Volume 29(1). Published May, 01, 2016
www.jnsciences.org
E-ISSN 2286-5314

Partial vacuum and active modified atmosphere packaging for keeping overall quality of dates

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Abstract - This study was carried out during two successive seasons 2011 and 2012 at the Aridlands and Oases Cropping Laboratory, Institute of the Arid Regions Medenine. The effect of several levels of partial vacuum (PV) and gas composition in modified atmosphere packaging (MAP) on overall quality of *Deglet Nour* date palm at Tamar stage during storage for 30 days at 20°C was studied. The following treatments were applied, unpackaged dates (Control), active MAP: 85 kPa CO_2, 60 kPa CO_2, 20 kPa CO_2, 85 kPa O_2, 60 kPa O_2, 20 kPa O_2, 100 kPa N_2, 60 kPa N_2 and 20 kPa N_2, balance air. In addition 100, 90, 80, 60 and 25% of vacuum packaging were applied. During storage physical and chemical attributes of dates as well as microbial growth were monitored. The results were clearly stated that there is a positive relationship between CO_2 concentrations and fruit physical and chemical properties. In addition, both MAP and VP induced a better quality of dates after storage compared to control. The CO_2 active MAP was more effective in keeping date's quality after storage than the others gases combinations and the vacuum, being 60 kPa CO_2 the best concentration.

Keywords: *Deglet Nour* date palm, quality, storage, CO_2 concentrations.

1. Introduction

The production of dates in Tunisia is increased every season. *Deglet Nour* cv. is the main cultivar produced in the south areas of Tunisia, being the pillar of the economy of Djerid and Nefzaoua regions. That increase is accompanied with several problems during storage. In fact, the careful harvesting and optimal horticultural maturity at harvest are the major factors responsible for extending the shelf life of dates. During the storage, bacteria, fungi, or yeasts growth are the most important responsible for the spoilage of dates.

Modified atmosphere packaging (MAP) technique alters the composition of normal air (78.9 kPa N_2, 21kPa O_2, 0.03 kPa CO_2 and traces of noble gases) to provide an optimum atmosphere for increasing the storage length and overall quality of the foods (Al-Ati and Hotchkiss 2002; Farber et al. 2003; Hamayouni et al. 2015). MAP combined with chilling temperatures could be used to enhance the safety and extending the shelf life of whole and fresh-cut fruit and vegetables (Al-Ati and Hotchkiss 2002; Toivonen and DeEll 2002; Farber et al. 2003; Sapers et al. 2006; Sandhya 2010; Martínez-Hernández et al. 2013; Oliveira et al. 2015). During storage, O_2 is consumed and CO_2 is generated by produce respiration. For that, O_2 concentrations are usually reduced below and CO_2 increased above atmospheric concentrations in order to reduce microbial activity. Microorganisms are affected indirectly by lowering ripening and senescence and directly by restriction of O_2 and antimicrobial activity of CO_2; superatmospheric O_2 has also been shown to be antimicrobial but is not yet employed at commercial scale (Sapers et al. 2006; Sandhya 2010; Martínez-Hernández et al 2013; Oliveira et al. 2015). Due to it, O_2 within the package, aerobic pathogens might grow. Then, the reduction in O_2 and/or the increase in CO_2 concentrations in the atmosphere reduce the growth of gram-negative and aerobic spoilage organisms, while growth of gram-positive, microaerophilic species increases. MAP

effect on yeasts is negligible, however, molds are aerobic microorganisms and therefore CO_2 can cause growth inhibition at 10% concentration, although the effect is fungistatic.
Generally, an atmosphere of 3-6 kPa O_2 and 2-10 kPa CO_2 achieves microbial control and extends the shelf-life of a wide variety of fresh-cut products (Oliveira et al. 2015). Others gases such as noble gases, nitrous and nitric oxides, sulfur dioxide, ethylene, chlorine, as well as ozone and propylene oxide have been investigated. However, due to safety, regulatory and cost considerations, they have not been commercially applied. These gases are combined in three ways for use in modified atmospheres: inert blanketing using N_2, semi reactive blanketing using CO_2/N_2 or $O_2/CO_2/N_2$ or fully reactive using CO_2 or CO_2/O_2 (Farber et al. 2003). CO_2 is the only one that has significant and direct antimicrobial activity. In general, CO_2 in MAP results in an increased lag phase and generation time during the logarithmic phase of growth of the organisms involved, with inhibition being concentration and temperature dependent (Al-Ati and Hotchkiss 2002; Farber et al. 2003; Oliveira et al. 2015).
Moderate vacuum packaging (VP) is another type of packaging of fresh fruit and vegetables. It reduces the amount of O_2 in the storage atmosphere, slows the respiration rate of the stored produce, and inhibits growth of microorganisms. Any O_2 consumed is converted to CO_2. This system also prevented enzymatic browning of cut vegetables and fruits and it controls the survival and growth of pathogenic bacteria (Gorris and Peppelenbos 1992).
In date fruit the declining use of postharvest pesticides due to their toxic effects on health, makes necessary to look for technically and economically feasible sustainable commercial alternatives for keeping their overall quality and safety (Jemni et al. 2014ab).
The objective of this study was to investigate the effects of MAP and VP on the physical, chemical and microbial quality of *Deglet Nour* date palm cultivar throughout shelf-life at 20°C. As far as we know no other studied on this purpose have been accomplished with that cultivar.

