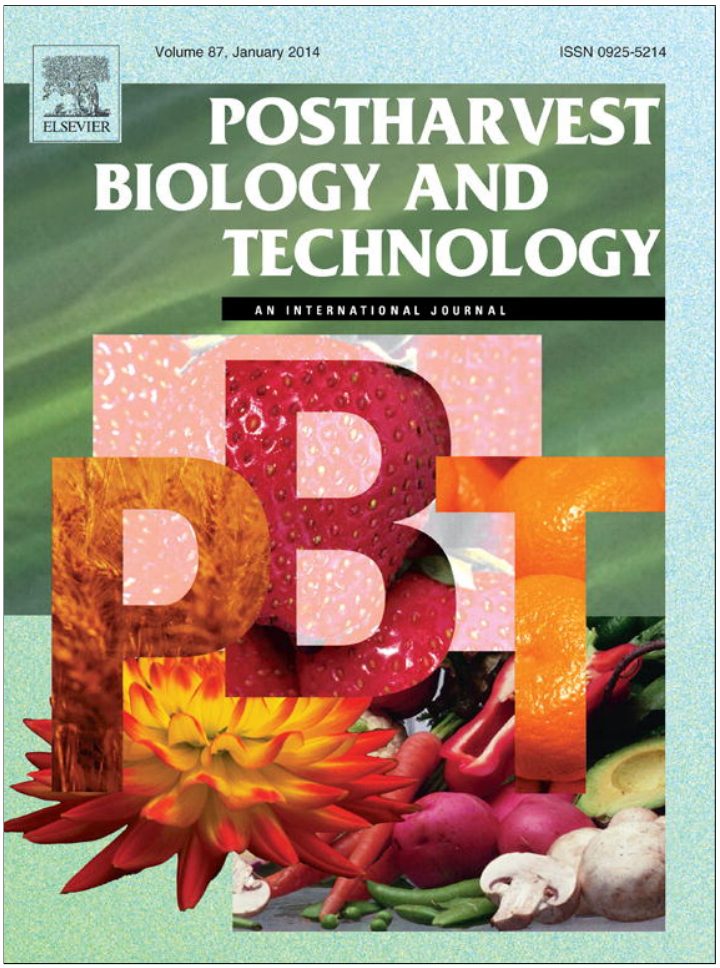

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Conventional and emergent sanitizers decreased *Ectomyelois ceratoniae* infestation and maintained quality of date palm after shelf-life

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ABSTRACT

Several methods have been used to prevent pest diseases and microbial contamination of dates, although their use is being restricted due to harmful effects on humans and/or to the environment. Sustainable sanitation techniques for keeping overall quality and safety of harvested dates should be developed and implemented. The current work studied the effect of NaClO, UV-C, ozonated water and alkaline and neutral electrolyzed water (NEW) on natural infestation by *Ectomyelois ceratoniae* or moth of pyrale, and on overall quality of 'Deglet Nour' dates stored for 30 days at the commercially used temperature of 20 °C. As controls, untreated samples were used. The skin color, firmness, pH, titratable acidity, total soluble solids content, sugar content, total polyphenols, antioxidant activity, microbial counts, sensory quality and moth infestations were monitored. Phenolics content increased after shelf-life. As expected, all sanitizers lowered microbial counts and moth infestation. A dose of 6 kJ UV-C m^{-2} was the most efficient treatment against yeast and molds (without differences with NaClO and O_3), and coliforms, maintaining overall quality of dates after shelf-life. UV-C and NEW (pH 7.2, ORP 814 mV, and 300 mg L^{-1} of free chlorine) were the most effective against moth proliferation, and could be considered as promising useful tools for commercial disinfection of fresh dates and extending shelf-life. As far as we know, no other comparative studies on these postharvest sanitizers on dates have been reported.

1. Introduction

Dates around the world are very often affected by insect infestation from coleopterons, lepidopterons and hymenopterons, as well as by bacteria (such as *Escherichia coli, Staphylococcus aureus* and *Bacillus cereus*), and by several mold and yeast genera (Warner et al., 1990). Among these biological attacks, the most common infestation of dates in Tunisia and Algeria is by the moth of pyrale (*Ectomyelois ceratoniae* Zeller), which causes in Morocco up to 30% date yield loss (Bouka et al., 2001). In Tunisia, it infests 20% of the harvestable dates annually, being the major insect pest both in field and in storage. It degrades the stored dates and causes weight loss, downgrading their commercial value (Haouel et al., 2010). The female moth of *E. ceratoniae* lays eggs on the skin of the date provided with a sticky substance that allows its attachment to the fruit. After hatching, the larvae feed and roam outside at the expense of the date skin, then attempt to enter inside, which can facilitate fungal growth. As penetration is more easy when the skin is thin and shriveled, the attack is more frequent on dried than on soft dates, in which the rate is lower due to the close attachment of the perianth on the skin, reducing access of the young larvae (Dhouibi, 2000).

Several methods have been developed for avoiding postharvest pest diseases and microbial attacks of dates, including fumigants (methyl bromide CH_3Br, phosphine PH_3, sulfur dioxide SO_2, carbon sulfate CS_2, or carbon dioxide CO_2 alone or mixed with ethylene oxide C_2H_4O, among others), vacuum storage, and application of microwaves, chlorine (NaClO), ozone (O_3), ultraviolet (UV) radiation, heat treatment, freezing or irradiation (Zouba et al., 2009; Dehghan-Shoar et al., 2010; Haouel et al., 2010; Ben-Lalli et al., 2013). There is also recent interest in the use of essential oils for postharvest control of the carob moth, but the high doses required

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have limited their eligibility (Ben Jemâa et al., 2013). Indeed, CH_3Br is the most widely used fumigant on stored dates, although due to its being harmful to human health as well as for the environment, its use is scheduled for worldwide withdrawal in 2015 under the Montreal Protocol of the United Nations Environment Program on ozone-depleting substances (UNEP, 1995; Bell, 2000). Because of this, a search of commercial alternatives is required.

Chlorine is a potent sanitizer with strong oxidizing properties, generally effective, comparatively inexpensive, and the most used agent by the food industry for sanitizing both products and equipment. NaClO in water increases pH and generates HOCl, which is the active disinfectant, being more efficient at a pH range of 6.5–7.5. However, NaClO may incompletely oxidize organic food constituents to produce toxic byproducts in processing water, such as chloroform, haloacetic acids or other trihalomethanes. Because of this, in some European countries such as Germany, The Netherlands, Denmark, Switzerland and Belgium, the use of NaClO in plant products has been forbidden. Consequently, alternative sanitizers to NaClO for the food industry should be explored (Betts and Everis, 2005; Artés et al., 2009).

