquot of 17 µL of the sample extracts was added to 198 µL of reagent solution. The mixture was incubated in darkness for 40 min at room temperature. After the 40 min incubation at room temperature, the decrease in absorbance at 515 nm was measured, using the above-cited multi-scan plate reader. Calibration curves were made for each assay using ascorbic acid (AA) as standard. The antioxidant activity was expressed as g of Ascorbic-Acid equivalents (AAE) antioxidant activity per kg fresh weight of date tissue.

Total phenolic content (TPC): This assay was performed using the Folin–Ciocalteu reagent. Briefly, an aliquot of 17.2 µL extract of the supernatant was mixed with 30 µL of Folin–Ciocalteu reagent (1:10, v/v diluted with MilliQ water) and 193 µL sodium carbonate (20%, w/v). The mixture was incubated for 40 min at room temperature in darkness, after which the absorption was measured at 750 nm. Total phenols were quantified using gallic acid (GA) as the standard and expressed as g Gacllic Acid equivalents (GAE) per kg fresh weight. For each repetition, both antioxidant and phenolic assays were carried out in triplicate.

2.2.2.3. Microbiological analysis

Date fruits were pitted aseptically using sterile forceps and scalpels. Three randomized samples from each treatment containing ten grams of date fruits were aseptically placed in a sterile stomacher bag and mixed with 90 mL of sterile tryptone-phosphate water (pH 7.0) by using a Masticator (Seward Medical, London, UK). Serial dilutions were prepared in tryptone-phosphate water. Plate count agar was used for counting of mesophilic aerobic bacteria, after the plates were incubated for 48 h at 30 °C. Rose Bengal Agar was used for yeast and molds, and the plates were incubated at 25 °C for 3 and 7 d, respectively. Microbial counts were expressed as $\log_{10}$ cfu/g (colony forming units per g of sample).

2.2.2.4. Sensory evaluation

Sensory analyses of samples from each sampling day were performed according to Ismail et al. (2001). The panel consisted of eight individuals aged 25-40, from the Food Engineering department at the Technical University of Cartagena, who had been previously screened for sensory ability (Table 2).

2.2.3. Statistical analysis

For the quality experiment, a one-way analysis of variance (ANOVA, $p < 0.05$) was carried out (Statgraphic Plus, version 5.1, 2001, Manugistic Inc, Rockville, MD, USA). Mean values were compared using an LSD test when significant differences among treatments were found.

3. Results and discussion

3.1. Experiment 1: Effect of freezing treatment on *Ectomyelois ceratoniae* mortality

Table 1 shows the effect of different freezing treatments at -18 °C (50, 77 and 125 h) on the different stages of *E. ceratoniae* (young instars, old instars and pupae). Results showed that all freezing treatments used in this experiment resulted in 100% mortality of all the development stages of *E. ceratoniae*. Therefore, as 50 h was enough time required to kill the *E. ceratoniae*, this treatment was selected to study the effects of a freezing treatment on the quality of the Deglet Noor palm date fruit variety.

Many studies using freezing treatments have shown that temperatures lower than 0 °C were able to achieve significant mortality of various insects at different growth stages. Boardman et al. (2012) reported that the larvae of the false codling moth, *Thaumatotobia leucotreta*, were killed by brief exposures to temperatures between -8 °C and -12 °C. Mullen and Arbogast (1979) showed that at -5 °C, 50% of *Tribolium castaneum* eggs survived for 0.3 days. Studies with the related species *Tribolium confusum* showed that 50% of the eggs exposed to 6 °C survived for 0.2 days (Nagel and Shepard, 1934). In this context, Johnson and Valero (2003) conducted studies in a commercial freezer set at -18 °C to examine the effects of freezing on the disinfestation of different life stages of the cowpea weevil, *Callosobruchus maculatus (F.)* present in bulk-stored garbanzo beans, and reported that egg mortality was estimated to be > 98% after just 7 d of exposure, and complete mortality of eggs occurred after 14 d of cold storage, with the egg stage being the most tolerant to -18 °C, and the adults being most susceptible at this temperature. Similar results were obtained by Flinn et al. (2015), reporting that treating flour pallets in commercial freezers for 5.5 days at -17.8 °C was a feasible method for achieving 100% mortality of *T. castaneum* eggs. Other techniques such as hot water treatment (50 °C for 10 min, 55 °C for 5 min and 60 °C for 3 min) (Ben-Amor et al., 2015) and hot air (at 55 °C for 30 min, 60 °C for 20 min and 60 °C for 15 min), have also obtained 100% mortality *E. ceratoniae* larvae (Ben-Amor et al., 2016). In contrast, chlorine, UV-C radiation, ozonated water and alkaline and neutral electrolyzed water only obtained between 8 to 15% of *E. ceratoniae* mortality (Jemni et al., 2014).