2. Materials and methods

2.1. Plant material

Date fruits (cv. *Deglet Nour*) were hand harvested by professional pickers at the end of October at full maturity (‘Tamar’) stage from a commercial farm located in an Oasis of the Governorate of Kebili (South of Tunisia). Date bunches were detached from the head of the tree palm and placed on the ground to avoid crushing and the abscission of dates. The bunch was then cut into spikelets and carefully selected. After arriving to the laboratory, they were manually detached from the spikelets, sorted looking for good visual quality and uniformity and those damaged were discarded.

2.2. Modified atmosphere and vacuum packaging of dates

200±10g of dates were distributed into 750 mL bags with a 30 µm thickness BPP film (CO_2/O_2 permselectivities is closer to 1). After some preliminary tests, the following treatments were applied:

- Unpackaged date fruits (Control)
- Active MAP: 85±0.1 kPa CO_2, 60±0.1 kPaCO_2, 20±0.1 kPa CO_2, 85±0.1 kPaO_2, 60±0.1% kPa O_2, 20±0.1 kPaO_2, 100±0.1 kPaN_2, 60±0.1 kPa N_2 and 20±0.1 kPaN_2, balance air.
- VP: 100, 90, 80, 60 and 25% of vacuum.

The introduction of gas and the packaging of dates were did by using an Orved Vacuum Packaging (Machine Model 200 A, Wolfertschwenden, Germany). Three replicates per treatment were prepared and then stored at 20ºC. This temperature was selected to simulate the most commonly used at commercial distribution and retail sale scale in Europe, which shorten the shelf-life of dates (Jemni et al. 2014a).

2.3. Microbial analysis

To determine microbial growth, three randomized samples from each treatment were taken at the beginning and at the end of storage. The colonies of mold, yeast and total mesophilic were determined according to NF V 08-059(1995) and NF V 08-05(1999) respectively. All microbial counts were reported as $\log_{10}$ colony forming units per g of sample (log cfu g^{-1}).

2.4. pH, titratable acidity and moisture content

After removing the pits, the dates were cut into small pieces, and ground into a uniform mash. The pH was potentiometrically measured using a digital pH-meter (Model Neo Met pH 200L ISTEK, Seoul, Korea) equipped with temperature control probe (NF V 05-108 1970). Titratable acidity (TA, g citric

acid 100 g^{-1} fw) was assessed by titrating the sample extract with 0.1N NaOH (NF V 05-101 1974). The moisture content was determined according to NF V 05-105 (1974).

2.5. Sugars concentration

The quantification of reducing sugars was determined according to the method of 3.5-dinitrosalycilique acid (DNS) (Miller 1956), in which1mLof diluted sample extract was mixed with 1.5mLof DNS. The mix was incubated at 100°C for 10 min in a water bath. After that, 10 mL of distilled water was added to the mix. The optical density (OD) was measured at 530 nm wave length using a spectrophotometer (Spectro UV-Vis Double PC 8 Auto Cell, UVD-3000, LABOMED, Inc. Los Angeles, U.S.A).

The determination of total sugars was based in the Dubois et al. (1956) method by using phenol and concentrated sulfuric acid as reagents. 200 µL of diluted sample extract was added with 200 µL of phenol (5%).The mix was homogenized by vortex and 1mLof concentrated sulfuric acid was added. After homogenization, the mix was incubated in a water bath at 100°C for 5 min. Then the tubes were cooled in an ice bath and placed for 30 min in darkness. The OD was subsequently measured at 492 nm.

The concentration of reducing and total sugars in the extract was calculated based on the calibration curve obtained from a glucose standard.

2.6. Total phenolics concentration

Frozen samples (0.5 g) were diluted with 3 mL MeOH and maintained in darkness for extraction during 1 h. The homogenates were centrifuged at 4ºC for 10 min at 15,000 × g to obtain the extracts. The amount of total phenolics was determined by using the Folin–Ciocalteu reagent based in Artés-Hernández et al. (2010) Briefly, an aliquot of 19.2 µL extract of the supernatant was mixed with 29 µL of Folin–Ciocalteu reagent (1:10, v/v diluted with MilliQ water) and 192 µL sodium carbonate (20%, w/v). The mixture was incubated for 1 h at room temperature in darkness, measuring the absorption at 750 nm (Hewlett Packard 8453, UV–vis spectrophotometer, Columbia, USA). Total phenolic content was expressed as gallic acid equivalents (GAE) in g 100 g^{-1} fw. All extracts were analyzed in triplicate.