The O_3 is a generally recognized as safe sanitizer in the food industry (FDA, 1997). It is a strong oxidizing agent, very effective on microorganisms through the progressive oxidation of vital plant cell components, preventing microbial growth and thus extending shelf-life (Guzel-Seydim et al., 2004; Artés et al., 2009; Gabler et al., 2010). In addition, a continuous O_3 enriched-air flow has been found to enhance total phenolics contents (Artés-Hernández et al., 2003, 2007). However, effectiveness depends on the application dose, temperature, duration, pH and soluble solids in water (Restaino et al., 1995), and even on the method of washing, as dips or drench (Aguayo et al., 2006).

Non-ionizing UV-C radiation at a wavelength of 190–280 nm is an efficient antimicrobial technique for surface decontamination of fruit and vegetables by reducing natural microflora, delaying senescence, and extending shelf-life. The UV-C damages microbial DNA at 0.5–20 kJ m^{-2}, inhibiting growth (Allende and Artés, 2003; Pan and Zu, 2012). But exposure of cells to visible light after UV-C treatment induces enzymatic photorepair and expression of excision-repair genes that may restore DNA integrity (Lado and Yousef, 2002). The main advantages of UV-C light is that it does not leave any residue, is lethal to most types of microorganisms, is easy to use without extensive safety equipment, and does not have legal restrictions (Artés et al., 2009; Artés-Hernández et al., 2009).

Electrolyzed water (EW) generated by electrolysis of aqueous NaClO is an emerging industrial food sanitizer, more eco-friendly than NaClO, not corrosive to skin, mucous membranes or organic material, and not potentially harmful for human health (Izumi, 1999; Ongeng et al., 2006; Huang et al., 2008; Artés et al., 2009). However, gas emission, metal corrosion, free chlorine content and possible by-products formation must be investigated (Tomás-Callejas et al., 2011). EW involves on-site production, which means there are no chemicals to store or handling costs for dealing with them (Artés et al., 2009). EW has a high oxidation reduction potential (ORP) which could alter metabolic fluxes and ATP production in cells, with strong effects against microorganisms (Rico et al., 2008).

Alkaline EW (AEW) has a pH greater than 11.3 and an ORP of −800 mV or less, with a strong reducing potential, which leads to the reduction of free radicals in biological systems (Huang et al., 2008). AEW has a surface-active effect due to dilute NaOH, dissolved H_2, and active H_2. Neutral EW (NEW) is not corrosive due to its neutral pH and does not affect tissue pH, surface color or appearance (Tomás-Callejas et al., 2011). But studies on the effects of EW against passive or pathogenic microflora in plant foods are still scarce.

The aim of the current work was to assess the effects of NaClO, UV-C, O_3, AEW and NEW treatments at harvest on survival of moths of pyrale and on natural microflora growth, as well as on quality attributes of 'Deglet Nour' dates throughout shelf-life. It was hypothesized that these treatments, applied in the frame of the hurdle technology, could have a synergistic effect on microbial growth while improving shelf-life (Martínez-Hernández et al., 2013). As far as we know, this is the first comparative report about these sanitizing treatments on dates.

2. Materials and methods

2.1. Plant material

The date palm (*Phoenix dactylifera* L.) ($2n = 36$) is a monocotyledon, dioecious and perennial, and the only cultivated species of the genus Phoenix family Arecaceae (Munier, 1973). 'Deglet Nour' dates, the most produced date palm cv. in Tunisia, were harvested at the end of October at fully mature ('Tamar' stage) from a farm located in an Oasis of the Governorate of Kebili (South of Tunisia). Professional pickers detached the bunches of dates from the head of the tree palm and by hand these were placed on the ground 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 at ambient temperature by car about 500 km to Tunis, then by plane to Madrid (Spain), and finally by car about 400 km to the Pilot Plant of the Technical University of Cartagena. Total transport duration was about 7 days. After arrival, dates were manually detached from the spikelets and inspected, damaged fruit were discarded, and sound dates were sorted to achieve uniformity.

2.2. UV-C treatment

The UV-C equipment consisted of two batches of 15 reflectors with unfiltered germicidal emitting lamps (TUV 36W/G36 T8, Philips, Holland) fixed to a chamber frame. The equipment is fully described in Artés-Hernández et al. (2010). According to our preliminary studies on dates, the selected UV-C radiation dose was 6 kJ m^{-2}.

2.3. Electrolyzed water production

In order to produce EW, an Envirolyte EL 400 device (Aquarioja S.L., Madrid, Spain) was used. The EW was generated by using a continuous supply of dilute salt (26% NaCl) in tap water. Two types of processing water with different characteristics were generated. AEW solution (pH 11.28 ± 0.1, ORP = -800 ± 1.5 mV, free chlorine = 290 ± 2.6 mg L^{-1}) was produced at the cathode side, while an electrolyzed acid solution (pH 3 ± 0.1 and ORP > 1100 mV) was produced at the anode side. To obtain a neutral EW solution (NEW), it was drained from the anode and from the cathode and mixed in correct proportions. NEW had a pH value 7.2 ± 0.1, ORP = 814 ± 0.9 mV, and 300 ± 3.2 mg L^{-1} of free chlorine. In order to achieve the desired 100 mg L^{-1} of free chlorine for both AEW and NEW, an appropriate proportion of distilled water was mixed with the original solutions (Tomás-Callejas et al., 2011). For all solutions free chlorine and pH were determined by using a photometer (HI 94771, Hanna Instruments, Eibar, Spain) and a pH meter (Basic 20, Crison, Barcelona, Spain), respectively.

2.4. Ozone production

O_3 was obtained from a commercial generator producing 0.4 mg L^{-1} O_3 (Ambicon S.L., Murcia, Spain). The O_3 level was monitored by using a sensor (EcoSensor, Inc., A-21ZX model, USA). O_3 flow was mixed with tap water and this ozonated water (Silveira

et al., 2010) with 814 mV of ORP and 0.6 ppm O_3 was used for washing the dates.

2.5. Sample preparation, treatments and storage conditions

The dates were processed in a disinfected area at room temperature (about 20 °C). The following treatments were applied:

(a) UV-C: A dose of 6 kJ UV-C m^{-2} was applied to dates in the equipment above described according to Martínez-Hernández et al. (2011). In this treatment no water washing was used.
(b) NaClO: dates were dipped for 4 min in a water solution at about 15 °C containing NaClO (100 mg L^{-1}, pH 6.0), followed by 1 min rinsing in tap water.
(c) O_3: dates were dipped for 2 min in ozonated water (ORP 814 mV and 0.6 ppm O_3) at about 15 °C, followed with 1 min rinsing in tap water.
(d) NEW: dates were dipped for 2 min in 100 mg L^{-1} free chlorine NEW at about 15 °C followed by 1 min dipping in tap water.
(e) AEW: the same treatment as NEW but using AEW.
(f) Control: dates were dipped in tap water at about 15 °C for 2 min.