3.2. Experiment 2: Overall date fruit quality after treating with the temperature that caused 100% mortality of *Ectomyelois ceratoniae.*

In this second experiment, the freezing treatment of 50 h at -18 °C was used as this was the shortest treatment time that effectively caused 100% *E. ceratoniae* mortality in infested date fruits.

3.2.1. Physical parameters

Color: All samples had a similar color (h°) without significant differences between treatments (Table 3). However, control and frozen dates suffered a similar and significant reduction of L* and Chroma. These luminosity and saturation changes led to a darker skin color. In agreement with our results, Garden-Robinson (2013) indicated that some fruits such as peaches, apples, pears and apricots darken quickly after freezing. The color changes could be attributed to the oxidized chemical compounds formed following freezing, such as water soluble polyphenols (Skrede, 1996). It is well known that polyphenol compounds are more sensitive to enzymatic browning reactions, thus explaining the pronounced color changes (to brown) observed in frozen date fruit (Chassagne-Berces et al. 2010).

Firmness: This is one of the main quality parameters used in sensory acceptance of date fruits by the consumers. Fruit firmness increased in frozen and control date fruits (Table 3). The quality demanded in frozen fruit products is mostly based on the intended use of the product. If the fruit is to be eaten without any further processing after thawing, texture characteristics are more important when compared to its use as a raw material in other industries (Barbosa-Cánovas et al., 2005). This increase of firmness in palm date fruit was previously described by Abboudi and Thompson (1998) who showed that dates stored at 0 °C had a lower textural firmness than dates stored at -

10 °C and -20 °C, and that might have been caused by the higher enzymatic activity at elevated temperatures. On the other hand, Afoakwa and Sefa-Dedeh (2001) indicated that the content of plant cell wall polysaccharide constituents had a high positive correlation with firmness of the samples. The main reason for the change of firmness of date fruits during the storage period was probably due to the increase in cell rigidity and subsequent strengthening of cell wall bonding. Low temperature storage could therefore be used to effectively increase the firmness of date fruits (Aleid et al., 2014).

TSS: The statistical analysis showed that this parameter was unaffected by storage at room temperature or freezing treatment and all of samples had a similar range of ºBrix, ranging from 57 to 62 (Table 3). This unchanged TSS was probably due to the harvesting of palm date fruit at the full maturity stage (Tamar), which is characterized by having the maximum TSS content (Ahmed et al., 1995).

3.2.2. Chemical measurements

Main free amino acids: Suliemen et al. (2012) reported that the date palm extract contains high amounts of essential amino acids, indicating that dates have a high nutritional value. The free amino acids analysis results are shown in Table 4, and they indicate that the freezing treatment did not affect the free amino acids contained in date palm fruit, as there were no significant differences as compared to the control treatment. Likewise, Lisiewska et al. (2007) reported that the differences in the content of amino acids between raw, cooked and frozen seeds were insignificant on broad bean seeds.