2.7. Flavonoids contents

The flavonoids contents were determined according to Chaira et al. (2009). Distilled water (4 mL) was added to 1 mL of sample extract. Then, 0.3 mL of 5% Na NO_2 was added followed by 0.3 mL of 10% $AlCl_3$. Test tubes were incubated at ambient temperature for 5 min, and then 2 mL NaOH 1 M were added to the mixture. The volume of reaction mixture was made to 10 mL with distilled water. The mixture was homogenized and the absorbance was measured at 510 nm using a spectrophotometer (UV_VIS UVD 3000; Labomed, Inc.,California, USA). A calibration curve was prepared with quercetin and the results were expressed as mg quercetin equivalent/100 g FW.

2.8. Statistical analysis

All parameters were determined by triplicate for each treatment and each replicate was analyzed one time. Statistical analysis was performed with Info Stat (version 1). An analysis of variance (ANOVA) and a LSD test were applied in order to evaluate the influence of treatment and storage time on microbial, physical and chemical analysis of dates. A least significant difference (LSD) multiple range test at 5% probability level was used to determine significant differences between means.

3. Results and discussion

3.1. Microbial analysis

As expected the MAP and VP treatments significantly affect the growth of yeast, molds and total mesophilic microorganisms in comparison with the control (Figure 1). The yeast and molds counts increased from 3.68±0.17 log cfu g^{-1} to 7.32±0.06 log cfu g^{-1} and 4.37±0.01 log cfu g^{-1} respectively after 30 days at 20°C for Control and 60 kPa O_2 samples, while decreased for all others treatments. No growth of yeast and molds was detected in samples under 100 and 60 kPa N_2 and in VP samples. Our results agree with those shown by Dehghan-Shoar et al. (2010) on *Sayer* date fruits stored at 30°C under different MAP conditions. In fact, they reported that in the MAP treated dates mold and yeast counts were lower than that of controls and detected a negative correlation between CO_2 levels and

mold and yeast counts. Al-Eid et al. (2012) reported values of yeast and molds for *Khales* dates sealed with 10% CO_2, balance air; 20% CO_2, balance air; 30% CO_2, balance air and with 20% CO_2, balance N_2 less than control sample stored for 27 days at 0°C. Al-Redhaiman (2004) proved that 20%CO_2 is the best method to conserve *Barhi* date at 0°C for 150 days.

The total mesophilic count was 3.82±0.2 log cfu g^{-1} at day 0 and increased with storage time for all types of packaging. This increase was accentuated in control (7.57±0.04 log cfu g^{-1}), 85kPa CO_2 (5.22±0.05 log cfu g^{-1}) and 60 kPa O_2 (4.95±0.09 log cfu g^{-1}), and was stable for samples kept at 60 kPa CO_2 (3.95±0.07 log cfu g-1). However, total mesophilic count decreased in dates under 100 kPa N_2, 60 kPa N_2 and 20 kPaO_2. These results are in agreement with those of Al-Eid et al. (2012) on *Khales* dates stored for 27 days at 0°C under 10, 20 and 30 kPa CO_2, balance air, and with 20 kPa CO_2, balance N_2 .They found the highest aerobic bacteria counts in Control sample when compared to all other MAP treated dates (at 0°C, temperature of long storage). As expected N_2 does not support the growth of aerobic microbes and therefore inhibits the growth aerobic spoilage but does not prevent the growth of anaerobic bacteria (Sandhya 2010).

Al-Ati and Hotchkiss (2002) reported that microbial growth under CO_2 enriched MAP are different from that found in air. CO_2 in excess of 5% v/v inhibits many refrigerated food spoilage bacteria growth, especially psychotropic species. Most mold species require O_2 and are sensitive to high CO_2 concentrations. Many yeast grow anaerobically and are relatively resistant to CO_2. The extent of CO_2 activity depends on the type, number and age of microorganisms, as well as CO_2 concentration, aw and pH of the product, and storage temperature.