After sanitizing treatments, dates were manually drained with absorbent paper to eliminate the excess of surface water. Then, 200 g of dates from each treatment were placed in 750 mL PP baskets that were thermally sealed at the top with a 30 μm thickness BPP film (Plásticos del Segura S.L., Murcia, Spain) which was perforated with a 0.7 mm ϕ needle in order to provide an air atmosphere with high relative humidity within packages.

Three replicates per treatment were prepared and then stored in darkness at 20 °C. This adverse temperature was selected to simulate the commonly used at commercial distribution and retail sale scale in Europe, which shortens the shelf-life of dates.

2.6. Detection of pyrale infestation and microbial analyses

The natural pyrale infestation was calculated by the ratio between the number of dates naturally infested by larvae of *E. ceratoniae* observed by eye, and the total number of dates of each replicate expressed as a percentage. The data were obtained from three replicates of 20 dates each.

To determine natural microflora growth on dates, three randomized samples from each treatment were taken on the processing day and after 30 days of shelf-life at 20 °C. Ten g of dates were blended (NF V 08-010, 1996) with 90 mL of sterile tryptone phosphate water (pH 7.0) (Scharlau Chemie S.A. Barcelona, Spain) for 1 min in a sterile stomacher bag (BA6/4/cpg, London, UK) by using a Masticator (Seward Medical, London, UK). Serial dilutions were prepared in 9 mL tryptone phosphate water (NF V 08-010, 1996). By using saboraud oxytetracycline agar base (Scharlau Chemie S.A. Barcelona, Spain), mold and yeast colonies were counted 3 days after incubation at 25 °C (NF V 08-059, 1995) and totals mesophilic were counted on plate count agar after 48 h of incubation at 30 °C (NF V 08-05, 1996). Coliforms bacteria were counted on violet red bile dextrose agar (VRBD, pH 7.2) (Scharlau Chemie S.A. Barcelona, Spain) after 24 h at 37 °C (NF V 08-015, 1991). All microbial counts were reported as $\log_{10}$ colony forming units per g of sample (log cfu g^{-1}).

2.7. Weight loss, firmness, pH, titratable acidity, dry matter and water activity

Weight losses were calculated as percent of the initial fresh weight (fw). Firmness was determined in the two flat sides of each date piece by mean of a texturometer (Ibertest, Madrid, Spain) equipped with a probe of 4.0 mm diameter at 30 mm min^{-1} and with depth of 3 mm. The results were expressed in Newtons (N).

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 (Crison 501, Barcelona, Spain) 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 0.1 N NaOH (NF V 05-101, 1974). Dry matter (dm) and moisture content were determined according to NF V 05-105 (1974).

The water activity (a_w) was determined based on the moisture content using an a_w-meter (Novasina Lab Master-aw, Swiss). Samples were ground into thin pieces and filled into a dried cup, about 2/3 of its capacity. The filled cup was then placed in the measuring cuvette. After removing the measuring head on the bowl the a_w value of the sample was read from the display of the device.

2.8. Sugars composition and concentration

Sugar composition was determined based on Aguayo et al. (2006) using an analytical HPLC (Merck Hitachi, Darmstadt, Germany) equipped with a refractive index detector (Hitachi, L-7490 model, Tokyo, Japan), L7100 pump, a Hitachi L7200 automatic sample injector and a LiChroCART 250-4 Purospher STAR NH 2 column (5 μ) (Merck, Darmstadt, Germany). A 20 μL extract sample was injected using a mobile phase of 85:15 acetonitrile:water (Merck, Germany) at a 1.5 mL min^{-1} flow. The sucrose, glucose and fructose concentrations were determined. Results were reported as g sugar 100 g^{-1} dm.

2.9. Color

For color monitoring, 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 CIEL*a^*b^* color space units. The mean values for the lightness (L^*), red/green coordinate (a^*) and blue/yellow coordinate (b^*) parameters were calculated for each fruit. The skin color was determined as L^*, or calculated as Chroma (C^*) and Hue angle (H°) according to CIE (1976). Color was measured on three randomized sides of 10 dates randomly selected from each treatment.

2.10. 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 4 °C for 10 min at 15,000 × g to obtain the extracts. The amount of total phenolics was determined using the Folin–Ciocalteu reagent based on 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 (Hewlet Packard 8453, UV–vis spectrophotometer, Columbia, USA). Total phenolic concentration was expressed as gallic acid equivalents (GAE) in g/100 g fw. All extracts were analyzed in triplicate.

2.11. Total antioxidant activity

The total antioxidant activity of dates was assayed based on the evaluation of the free radical scavenging capacity, according to Artés-Hernández et al. (2010). A solution of 0.7 mM 2,2-diphenyl-1-picrylhydrazil (DPPH) radical in methanol prepared 2 h before the assay was adjusted to 1.1 ± 0.02 nm immediately before use. An aliquot of 21 μL of the extracts obtained from the preparation of

Table 1
Morphological and physical characteristics of fresh 'Deglet Nour' date fruit. Data are means ($n = 20$) ± SD.

	Weight (g)			Length (mm)		Diameter (mm)		Pulp thickness (mm)	Pulp/fruit ratio (%)	Color		
	Fruit	Seed	Pulpe	Fruit	Seed	Fruit	Seed			L^*	Chroma	Hue°
Mean	11.9	0.8	11.1	44.0	27.4	22.1	9.5	5.7	93.2	32.9	19.9	61.8
Max.	15.7	1.1	14.6	50.0	31.0	26.0	10.0	8.0	95.4	34.5	21.3	63.9
Min.	8.4	0.5	7.9	40.0	24.0	20.0	6.0	4.0	91.6	32.6	18	60.2
SD	1.7	0.2	1.6	3.0	1.8	1.6	1.0	1.1	1.0	0.8	1.0	1.2

phenolic compounds was added to 194 μL of this solution. The mixture was incubated in darkness for 50 min at room temperature. The antioxidant activity was measured by decreasing the absorbance at 517 nm (Tecan Infininte M200, Männedorf, Switzerland). The results were expressed as g ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g fw. All measurements were made in triplicate.

2.12. Sensory evaluation

Sensory analyses were performed according to international standards on the processing day and after 30 days of shelf-life at 20 °C. The panel consisted of five assessors (three women and two men, aged 25–65 years) screened for sensory ability (Martínez-Hernández et al., 2013). Overall quality, color, texture and flavor were evaluated on a five-point hedonic scale (1: extremely poor, 2: poor, 3: acceptable and limit of usability, 4: good; 5: excellent).