Composition and concentration of sugars: Date fruit is unique in relation to freezing treatments, as it has a very high sugar content. Freezing characteristics, such as freeze-thaw temperatures, ice crystal growth, freeze-concentration and freeze-drying are functions of solute concentration (Shomer et al., 1998). The sugar analysis on date fruit showed that they are rich in glucose, fructose and sucrose, which are easy to digest by human cells and useful for getting the energy required for metabolic processes (Sulieman et al., 2012). Sucrose is one of the main sugars that is characteristic of the Deglet Noor cultivar and one of the main reasons behind the very pleasant taste of the fruit (Mrabet et al., 2008, Guizani et al., 2010). The freezing treatments increased the content of glucose and fructose as compared to day 0, maintaining the sucrose and the total sugar content to similar levels as compared to the rest of the samples (Table 5). This increase in monosaccharides recorded during the freezing treatment was previously observed in frozen date fruits at -20 °C (Alhamdan et al., 2015). This could be due to the effect of freezing on the concentration of solutes more than the effect of invertase hydrolyzation during storage of date fruits, when the concentration of sucrose decreases as it is converted to glucose and fructose, with the concentration of these monosaccharides increasing as a consequence (Chang et al., 1998). In agreement with our results, Chung et al. (2013) reported that the freezing pretreatment can increase the glucose and fructose content in the juices extracted from *Prunus mume* fruit with no significant difference in sucrose content.

Antioxidant activity: The freezing treatment significantly reduced the antioxidant activity of the fruits samples as measured by FRAP and DPPH (Table 6). In accordance with our results, storage at -18 °C decreased antioxidant activities in fresh spring onions (Hadidi et al., 2014), peas and spinach (Hunter and Fletcher, 2002). Moreover, Jeusti Bof *et al.* (2012) observed that the antioxidant activity of pear pulp stored at -15 °C for 90 days resulted in a significant decrease as compared to fresh fruit. Similarly, Shofian *et al.* (2011) reported that fresh starfruit and mango exhibited significantly higher FRAP and DPPH values as compared with that of freeze-dried samples at -20 °C for 24 hours.

Total Phenolic Content: Palm date fruit are rich in phenolic compounds, and Tunisian dates, especially, may be considered to be a good source of these compounds (Benmeddour et al., 2012). Therefore, date fruit can be considered a potential natural source of bioactive phytochemicals that play major roles in human health as free radical scavengers (Louaileche et al., 2015). The sample's phenolic content averages ranged from 0.81 to 0.96 g GAE kg^{-1} (Table 6). Results obtained in this study were higher than those of Algerian date varieties, 0.02 to 0.08 g GAE/ kg fw, as analyzed by Mansouri *et al.* (2005). Closer to our results, Kchaou *et al.* (2013) showed average levels for Tunisian date varieties to be 1.6 to 2.2g GAE/ kg fw. The freezing treatment did not cause any significant changes in TPC in date fruit, as previously reported by Rickman *et al.* (2007), who informed that freezing caused minimal destruction of phenolic compounds in fruits, with retention levels dependent on cultivar. Our results agree with those reported for raspberry (De Ancos *et al.*, 2000, Mullen et al. 2002) when fruits were stored at - 20 °C for 12 months and highbush blueberries (Scibisz and Mitek, 2007) when fruits were stored at -18 °C for 6 months .

3.2.4. Microbiological analysis

The microbial load of date samples are shown on Figure 2. The initial microbial counts for untreated samples were 2.7 ± 0.1 log cfu g^{-1} for mesophilic bacteria, 4.2 ± 0.2 log cfu g^{-1} for yeast and 4.0 ± 0.2 log cfu g^{-1} for molds. These microbial counts remained virtually unchanged in both samples stored for 3 d at room temperature (control) and those treated for 3 d with the freezing treatment. Our results agree with the UNIDO report (2004), which states that freezing processes are not suitable for the destruction of the microorganisms present on fruits and vegetables. In this context, Garden-Robinson (2014) informed that microorganisms do not grow at freezing temperatures, but most are not destroyed and will multiply as quickly as ever when the frozen food is thawed and allowed to be kept at room temperature.

3.2.5. Sensorial analysis

Sensorial quality is an important parameter used by producers to determine shelf-life and to establish consumption guidelines for marketing purposes (Liang et al., 2015). Freezing is one of the most important methods for retaining quality during long-term storage (Barbosa-Cánovas et al. 2005). The sensorial quality scores of palm date fruit were absolutely not affected by the freezing treatment, as all the sensorial attributes such as color, flavor and texture remained stable (with mean scores about 3.2, 3.3 and 3.8, respectively). This was also true for the overall acceptance (Table 6) of the fruit before as well as after treatment. These results qualify the freezing treatment as a good tool for the preservation of sensorial quality parameters. Thus, in all the samples, the sensorial parameters scores were above the limit of marketability (> 3). Similar overall quality results for frozen storage of raspberry fruit were reported by De Ancos et al. (2000). However, Shomer et al. (1998) reported that date fruits stored at -18 °C for 10 months developed tissue injuries, evidenced as yellow-brown spots, pale color and cell sap leakage. In our experiment, these chilling injuries were not seen, which was probably due to the short treatment time used.