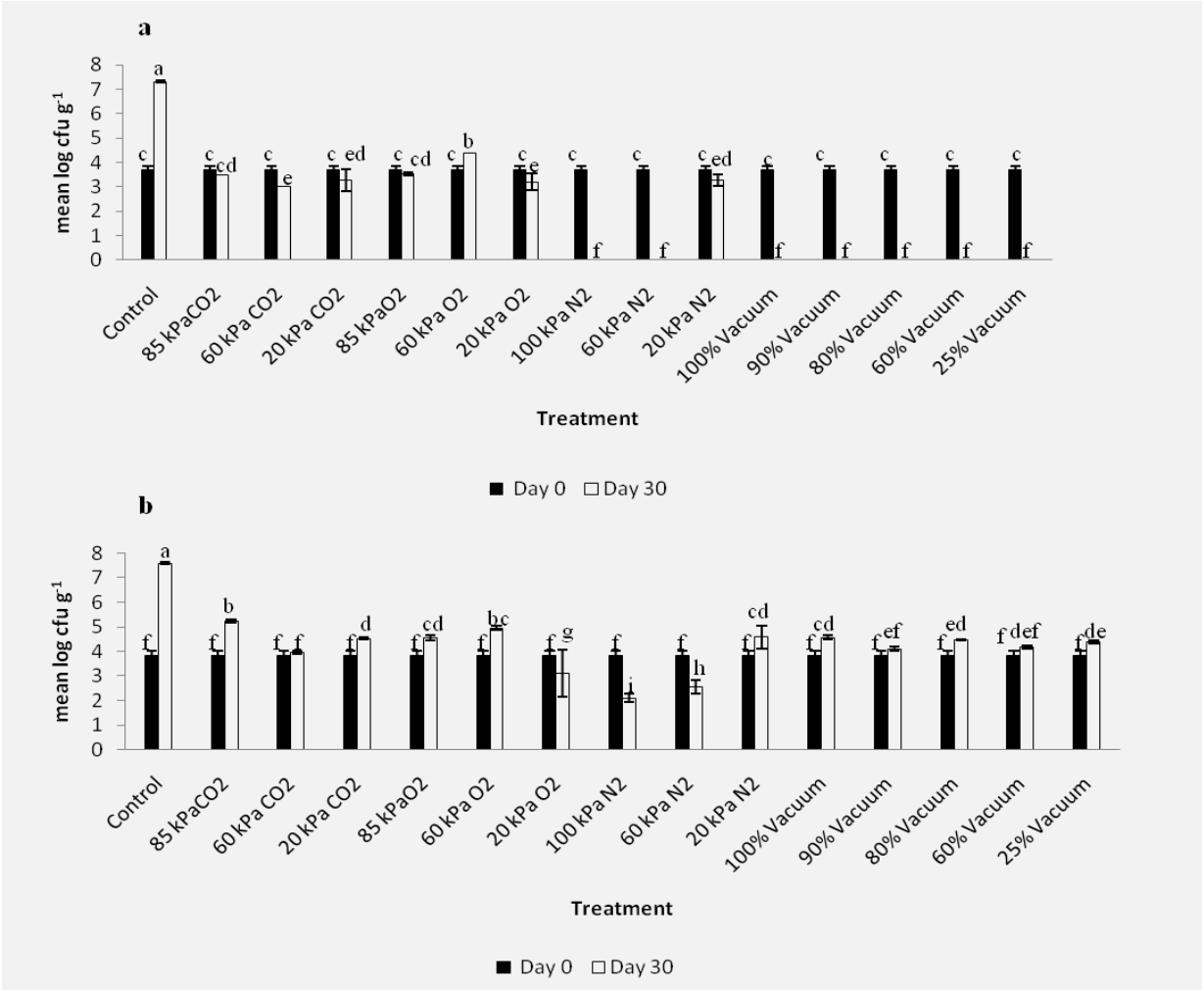

Figure 1. Effect of modified atmosphere and vacuum packaging on yeast and molds (a), mesophilics bacterial (b) counts (mean log cfu g^{-1}) of *Deglet Nour* date after a shelf life of 30 days at 20°C. Data are means (n = 3) ± SD. Data within a bar followed by different letter are significantly different ($p \leq 0.05$) according to LSD test.

3.2. Changes in pH and titratable acidity

The MAP and VP affect significantly the pH and the TA of dates during storage (figure 2). It was stable for samples stored under 85, 60and 20 kPaCO_2, and under 85$_2$ and 60kPa O_2. However, the pH decreased and the TA increased in samples stored at 20 kPa O_2, 100, 60 and 20 kPa N_2, VP and Control. The highest decrease of pH and increase of TA were found under 60 kPa N_2, 25% vacuum and Control. They respectively decreased for pH from 5.4±0.08 to 4.97±0.02 and increased for TA from 0.175±0.031 to 0.229±0.017 g citric acid 100g^{-1}fw. According to Baloch el al. (2006), the pH of *Dhakki* dates stored at 40°C for 4 months gradually declines in samples stored under air, O_2 or N_2. Dehghan-Shoar et al. (2010), showed a pH decrease of *Sayer* dates stored at 30°C under different MAP conditions less pronounced than that obtained in control sample. According to these authors, the pH reduction was mostly due to CO_2 solubility in the fruits' flesh, and to the activity of microorganisms and insects.

