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Original article

Effect of gaseous pretreatment on enzymatic browning of mature date after cold storage

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Summary

Introduction – **Dates are usually stored at room temperature, which results in their dehydration; this can be avoided by cold storage but the subsequent passage at a higher temperature accelerates the enzymatic browning of these fruits. Some positive effects of a gaseous pretreatment on the browning process and quality parameters of 'Deglet Nour' date after cold storage were expected.** *Materials and methods* – **After cold storage, mature 'Deglet Nour' dates were distinctly subjected to one of the two gaseous pretreatments low in oxygen, and stored at 35 °C in polyethylene low density pouches. The injected gas mixtures were: (T_I) 2% O_2, 20% CO_2 and 78% N_2 for the first treatment; and (T_{II}) 2% O_2, 5% CO_2 and 93% N_2 for the second one. Enzymatic browning and nutritional quality parameters of dates were studied for one month.** *Results and discussion* – **Until 14 days of storage a significant decrease in polyphenoloxidase (PPO) and peroxidase (POD) activities and an increase in phenolics and ascorbic acid contents were observed in the treated samples. The control samples under ambient air were the lowest in weight and water content, with comparable pH, titratable acidity and total soluble solids to the treated samples.** *Conclusion* – **Both treatments maintained fruit quality by slowing down enzymatic browning compared to the control. T_{II} was more efficient than T_I.**

Keywords
Algeria, date palm, *Phoenix dactylifera*, 'Deglet Nour', modified atmosphere, fruit quality, phenolics, postharvest management

Résumé

Effet d'un prétraitement gazeux sur le brunissement enzymatique de la datte mûre après stockage au froid.

Introduction – **Les dattes sont généralement stockées à température ambiante, ce qui entraîne leur déshydratation, qui peut être évitée par stockage au froid; mais le retour aux températures plus élevées accélère le brunissement enzymatique des fruits. Cette étude envisageait les effets positifs d'un prétraitement gazeux sur le processus de brunissement et les paramètres de qualité de la datte 'Deglet Nour' après une période de stockage au froid.** *Matériel et méthodes* – **Après stockage au froid, les dattes 'Deglet Nour' matures ont été soumises distinctement à l'un des deux prétraitements gazeux pauvres en oxygène, puis stockées à 35 °C sous emballage polyéthylène basse densité. Les mélanges gazeux injectés étaient: (T_I) 2% O_2, 20% CO_2 et 78% N_2 pour le premier traitement ; et (T_{II}) 2% O_2, 5% CO_2 et 93% N_2 pour le second. Le brunissement enzymatique et les paramètres de qualité nutritionnelle des dattes ont été étudiés pendant un mois.** *Résultats et discussion* – **Jusqu'à 14 jours de stockage, une diminution significative d'activité de la polyphénol oxydase (PPO) et de la peroxydase (POD) et une augmentation des teneurs en composés phénoliques et en acide ascorbique ont été observées chez les échantillons traités. Les échantillons témoins sous air ambiant ont présenté les masses et teneurs en eau les plus faibles, avec des valeurs de pH, acidité totale et teneur en matières solubles totales comparables à celles des échantillons traités.**

Significance of this study

What is already known on this subject?
- Cold storage of 'Deglet Nour' dates avoids dehydration, but accelerates browning when back to room temperature.
- Several methods attempted to reduce this browning process of dates but never gaseous pretreatment.

What are the new findings?
- An initial atmosphere low in O_2 can reduce the date fruit browning.
- It increases both phenolic and ascorbate contents and decreased PPO and POD activities.

What is the expected impact on horticulture?
- Gaseous pretreatment can be envisaged as a solution to market mature dates after cold storage by preventing fruit browning.

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Conclusion – **Les deux prétraitements gazeux ont permis de maintenir la qualité du fruit tout en ralentissant le brunissement enzymatique par rapport au témoin. T_{II} s'est montré plus efficace que T_I.**

Mots-clés
Algérie, palmier dattier, *Phoenix dactylifera*, 'Deglet Nour', atmosphère modifiée, qualité des fruits, composés phénoliques, gestion post-récolte

Introduction

Date fruit (*Phoenix dactylifera* L.) has a great nutritional importance in many subtropical areas. The variety 'Deglet Nour' is much appreciated for its exquisite taste, its translucent aspect and high nutritional value. At full maturity stage it is rich in functional components, including phenolic compounds. Several biological effects are attributed today to phenolics, such as antioxidant and antibacterial activities (Daas Amiour *et al.*, 2014). Moreover, phenols add taste and organoleptic properties to dates.

Unfortunately, phenolics are subject to oxidation inducing enzymatic browning or darkening. It is one of the most important physiological disorders of dates after harvest. Although browning is desirable during fruit development, its continued process after maturation and harvesting leads to damage and wastage (Daas Amiour and Hambaba, 2016), as browning of harvested date fruit decreases their nutritional quality and appreciation by the consumers.

Semi-soft dates, such as 'Deglet Nour', have longer shelf life than soft dates. For these longer storage durations, temperatures below the highest freezing temperature of -15.7 °C are used (Kader and Hussein, 2009). In Algeria, producers of 'Deglet Nour' keep fruit after harvest in cold storage (-18 °C) for several months to prevent drying, control insect infestation and maintain quality. Before marketing, fruits are placed back to room temperature and they quickly turn brown with a deterioration of their taste and thus great loss of market value. In this context, Mutlak and Mann (1984) have noticed that in frozen dates the darkening occurred slightly during storage, and rapidly during thawing. That browning resulted of an enzymatic oxidative process.