4. Conclusions

All the freezing treatments studied (-18 °C for50 h, 77 h and 125 h) lead to 100% mortality of all the stages of *E. ceratoniae* in date fruit, and these treatments can be used as an alternative tool to chemical treatments. The use of a freezing treatment of 50 h maintained all the physico-chemical studied parameters stable, with only a significant reduction on the antioxidant activity and an increase of monosaccharides. The microbial and sensorial qualities were not affected by the freezing treatment.

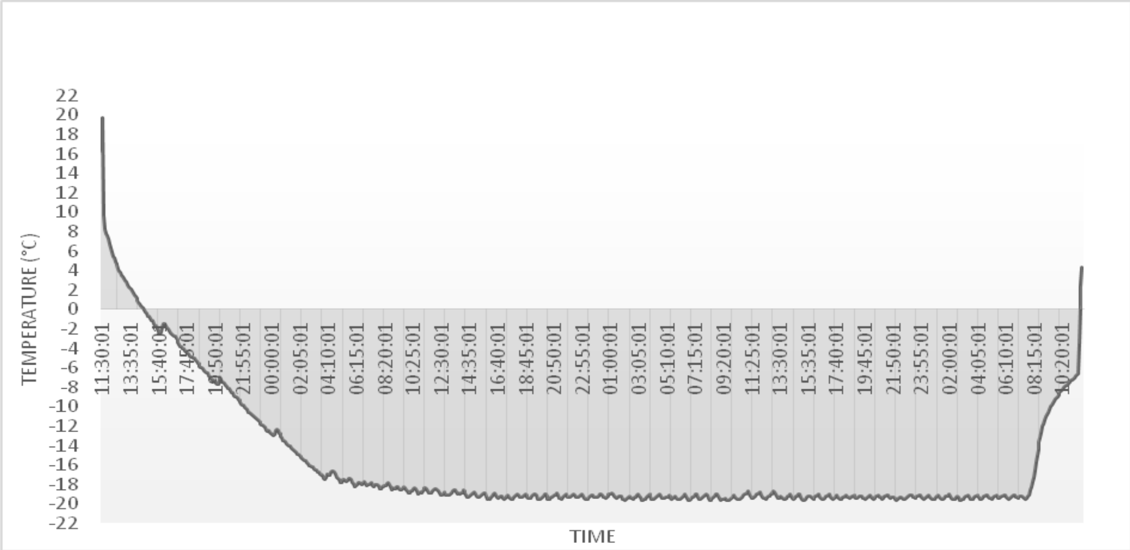

Figure 1: Total duration of freezing treatment process (3 days) of the shorter treatment that provided 100% *Ectomyelois ceratoniae* mortality (50 h at -18 °C).