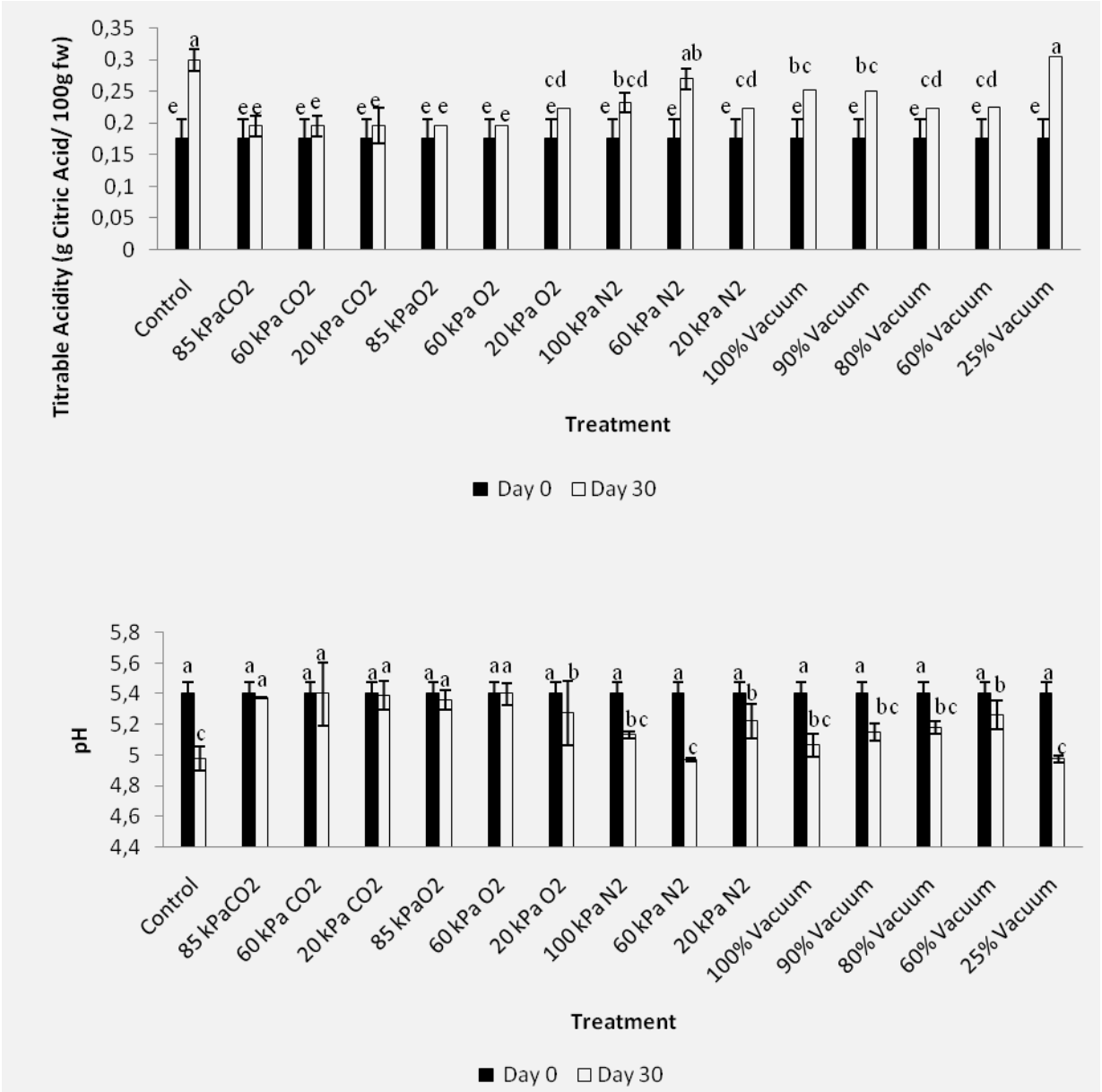

Figure 2. Effect of modified atmosphere and vacuum packaging on pH and titratable acidity of *Deglet Nour* date after a shelf life of 30 days at 20°C. Data are means (n = 3) ± SD.

3.3. Moisture content

The moisture of samples was kept after 30 days at 20°C under 25, 60 and 80% vacuum, 85, 60 and 20 kPa CO_2 and 20% O_2 (figure 3). The moisture decrease was more pronounced in Control from 17.10±0.58% at day 0 to 12.87±0.025% without significant differences with 60 and 100 kPa N_2. Toivenen et al. (2009) showed that water is essential for all metabolic activity in a living cell and hence decline in levels will lead to some form of metabolic impairment or even permanent injury if the water loss is great enough and the moisture is essential on the control of spoilage microorganisms. In addition, high humidity within packages leads to increased risks in water condensation and growth of spoilage microorganisms.