2.13. Statistical analysis

An ANOVA for each quality attribute was performed and values reported for treatment and storage period were compared to find significant differences. By the use of Info Stat (version 1), the least significant difference multiple range test at $p < 0.05$ was conducted.

3. Results and discussion

3.1. Morphological and physical characterization of 'Deglet Nour' dates

The weight, length, diameter and color of dates at 'Tamar' maturity stage are reported in Table 1. The weight ranged between 8.39 ± 1.74 g and 15.73 ± 1.75 g with a high ratio of pulp/fruit (91.62 ± 0.98% to 93.17 ± 0.98%). Chaira et al. (2009b) who studied 10 other Tunisian cvs. reported that fruit weights varied from 4.34 to 11.28 g, which are lower than that of 'Deglet Nour' fruit. The weights of fruit, pulp and seed and their color parameters were quite similar to those showed for this cultivar by Elleuch et al. (2008) and El Arem et al. (2011).

3.2. Pyrale infestation and microbial analysis

The infestation level of date fruit by *E. ceratoniae* was significantly affected by the sanitizing treatments and the storage period (Fig. 1). As expected, natural infestation increased over the storage period, and all sanitizing treatments lowered the natural infestation. In particular, UV-C and NEW were the most effective against moth proliferation (8.33 ± 2.89% and 9.00 ± 0.50% infestation after treatments, respectively, without significant difference) compared to the control (26.67 ± 2.98% infestations). No differences among NaClO, O_3 and AEW against pyrale infestation were found. According to Dhouibi (2000), the eggs of pyrale are attached to the surface of dates, and so it could be expected that the UV-C inhibited their growth. In a similar way, Vieira et al. (2009) have eliminated *Ceratitis capitata* eggs in infested guavas at doses of 16 kJ m^{-2} UV-C and *in vitro* at 1.4 kJ m^{-2} UV-C.

Immediately after all sanitizing treatments, the microbial counts of molds, yeasts, total mesophilic and coliforms were significantly lowered with respect to the control (Fig. 2). This effect was more pronounced for mesophilic and yeast and molds counts, with a reduction of around 2.5 log cfu g^{-1} for yeast and molds washed with NEW, 1.4 log cfu g^{-1} for total mesophilics treated with AEW, and 1 log cfu g^{-1} for coliforms sanitized with both NEW and AEW. On the processing day, NEW was the most efficient treatment against yeast and molds, total mesophilics (without differences with NaClO, AEW and O_3) and against coliforms (without differences with AEW). However, after shelf-life, UV-C showed the most efficient antimicrobial effect for yeast and molds with a reduction of 1.6 log cfu g^{-1} (without differences with NaClO and O_3), total mesophilics with 2.5 log cfu g^{-1} (without differences with O_3) and coliforms with 1.3 log cfu g^{-1} with respect to the control samples (Fig. 2). No differences in growth of coliforms between NaClO and NEW samples were found. In this way, UV-C and EW seem to be promising alternatives to NaClO for commercial disinfection of fresh dates.

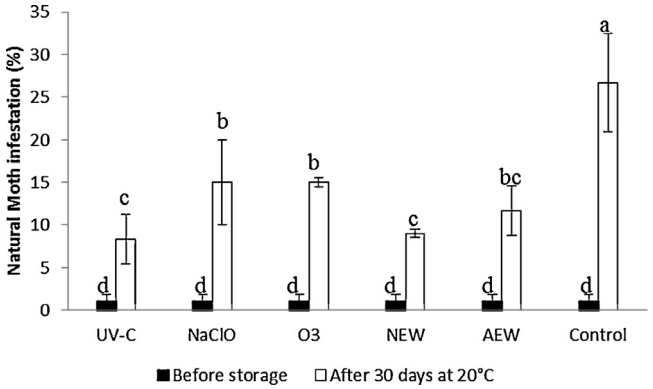

Fig. 1. Effect of several sanitizing treatments on infestation by moths of pyrale of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 60$) ± SD. Data within a bar followed by different letter are significantly different ($p \leq 0.05$) according to LSD.

The results reached with O_3 (about 1.5–2 log cfu g^{-1} reductions for different microbial groups) agree with those reviewed by Artés et al. (2009), who reported that similar treatments commonly lowered the microbial load on the surface of fruit and vegetables. Likewise, the results obtained with EW agree with those recently reported in other horticultural products (Tomás-Callejas et al., 2011; Martínez-Hernández et al., 2013).

3.3. Weight loss and firmness

The weight loss was significantly affected by sanitizing treatments. After shelf-life, dates treated with UV-C and with NaClO showed respectively the lowest (1.49 ± 0.55%) and the highest (2.94 ± 0.43%) weight losses (Table 2). This can be explained by the fact that UV-C was the only treatment which did not include a water bath. This wash normally induces a slight gain of fruit weight immediately after treatment, which is afterward lost due to water evaporation during storage at 20 °C (Artés et al., 2007). Khali and Selselet-Attou (2008) also showed that dates commonly suffer weight loss during storage, mainly at room temperature. The O_3 and EW treatments did not affect weight loss compared to the control, confirming results reported in other plant produce (Palou et al., 2002; Forney et al., 2007; Rico et al., 2008; Tomás-Callejas et al., 2011).

The sanitizing treatments and storage time did not promote significant changes in firmness and only a slight trend to a decrease after storage was found (Fig. 3). This was very probably due to firmness variability among date fruit and could be also attributed to the low water loss which kept the firmness. These results agree with those reported for dates by Falade and Abbo (2007).

Table 2
Effect of several sanitizing treatments on weight loss of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD. Means in the column followed by different letters are significantly different ($p \leq 0.05$) according to LSD test.