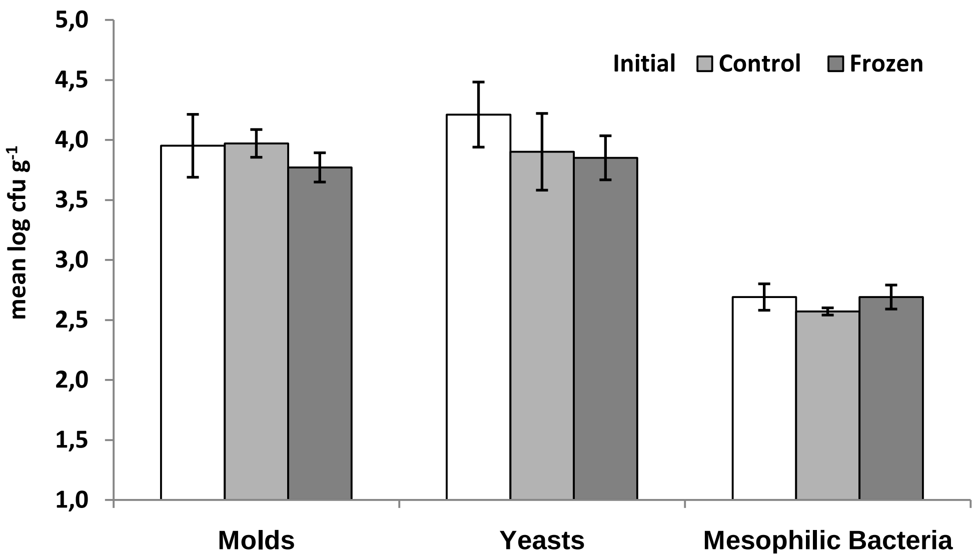

Figure 2. Microbial quality (log cfu g $^{-1}$) of Deglet Noor date fruits on control or frozen treatment. Data are means (n = 3) ± ES.

Table 1. Effect of freezing treatments (-18 ºC) on *Ectomyelois ceratoniae* mortality in infested Deglet Noor date fruits.

Treatments	Samples	N ºdates	Non infested dates		Natural infested dates		Stage of E. *ceratoniae*						Other pests		Feces and webbing larvae
							Young Instars		Old Instars		Pupae				
		N	N	%	n	%	n	Mortality	n	Mortality	n	Mortality	n	%	n
50 h	**Untreated**	300	131	44	169	56	36	0	56	0	8	0	5	0	64
	Treated	300	141	47	159	53	38	100	63	100	7	100	3	100	48
77 h	**Untreated**	300	76	25	224	75	68	0	54	0	8	0	8	0	86
	Treated	300	75	25	225	75	52	100	72	100	10	100	22	100	69
125 h	**Untreated**	300	63	21	237	79	89	0	59	0	16	0	18	0	55
	Treated	300	66	22	234	78	70	100	50	100	11	100	17	100	86

Untreated samples: Date fruits kept at room temperature (20 ºC) and treated date fruits were kept at -18 °C. n: number.

Table 2. Guide of sensory evaluation for date fruits.

	1	2	3	4	5
Color	Very dark/Very yellow	Dark brown/ Yellowish brown	No uniform brown	Caramelized brown	Translucent brown uniform and bright color
Texture	Very soft, deformed and glued/very firm and wrinkled	Quite soft, skin separated from pulp and sticky	Soft but keeps its shape	Uniform firmness	Firmness uniform with its original shape when gently pressed with your fingers, not too dry, not too sticky
Flavor	High acidity/High fermentation/Bitterness	Slightly acid/fermented, strange taste	Sweet just enough without foreign flavors	Sufficiently sweet	High perception of sweetness
Overall quality	Extremely poor	Poor	Acceptable and limit of usability	Good	Excellent

Table 3. Physico-chemical parameters of Deglet Noor date fruits on control or frozen treatment.

Treatment	hº	L*	Chroma	Firmness (N)	TSS (%)
Initial	65.92[y] a	38.18[y] a	19.25[y] a	9.76[x] b	58.33[z] a
Control	65.88 a	31.07 b	12.81 b	11.78 a	62.33 a
Frozen	63.32 a	29.87 b	12.95 b	13.51 a	57.67 a

[z] Data are the means of 3 replicates. [y] Data are means of 5 replicates. [x] Data are means of 12 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to LSD test.

Table 4. Free amino acids (mg kg^{-1}) of Deglet Noor date fruits on control or frozen treatment.

Treatment	Alanine	Arginine	Aspartic acid	Glutamic acid	Methionine	Proline
Initial	5.43[z] a	4.26 a	7.66 a	8.54 a	10.21 a	14.50 a
Control	4.48 a	4.16 a	7.10 a	8.38 a	9.82 a	13.93 a
Frozen	4.45 a	3.81a	8.64 a	8.85 a	9.92 a	12.83 a

[z] Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to LSD test.

Table 5. Individual sugars contents (g kg^{-1}) of Deglet Noor date fruits on control or frozen treatment

Treatment	Glucose	Fructose	Sucrose	Total sugars
Initial	138.7[z] b	171.3 b	484.9 a	794.9 a
Control	160.3 a	196.8 ab	409.6 a	778.9 a
Frozen	162.3 a	208.9 a	414.7 a	773.8 a

[z] Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to LSD test.