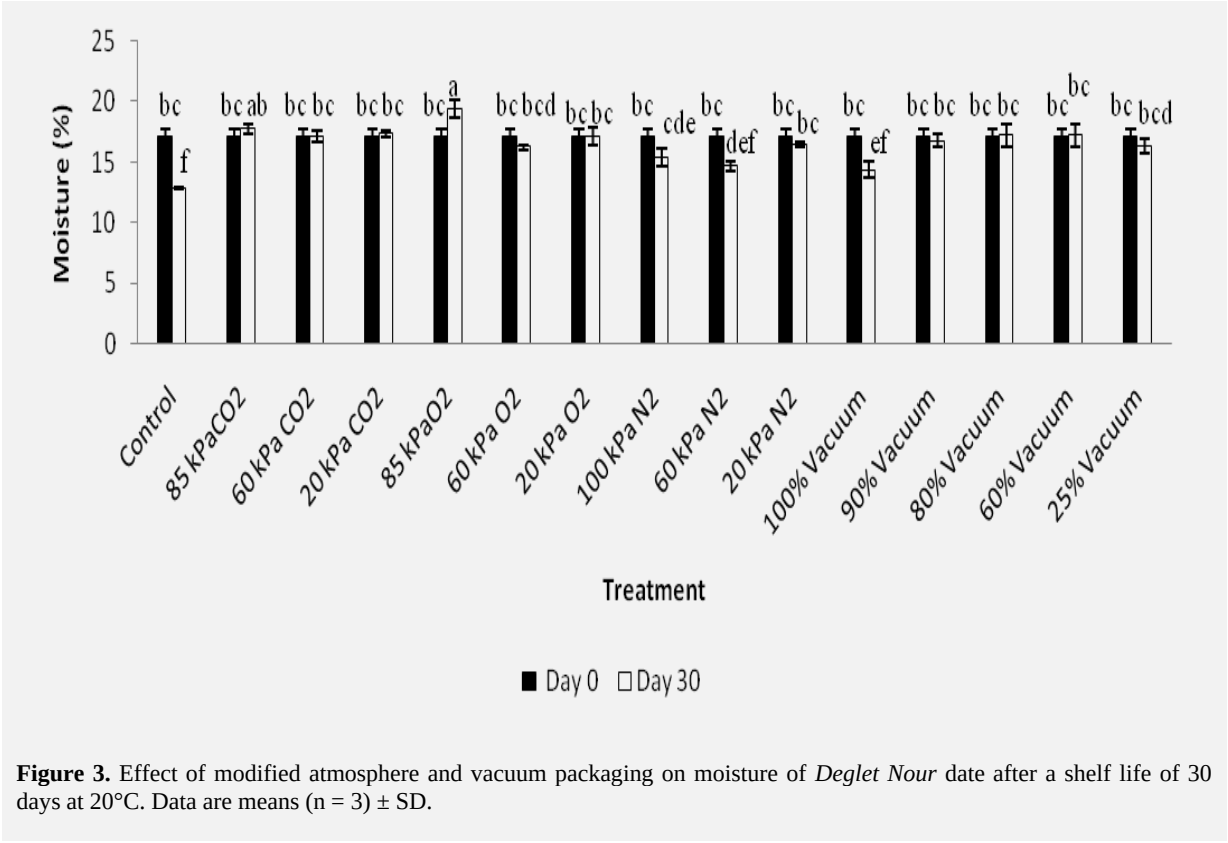

Figure 3. Effect of modified atmosphere and vacuum packaging on moisture of *Deglet Nour* date after a shelf life of 30 days at 20°C. Data are means (n = 3) ± SD.

3.4. Sugars concentration

The MAP and VP treatments had a significant effect on concentration of total sugars, reducing sugars and sucrose of dates stored at 20°C for 30 days (figure 4).After storage, it was found a decrease of total sugars and sucrose content, and an increase of reducing sugar content were found for the following samples: 85 and 20 kPa O_2, kPa100N_2 and 20 kPa N_2, and 60 and 25% vacuum. In fact, for these samples, the total sugar concentration decreased from 83.38±0.41 g$100g^{-1}$fw to a mean of 59.57±7.36 g $100g^{-1}$ fw, the reducing sugar increased from 21.91±0.71 g $100g^{-1}$ fw to a mean of25.95±1.33 g $100g^{-1}$ fw and the sucrose decreased from 61.47±0.71 g $100g^{-1}$ fw to a mean of 33.61±8.13 g $100g^{-1}$ fw. Our results in total sugar concentration are in contrast with those of Al-Redhhaiman (2004, 2005) which found an increase of total sugar in *Barhi* dates stored at 0°C under 5, 10 and 20 kPa CO_2 concluding that 20 kPa CO_2 was the best storage condition. El-Rayes (2009) found a slight increase in total sugar contents of *Barhy* date fruits stored at 6°C under MAP with 0.03, 5, 10, or 20 kPa CO_2 (balance air) and lowest rate of total sugar increment in fruits stored with 20 kPa CO_2 at 0°C. These differences with our results could be very probably due to different storage temperature, being more stimulated the respiration rate and global metabolism in our experiment at a considerably higher temperature. In fact, it is well known that sugars are excellent respiratory substrates.