Treatment	Weight loss (%)
UV-C	1.49 ± 0.55 (c)
NaClO	2.94 ± 0.43 (a)
O_3	2.05 ± 0.60 (bc)
NEW	2.11 ± 0.37 (bc)
AEW	2.53 ± 0.52 (ab)
Control	2.13 ± 0.69 (bc)

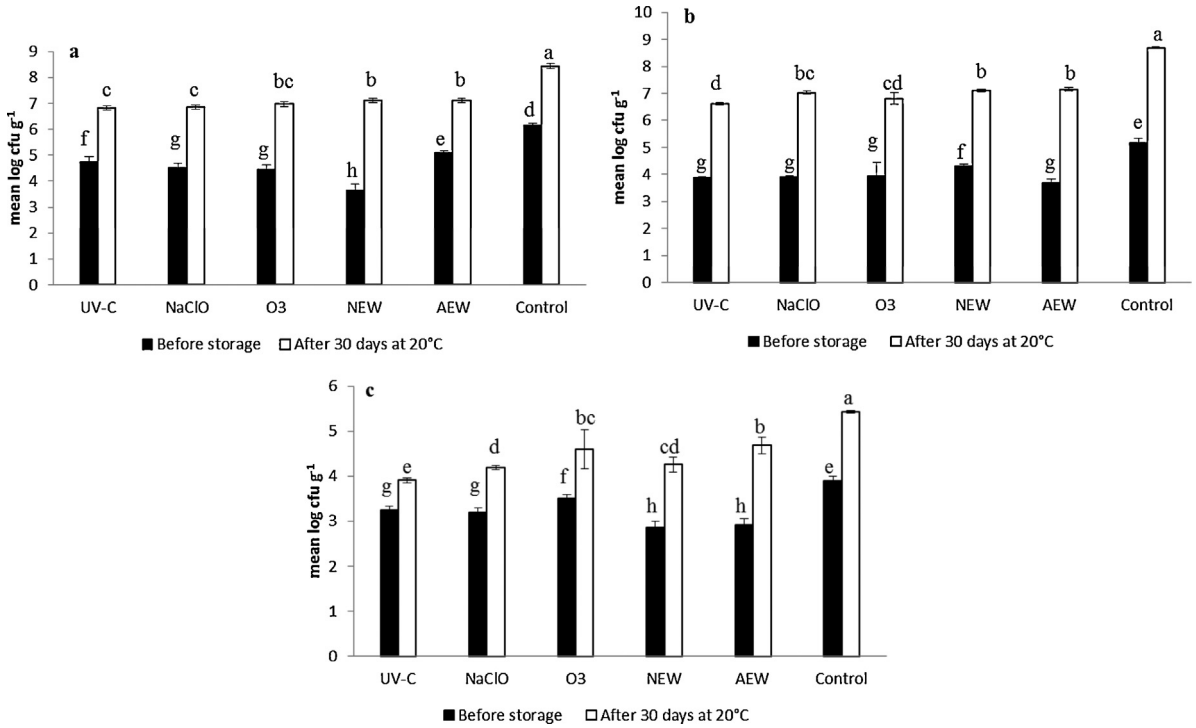

Fig. 2. Effect of several sanitizing treatments on yeast and molds (a), mesophilics (b) and total coliforms (c) counts (mean log cfu g^{-1}) of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD. Data within a bar followed by different letter are significantly different ($p \leq 0.05$) according to LSD test.

3.4. Titratable acidity and pH

No noticeable differences in TA (about 0.10 ± 0.02 g citric acid 100 g^{-1} fw on the processing day) among sanitizing treatments and storage time were found (data not shown). The storage period showed a significant effect on slightly lowering the pH values (about 5.60 ± 0.10 on the processing day) without differences among treatments (data not shown).

The pH values agree with those found by Besbes et al. (2009). In addition, our results confirm that the storage time induced a slight decrease in the pH values of dates (Dehghan-Shoar et al., 2010), without significantly changing the TA. Azelmat et al. (2006) reported that the pH decrease could be due to the activity of microorganisms and insects throughout shelf-life of dates.

3.5. Dry matter

The storage period was associated with a slight decrease of the date moisture. The initial moisture was between 19.40 ± 0.60% and 22.67 ± 1.04%, and after 30 days at 20 °C it ranged between 18.77 ± 0.37% and 20.59 ± 0.95%. These values agree with the range reported by El Arem et al. (2011) and with those of the Codex Standard for dates (1985), which specified that the moisture of 'Deglet Nour' fruit should be less than 30%. As expected, subsequent to the slight decrease of the date moisture, a relative increase in dry matter of dates after storage was found (Fig. 4), in agreement

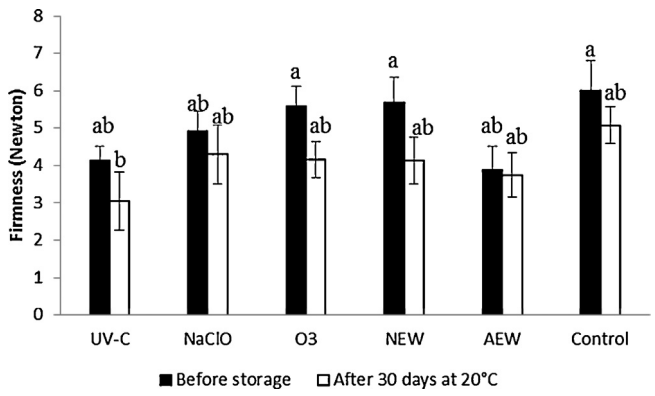

Fig. 3. Effect of several sanitizing treatments on firmness of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD.

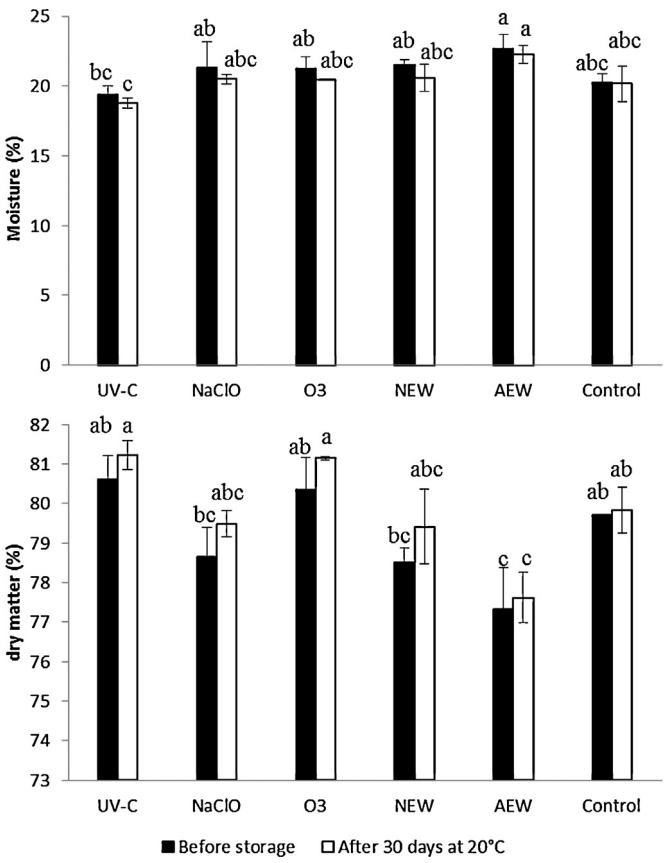

Fig. 4. Effect of several of sanitizing treatments on dry matter and moisture of 'Deglet Nou'r dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD.