Table 6. Antioxidant activity, total phenolic compounds and overall quality of Deglet Noor date fruits on control or frozen treatment.

Treatment	FRAP (g AAE kg^{-1})	DPPH (g AAE kg^{-1})	TPC (g AAE kg^{-1})	OQ (1 to 5)
Initial	1.09[z] a	0.86 a	0.96 a	3.65 a
Control 3 d	1.01 a	0.82 a	0.84 a	3.60 a
Frozen 3 d	0.67 b	0.48 b	0.81 a	3.30 a

[z] Data are the means of 3 replicates. Means within the same column with different letters are significantly different ($p \leq 0.05$) according to LSD test.

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GENENRAL CONCLUSIONS

GENERAL CONCLUSIONS

The general conclusions of the current PhD Thesis were as follows:

For the analysis of competitiveness and sectorial position of Tunisian palm date fruit Deglet Noor cv. in the Mediterranean basin:

1. The market share indices (constant part of the market) are more stable, with a slight upward trend. This indicates that Tunisia's Mediterranean market is stable, and is well above the index of all competing countries throughout the period analyzed. This highlights the importance of the Tunisian date in the Mediterranean Basin and Iran.
2. The Specialization index average (BIS = 6405.99) and the Dependency index (DI = 17.38), showed Tuninia's high specialization in the date palm sector, and independence to satisfy their supply, situating Tunisia ahead of its traditional competitors such as Algeria and Iran, specifically for the Deglet Noor exports sector.
3. The Tunisian trade balance index remained stable too, being close to 100 for the 20 years analyzed, indicating that the country continues to be a net exporter of dates. Conversely, imports in this sector are negligible, as in Iran, Algeria and Israel, its main competitors.
4. The competitive price index for Tunisia was very unstable from one country to another over the period analyzed. However, we can conclude that Tunisia is gaining a competitive position in terms of date export prices, since the competitive price index (CPI) for exports were significantly reduced as compared to the other countries studied for the period 2001-2010, except for Iran and Egypt, which have had very variable export CPIs, with significant reductions since 2007.

For the efficacy of physical postharvest treatment (hot water treatments-HWTs, hot air treatments-HATs and freezing), on *Ectomyelois certaniae* (Zeller) mortality and palm date fruit quality, we concluded that:

5. All HWTs, 50 °C 10 min, 55 °C 5 min and 60 °C 3 min, studied in laboratory conditions, achieved 100% *E. ceratoniae* larvae mortality.
6. Regarding quality parameters, the HWTs used did not cause any heat damage and maintained the fruits at optimal sensorial quality during the storage period, 30 d at 2 °C plus 4 d at 23 °C, although a reduction in color parameters and bioactive compounds such as antioxidant activity and total phenolic compounds was measured.
7. Concerning HATs, all the applied treatments, 55 °C 30 min, 60 °C 15 min and 60 °C 20 min, resulted in 100% *E. ceratoniae* larvae mortality.
8. The HATs did not affect the quality of the fruit, but reduced mesophilic bacterial counts. HATs, especially the combination of highest temperature and longer time of application (60 °C for 20 min), decreased the antioxidant activity (25% in DPPH and 14% in FRAP assay). The combination 60 °C for 15 min was the treatment that best maintained fruit quality for 45 d at 2 °C plus 4 d at 23 °C.
9. The results of the freezing treatment showed that all the studied treatment times (50 h, 77 h and 125 h at -18 °C) led to 100% mortality of all stages of *E. ceratoniae* in date fruits.
10. The use of a freezing treatment of -18 °C for 50 h maintained all the physico-chemical parameters stable, with only a significant reduction on the antioxidant activity. Microbial and sensorial quality were not affected by freezing treatment.

The current work confirms that Tunisia has a highly important trade position in the palm date fruit sector compared to the rest of the Mediterranean basin. On the other hand, this Thesis has revealed that all the studied physical postharvest treatments resulted in 100% mortality of *E. ceratoniae* found in date fruit Deglet Noor and these treaments can be used as an alternative to chemical treatments whilst maintaining postharvest fruit quality.