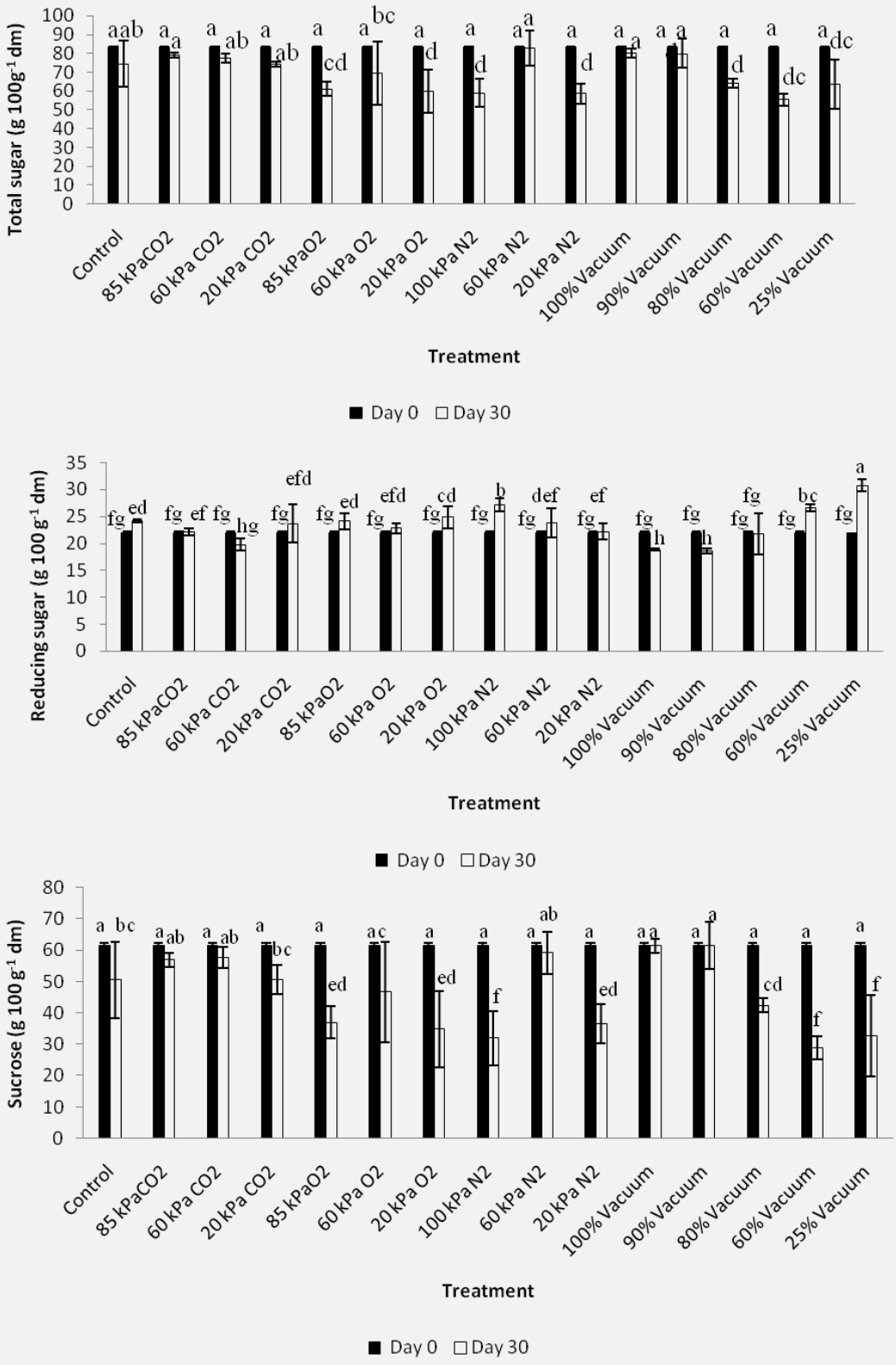

Figure 4. Effect of modified atmosphere and vacuum packaging on total sugars (a), reducing sugars (b) and sucrose (c) contents of *Deglet Nour* date after a shelf life of 30 days at 20°C. Data are means (n = 3) ± SD.

3.5. Total polyphenols changes

The total polyphenols concentration was affected by method of packaging (figure 5). In fact, levels were quite similar to the initial value 0.057±0.002 g GAE $100g^{-1}$ fw for MAP stored samples at

different of CO_2 and O_2 concentrations and for Control. However, it increased for samples packaged under N_2 and vacuum with a maximum value of 0.106±0.002 g GAE $100g^{-1}$ fw. El-Rayes (2009) found significant differences in total phenolic values of *Barhy* date fruits stored at 0°C for 173 days under different MAP (0.03, 5, 10, or 20 $kPaCO_2$, balance air) and cold storage treatments keeping the total phenolic values under 20 $kPaCO_2$. The total polyphenols concentration obtained in our study for *Deglet Nour* cv. was higher than those obtained by Mansouri et al. (2005) (*Deglet Nour* cv. 6.73 ± 0.27 mg GAE100 g^{-1} fw) and lower than those obtained by Besbes et al. (2009) (*Deglet Nour* cv. (493.5 mg GAE 100 g^{-1}fw). This variation of concentration could be explained by several factors such as cv., growing and cultivation conditions (geographic origin, season, climate, fertilizer, soil type, amount of sunlight received), methods, process and stabilization conditions, storage parameters, use of different analytical methods and use of different phenolics standards.

3.6. Flavonoids contents

The concentration of flavonoids decreased for all samples throughout storage with the highest decrease under 85 and 20 $kPaCO_2$, 85 $kPaO_2$ and 20 $kPaN_2$ (figure 5). The flavonoids content decreased from a value of 3.31±0.48 mg quercetin equivalent $100g^{-1}$ fw to about 1.5±0.5 mg quercetin equivalent at $100g^{-1}$ fw. Our results are in contrast with those reported by El-Rayes (2009) which found a slight increase in flavonoids contents in Barhy date during the storage period without significant difference among MAP treatments and cold storage conditions.