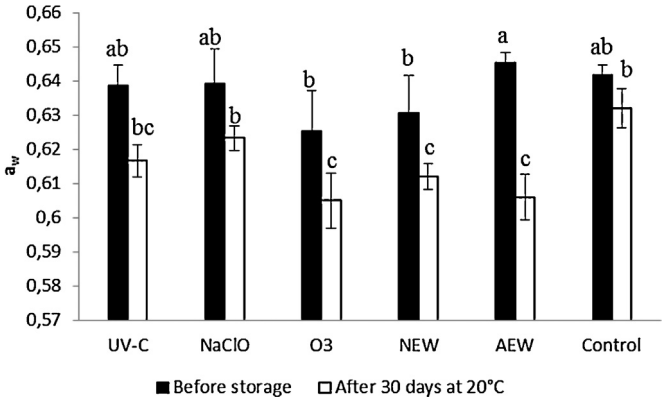

Fig. 5. Effect of several sanitizing treatments on water activity of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD.

with Azelmat et al. (2006), and this could be attributed to water evaporation after several months of storage at room temperature.

The moisture content is considered the most significant quality attribute to estimate the quality of 'Deglet Nour' fruit. When the level is lower than 18%, the dates of this cultivar lose their commercially accepted sensory quality and are downgraded (Achour et al., 2003).

3.6. Water activity

The a_w after sanitizing treatments was similar in all treated samples, with values ranging between 0.625 and 0.645. These values slightly decreased after storage at 20 °C, which could be explained by the partial dehydration of dates (Fig. 5). According to Besbes et al. (2009), 'Deglet Nour' fruit have a low a_w of 0.662 which protects them against all bacterial development. However, inadequate storage conditions could facilitate yeast proliferation.

3.7. Sugar concentrations

The treatments did not affect the sugars concentrations (Table 3), which were about 78.25 ± 0.71 g 100 g^{-1} dm. Sucrose was the predominant sugar with 39.95 ± 1.13 to 45.75 ± 3.71 g 100 g^{-1} dm. The sugar concentrations varied with ripening stage, firmness and water content. Therefore, different sugar levels have found by various authors. Besbes et al. (2009) reported 87.55 ± 0.10 g 100 g^{-1} dm, 53.59 ± 0.13 g 100 g^{-1} and 33.96 ± 0.23 g 100 g^{-1} for total sugars, sucrose and reducing sugars respectively. Chaira et al. (2007) found a total sugars concentration in 'Deglet Nour' date flesh of 72.82 ± 0.25%, sucrose 55.08 ± 0.14% and reducing sugars 17.74 ± 0.33%. Elleuch et al. (2008) reported similar values with total sugars 79.1 ± 0.8%, sucrose 52.7 ± 0.15%, glucose 13.7 ± 0.5% and fructose 12.6 ± 0.2%. More recently El Arem et al. (2011) reported a total sugars concentration of 63.16 ± 1.59%, sucrose 33.32 ± 1.91% and reducing sugars 29.79 ± 0.35%.

As expected the total sugars concentrations decreased after shelf-life from a range between 76.20 ± 0.71 g 100 g^{-1} and 79.58 ± 0.09 g 100 g^{-1} to between 58.51 ± 0.83 g 100 g^{-1} and 66.19 ± 0.98 g 100 g^{-1}. A degradation of sucrose and reducing sugar was also found. This trend was very probably due to consumption of sugars as respiratory substrates. These findings confirm those of Khali et al. (2007) who found decreased values of sugar concentrations in 'Deglet Nour' date flesh from 70.5 to 45.75% fw after 5 months at room temperature.

3.8. Color

Fruit color changes from light brown to dark brown after storage were well reflected by combined decreases of L^*, Chroma and H° values, slightly accentuated in the last two parameters. Compared to the control, both at the processing day and after shelf-life, no noticeable changes in L^*, Chroma and H° values due to sanitizing treatments were found (Table 4). It could be just a trend, without significant differences among treatments, with UV-C and AEW treated dates showing high L^* and Chrome values at the beginning and at the end of the storage period. The L^* value found in the current work (33.58 ± 2.05) agrees with that reported by Elleuch et al. (2008) which showed that 'Deglet Nour' fruit (L^* = 31.71 ± 0.57) were lighter than 'Allig' fruit. (L^* = 22.89 ± 0.45).

Results in color changes regarding sanitizing treatments and storage period generally agree with those early reported for other plant products. Kim et al. (2010) showed that NaClO did not affect strawberry color, although Martin-Diana et al. (2007) found that NaClO reduced L^* of fresh-cut lettuce. Fresh processed lettuce tissue became shinier when 8.14 UV-C kJ m^{-2} was applied, possibly due to induction of lignification-like processes in the lettuce tissue for protection against UV-C stress (Allende and Artés, 2003). However, Artés-Hernández et al. (2010) showed that low to moderate UV-C radiation for up to 7.2 kJ m^{-2} had no noticeable effect on color

Table 3
Effect of several sanitizing treatments on sugars concentration of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD.

	Total sugars (g 100 g^{-1} dm)		Sucrose (g 100 g^{-1} dm)		Glucose (g 100 g^{-1} dm)		Fructose (g 100 g^{-1} dm)	
	Before storage	After 30 d at 20 °C	Before storage	After 30 d at 20 °C	Before storage	After 30 d at 20 °C	Before storage	After 30 d at 20 °C
UV-C	79.07 ± 0.68 (a)	59.08 ± 0.62 (d)	45.75 ± 3.71 (a)	32.04 ± 1.23 (cd)	19.32 ± 2.86 (abc)	16.8 ± 0.78 (bc)	14.01 ± 1.80 (ab)	10.24 ± 0.49 (cd)
NaClO	79.56 ± 1.26 (a)	59.10 ± 0.93 (d)	44.95 ± 2.44 (a)	26.07 ± 1.06 (d)	21.11 ± 1.77 (ab)	21.52 ± 3.98 (ab)	13.50 ± 1.15 (abc)	11.52 ± 1.36 (bcd)
O_3	76.20 ± 0.71 (ab)	66.19 ± 0.98 (cd)	43.29 ± 3.73 (ab)	32.06 ± 1.95 (dc)	20.28 ± 0.64 (abc)	21.99 ± 0.76 (ab)	12.64 ± 0.12 (abcd)	12.14 ± 0.36 (abcd)
NEW	78.56 ± 0.09 (a)	62.08 ± 1.54 (cd)	44.73 ± 0.94 (a)	32.12 ± 3.03 (cd)	21.54 ± 3.6 (ab)	18.87 ± 2.79 (bc)	12.28 ± 1.21 (abcd)	11.09 ± 0.77 (bcd)
AEW	76.55 ± 1.43 (ab)	64.61 ± 2.06 (cd)	39.95 ± 1.13 (abc)	33.44 ± 3.57 (bcd)	23.76 ± 2.53 (a)	18.66 ± 0.84 (bc)	12.83 ± 2.18 (abcd)	12.51 ± 0.37 (abcd)
Control	79.58 ± 0.09 (a)	58.51 ± 0.83 (d)	41.07 ± 0.36 (abc)	32.60 ± 1.12 (cd)	23.58 ± 0.98 (a)	15.61 ± 1.45 (c)	14.93 ± 0.65 (a)	10.30 ± 0.61 (d)

Alphabets 'a' to 'd' denotes $P < 0.05$.