SCIENTIFIC PRODUCTION

SCIENTIFIC PRODUCTION DURING PREDOCTORAL PERIOD

a. Original papers published in peer-reviewed journals (ISI, Institute for Scientific Information):

Ben-Amor, R., Aguayo, E., Miguel-Gómez, M.D. 2015. The competitive advantage of the Tunisian palm date sector in the Mediterranean region. Spanish Journal of Agricultural Research, DOI: 10.5424/sjar/2015132.6390.

Ben Amor, R., Dhouibi, M.H., Aguayo, E., 2016. Hot water treatments combined with cold storage as a tool for on *Ectomyelois ceratoniae* mortality and maintenance of Deglet Noor palm date quality. Postharvest Biology and Technology, DOI: 10.1016/j.postharvbio.201509.005.

Ben Amor, R., Miguel-Gómez, M.D., Martínez-Sánchez, A., Aguayo, E., 2016. Effect of hot air on *Deglet Noor* palm quality parameters and on *Ectomyelois ceratoniae.* Journal of Stored Products, DOI: 10.1016/j.jspr.2016.03.001.

b. Original papers submitted to peer-reviewed journals (ISI, Institute for Scientific Information):

Ben Amor, R., Miguel-Gómez, M.D., Dhouibi, M.H., Hermi, N., Aguayo, E., 2016. Freezing treatments for *Ectomyelois ceratoniae* mortality and maintenance of *Deglet Noor* palm date quality. Submitted to Journal of the Science of Food and Agriculture.

c. Congress

Ben Amor, R., Miguel-Gómez, M.D., Aguayo, E., 2013. "Tratamientos de aire caliente para la conservación de dátiles "*Deglet Noor*" como alternativa al bromuro de metilo " 64º

Congreso Sociedad Agronómica de Chile y XXII Congreso Chileno de Fitopatología. Viña del Mar. Chile, 23-26th September. Poster Communication. Book of Abstracts, p. 249.

Ben Amor, R., Aguayo, E., Miguel-Gómez, M.D., 2015. Calidad de dátiles tunecinos "Deglet Noor" sometidos a baños térmicos como alternativa al bromuro de metilo. 3º Workshop en Investigación Agroalimentaria – WiA13, Actas del III Workshop en Investigación Agroalimentaria. Cartagena, 12-13th May. Oral Communication. Book of Communications, p. 38-41.

Ben Amor, R., Aguayo, E., 2014. "Tratamientos de agua caliente para la conservación de dátiles tunecinos "*Deglet Noor*" como alternativa al bromuro de metilo". XI Simposio Nacional y VIII Ibérico Sobre Maduración y postcosecha. Valencia, 21-23th October. Poster Communication. Book of Abstracts, p. 80.

Ben Amor, R., Miguel-Gómez, M.D., 2014. "La competitividad del dátil tunecino "*Deglet Noor*" en la Cuenca del Mediterráneo". XI Simposio Nacional y VIII Ibérico Sobre Maduración y postcosecha. Valencia, 21-23th October. Poster Communication. Book of Abstracts, p. 160.

Ben Amor, R., Aguayo, E., Miguel-Gómez, M.D., 2015. Competitiveness of palm dates fruit of Tunisia in the Mediterranean region. 4º Workshop en Investigación Agroalimentaria – WiA15, Proceedings of the 4th Workshop on agri-food research. Cartagena, 11-12 th May. Oral communication. Book of Communications, p. 224-227.

Ben Amor, R., Miguel-Gómez, M.D., Aguayo, E., 2015. "Aplicación de atmósferas controladas para la conservación de dátiles tunecinos *Deglet Noor*." XIV Congreso Nacional de Ciencias Hortícolas, Retos de la Nueva Agricultura Mediterránea. Orihuela, 3-5th June. Oral Communication. Book of Abstracts, p. 168.

Ben Amor, R., Miguel-Gómez, M.D., 2015. "Análisis de la competitividad del sector del dátil tunecino en el entorno mediterráneo." X Congreso Nacional de Economía Agraria. Córdoba, 9-11th September. Oral Communication. Book of Communications, p 163-168.

Ben Amor, R., Miguel-Gómez, M.D., 2016. "The competitive advantage of the Tunisian palm date sector in the Mediterranean region." 1st European Conference of Post graduate Horticultural Scientists (ECPHS). Palermo, 12-13th May.