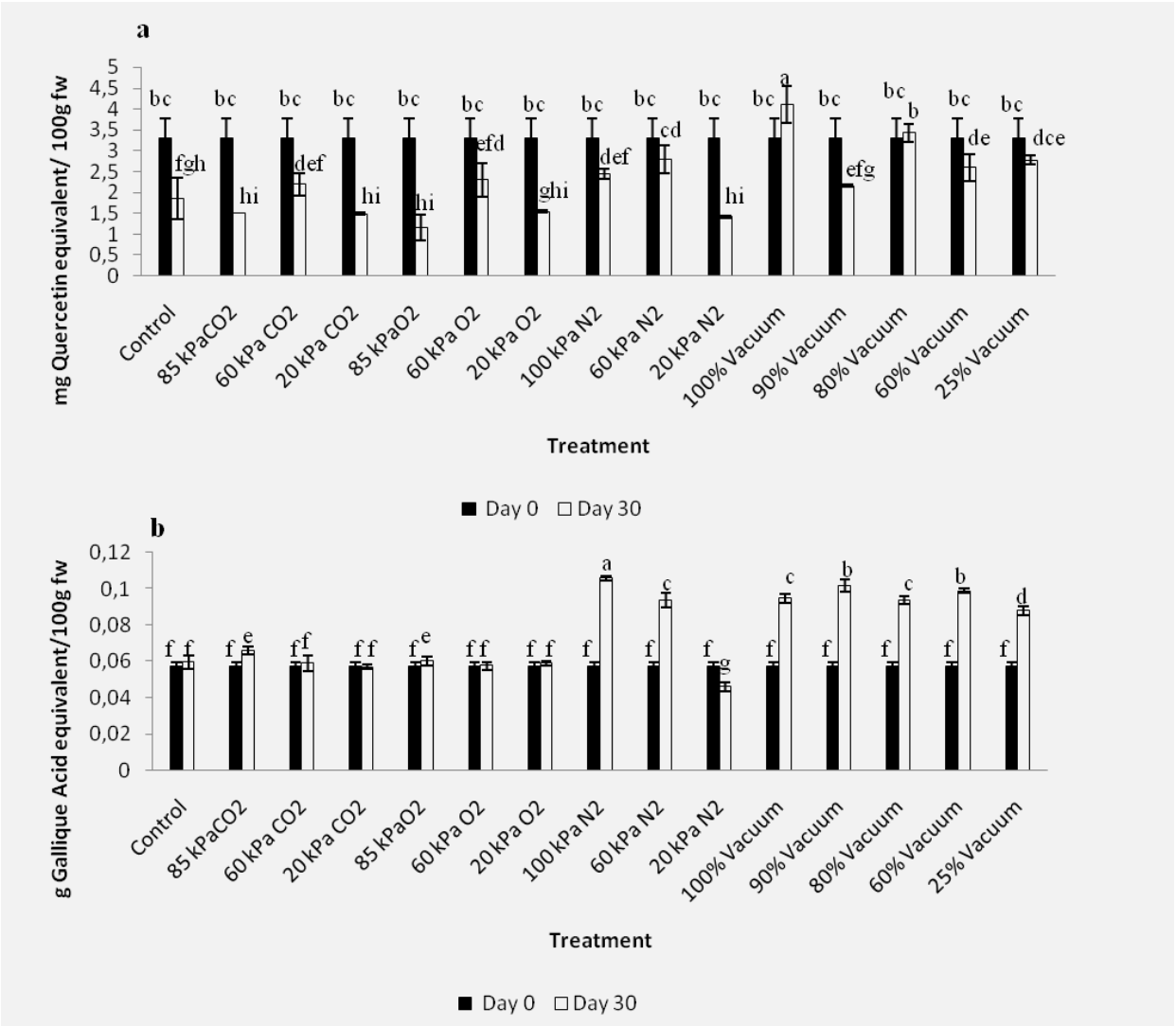

Figure 5. Effect of modified atmosphere and vacuum packaging on flavonoids contents (a) and total polyphenol (b) of *Deglet Nour* date after a shelf life of 30 days at 20°C.Data are means (n = 3) ± SD.

4. Conclusion

The method of packaging is very important for the preservation of quality of fruits, becoming the application of MAP and VP techniques very interesting for dates. As main conclusion of the current work it was found that both techniques are able to lead to a better quality of dates after a commonly used transportation and distribution period in Europe. The CO_2 was the gas that showed the most beneficial effect on keeping overall quality and safety of dates mainly at 60 kPa under active MAP. This method could be considered technically and economically feasible as well as a sustainable alternative for commercialization of *Deglet Nour* date. This work should be achieved by following the variation of gases under the packaging during the storage and by sensorial analysis to evaluate the global quality and to test if there is a presence of rear tastes.

Acknowledgement

The authors are grateful to Institute of the Arid Regions Medenine, Tunisia for providing some facilities.

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