Table 4
Effect of several sanitizing treatments on skin color parameters after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD. Means with different letters are significantly different ($p \leq 0.05$) according to LSD test.

	L^*	Chroma	Hue°
Before storage			
UV-C	34.55 ± 1.78 (a)	20.01 ± 2.64 (ab)	61.85 ± 4.24 (ab)
NaClO	32.60 ± 2.77 (abcd)	20.37 ± 4.71 (ab)	61.57 ± 5.56 (ab)
O_3	33.06 ± 2.81 (abc)	17.97 ± 4.60 (bc)	60.92 ± 7.56 (ab)
NEW	32.28 ± 2.67 (bcd)	19.79 ± 2.90 (ab)	60.17 ± 4.53 (abc)
AEW	32.45 ± 2.97 (abcd)	21.25 ± 2.95 (a)	63.93 ± 5.64 (a)
Control	32.78 ± 2.39 (abc)	20.04 ± 2.72 (ab)	62.29 ± 4.79 (ab)
After 30 days at 20 °C			
UV-C	33.65 ± 2.27 (ab)	15.98 ± 3.05 (cd)	58.31 ± 5.72 (bc)
NaClO	30.98 ± 1.28 (cd)	15.65 ± 2.30 (cd)	59.34 ± 5.98 (abc)
O_3	30.54 ± 3.04 (d)	15.85 ± 4.05 (cd)	60.05 ± 7.45 (abc)
NEW	32.04 ± 1.00 (bcd)	14.74 ± 1.60 (d)	59.96 ± 4.72 (abc)
AEW	33.64 ± 1.86 (ab)	15.26 ± 2.74 (cd)	57.81 ± 5.72 (abc)
Control	32.07 ± 2.92 (bcd)	15.60 ± 3.12 (cd)	55.30 ± 4.45 (c)

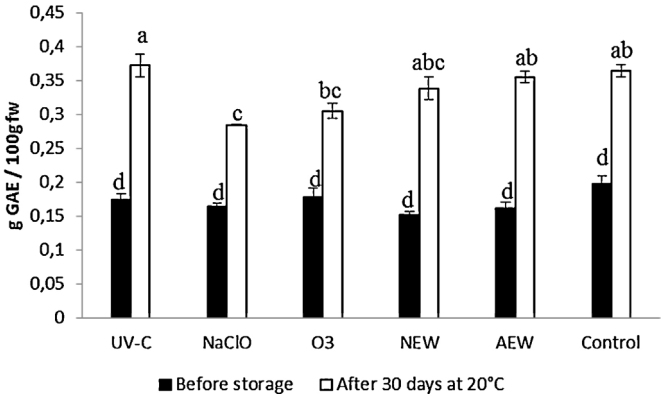

Fig. 6. Effect of several sanitizing treatments on polyphenols of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD. Data within a bar followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test.

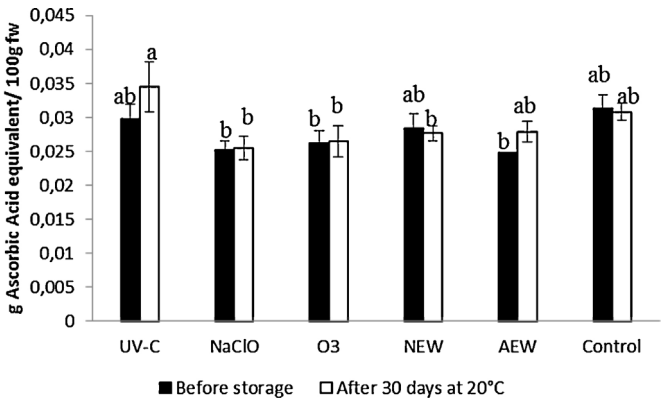

Fig. 7. Effect of several sanitizing treatments on the antioxidant activity of 'Deglet Nour' date after a shelf-life of 30 days at 20 °C. Data are means ($n = 3$) ± SD. Data within a bar followed by the same letter are not significantly different ($p \leq 0.05$) according to LSD test.

parameters of watermelon pieces. O_3 did not affect the skin color of whole tomatoes, which was only affected by storage time (Aguayo et al., 2006). Izumi (1999) and Tomás-Callejas et al. (2011) reported that NEW does not affect the surface color or visual appearance of fresh-cut produce. However, a^* values increased during storage in fresh-cut lettuce, without differences between NaClO and NEW treatments (Rico et al., 2008)

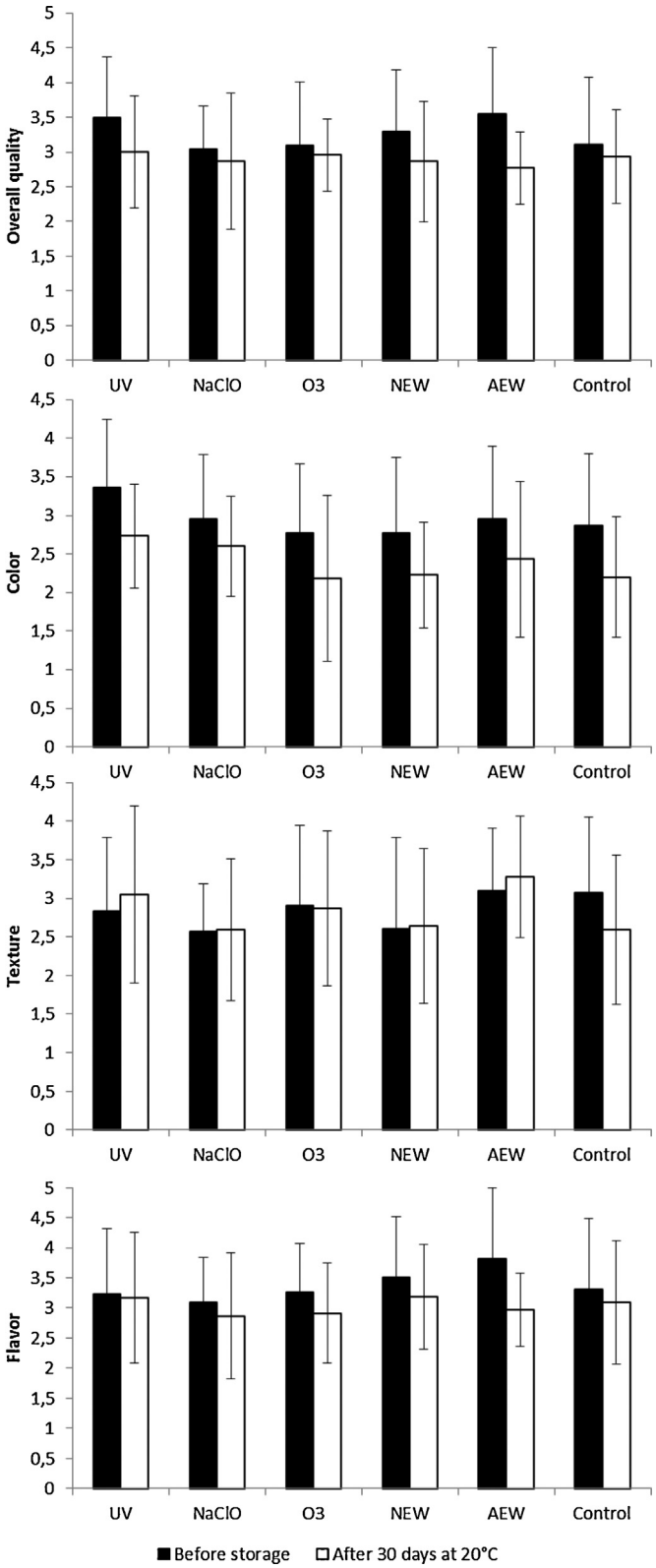

Fig. 8. Effect of several sanitizing treatments on flavor, texture, color and overall quality of 'Deglet Nour' dates after a shelf-life of 30 days at 20 °C. Data are means ($n = 5$) ± SD.

3.9. Total phenolics compounds concentration

The sanitizing treatments did not show a differential effect on the total phenolics concentrations which significantly increased after storage. In fact, they changed from a mean value of 0.172 ± 0.008 g GAE 100 g^{-1} fw to a mean of 0.336 g GAE 100 g^{-1} fw after 30 days of storage (Fig. 6).

Mansouri et al. (2005) reported in several Algerian date cultivars phenolic concentrations in a range from 2.49 to 8.36 mg GAE 100 g^{-1} fw (in 'Deglet Nour' 6.73 ± 0.27 mg GAE 100 g^{-1} fw). Moreover, Chaira et al. (2009b) reported values for different Tunisian cultivars from 3.57 ± 1.11 to 31.82 ± 1.29 mg GAE 100 g^{-1} fw. However, Besbes et al. (2009) showed higher values in 'Deglet Nour' fruit (493.5 mg GAE 100 g^{-1} fw). These diverse ranges could be explained by several factors such as cultivar, growing and cultivation conditions (geographic origin, season, climate, fertilizers, soil type, amount of sunlight received), maturity stage, methods, process and stabilization conditions, storage parameters, use of different analytical methods and use of different phenolics standards.

Biglari et al. (2009) found that total phenolic concentrations in dates increased with storage time. This increase is a usual response of plants to wounding and microbial attacks. The polyphenols levels depend on date cultivars and storage duration. In addition, polyphenol levels changed for fresh and dry dates from 3.49 and 145.70 mg GAE 100 g^{-1} dm respectively at the beginning of storage to 11.30 and 197.41 mg GAE 100 g^{-1} dm after 6 months at 4 °C. In the same way, the general trend throughout cold storage of mizuna baby leaves was of a slight increase in the initial polyphenols concentration with no remarkable differences among deionised water, EW and NaClO treatments (Tomás-Callejas et al., 2011). In contrast, the current results regarding the effect of O_3 treatment did not confirm those showing enhanced total phenolics concentrations in table grapes (Artés-Hernández et al., 2003, 2007). This could be due to different methods of application in a continuous O_3 gas flow and in a dip of ozonated water.

Martínez-Hernández et al. (2011) found that 6 kJ UV-C m^{-2} caused an initial stress on kaylan-hybrid broccoli cells, inducing an increase in the total phenolic levels. In contrast, our results showed that the same moderate UV-C treatment did not increase the phenolics concentrations, compared to those in dates washed with water.

3.10. Antioxidant activity

Immediately after sanitizing treatments, the antioxidant activity ranged between 0.024 ± 0.001 and 0.031 ± 0.002 g AEAC 100 g^{-1} fw, with no remarkable differences among treatments (Fig. 8). In the same way, no influence of several sanitizing treatments on the antioxidant activity in mizuna baby leaves was found by Tomás-Callejas et al. (2011). According to Chaira et al. (2009a), the antioxidant activity depends on date cultivars, ranging between 17.77 for 'Nefzaoui' and 31.86 mg AEAC 100 g^{-1} fw for 'Garn ghzal'. Our results for 'Deglet Nour' are in the high level of this range.

Increased antioxidant activity throughout storage of dates was reported by Biglari et al. (2009) probably due to ethylene action. This hormone stimulates phenylalanine ammonia lyase activity, a key enzyme in the biosynthesis and accumulation of phenolic compounds. In contrast, in the current experiments, compared to initial levels, no changes in antioxidant activity after storage have been found (Fig. 7). In fact, vitamin C and carotenoid losses after storage should be expected. Consequently more studies on this should be carried out.

3.11. Sensory analysis

At the end of the storage, a slight but not significant trend of a decrease in different sensory scores of the dates for all treatments, without differences among them, was found (Fig. 8). These results agree with those obtained on Tunisian dates by Khali and Selselet-Attou (2008). Storage conditions (duration, temperature, and RH) are very important factors for keeping sensory quality of dates (Ismail et al., 2008). In particular, the dark color of date fruit after storage, due to polyphenoloxidase enzyme activity on polyphenols, depends on the above mentioned conditions. Besides fruit color, appearance, texture, and flavor can commonly and progressively deteriorate during prolonged storage.

4. Conclusions

The treatment of 'Deglet Nour' dates with NaClO, UV-C, O_3 and EW showed a positive effect for lowering their natural infestation by *E. ceratoniae* as well as the microbial growth after 30 days of storage at 20 °C. Particularly, UV-C and NEW were the most effective against moth proliferation without adversely affect objective and subjective quality attributes. Independently of sanitizing treatments the phenolics concentration significantly increased throughout storage while the antioxidant activity determined by the DPPH method remained quite constant. After 30 days at 20 °C the quality attributes of dates were maintained. The beneficial results reached with the selected sanitizing treatments, not yet widely studied on dates, should be confirmed and optimized in further studies.

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