Polyphenoloxidase (PPO) and peroxidases (POD) are common enzymes widespread in plants. They are involved in various physiological processes that are not entirely elucidated, although their role in phenol oxidation was well studied. PPO catalyzes the oxidation of phenolic constituents to quinones, which finally polymerize to colored melanin. The formation of yellow and brown pigments in fruit products during enzymatic browning reactions is controlled by the levels of phenols, the amount of PPO activity, and the presence of oxygen (Lozano, 2006). During the ripening of date fruit, PPO activity is highest at the *kimri*, followed by *khalal* and *tamer* stages (Sidhu, 2006). POD catalyzes the H_2O_2 dependent oxidation of a wide variety of substrates, including phenolics (Al-Senaidy and Ismael, 2011).

To prevent enzymatic browning, several physical and chemical methods have been developed. They either inactivate enzymes or trap substrates or products of the reaction. Gaseous pretreatments are sometimes used to control fruit quality. In table grapes, short-term high CO_2 treatment has shown a residual effect and significantly reduced decay incidence (Romero *et al.*, 2006). In apples, a simple pretreatment with low O_2 reduced scald development (Pesis *et al.*, 2007). However, few work studied the impact of gaseous pretreatment. Most of the time, modified atmosphere packaging was studied because of its great potential to extend postharvest fruit and vegetable life (Vermeiren *et al.*, 1999). The main objective of this work was to study the impact of gaseous pretreatment on the browning process and quality of 'Deglet Nour' dates after cold storage.

Materials and methods

Plant material and experimental design

The plant material used in this study consisted of a semi-soft date fruit, the cv. Deglet Nour, which was harvested at full maturity, at the *tamar* stage of ripening. All samples came from a 10-year-old orchard where the palm trees derived from clones grown in the same area, in the South East of Algeria, the most important area of date production of the country. The trees had the same agronomic history. Homogeneous color dates (104 in total) were chosen and stored at -18 °C for six months before the experiment.

Pouches of polyethylene low density (LDPE) (CFS, France) were used. Initial gas mixtures were injected: (T_I) 2% O_2, 20% CO_2 and 78% N_2 for the first pretreatment, and (T_{II}) 2% O_2, 5% CO_2 and 93% N_2 for the second one. These gas mixtures were obtained using a gas mixer (Witt Gasetechnik). In preliminary tests we observed that gas transfers were very fast, so that to be sure to have ambient air around samples during all the storage time, at 35 °C we preferred to use macro perforated LDPE instead of glass jars for the control. Eight pouches or jars were used for each atmosphere, each containing four dates. This small size of packed samples was used to reduce the marketing ratio fruit number/packaging size and so increase the browning process.

All samples were stored at 35 °C in a Sanyo versatile environmental test chamber, which is a suitable temperature for the installation of the enzymatic browning. Follow up analyzes of the color (L*, a*), the quality (fruit weight, water content, total soluble solids, pH, titratable acidity and ascorbic acid content) as well as the phenolic oxidation (total phenol content, DO at 430 nm, PPO and POD activities) were carried out every seven days for one month (D_7, D_{14}, D_{21}, D_{28}). The first day (D_0) was devoted to the samples before packaging and storage. For every test, four replicate samples were taken from different fruit chosen randomly.

Gas analysis

A combined CO_2/O_2 analyzer (CheckMate 9900, PBI Dansensor) was used for carbon dioxide and oxygen content determination. The gas levels were measured daily during the first two weeks of storage and every two days thereafter.

Determination of weight loss and water content

Weight loss (ΔW) was calculated and expressed as a percentage of weight loss compared to the initial weight of samples. The water content (WC) was determined by drying a known weight of the sample in an isothermal oven at 80 ± 2 °C and at atmospheric pressure until getting a constant mass sample. The water content is equal to the loss of fruit mass in the measurement conditions.

Determination of total soluble solids, titratable acidity and pH

Total soluble solid content (TSS) was determined with a digital refractometer PAL-1 (ATAGO, Japan) and expressed in °Brix. Samples were obtained by adding 2 mL distilled water to 1 g of date ground in liquid nitrogen.

Titratable acidity (TA) was determined according to the French standard AFNOR (1982) by titration of an aqueous solution of date with a sodium hydroxide solution (100 mmol L^{-1}) in the presence of phenolphthalein indicator. Results were expressed as % citric acid of date pulp weight. The pH was measured using the solution previously obtained for the titratable acidity test.

Color analysis

Color analysis was conducted with a Konica Minolta colorimeter (CR-400, Japan). The colorimeter was calibrated with a white ceramic plate before each measurement time. Color was expressed as luminance (L*), and two color channels (a*, and b*). L* (lightness) and a* (varies from green to red color) were good indicators of browning and were considered in this study.

The color was also analyzed by homogenizing 5 g of 'Deglet Nour' powder in 10 mL of methanol. The homogenate was filtered and centrifuged at 10,000 ×*g* for 15 min. The supernatant was used directly to measure absorbance at 430 nm, as a browning index per gram of fresh weight.

Extraction and determination of enzymes activities

The enzymatic oxidative browning process was analyzed through the evaluation of the polyphenoloxidase (PPO) and peroxidase (POD) activities during the storage. The gas content was modified, creating two initial atmosphere conditions and samples were stored at 35 °C which is the optimum temperature of oxidative enzyme activities (Daas Amiour and Hambaba, 2016). The preparation of crude enzyme extracts of PPO and POD was realized using the method described by Lichanporn and Techavuthiporn (2013), 1 g of date fruit powder was homogenized in 10 mL of 50 mmol L^{-1} phosphate buffer (pH 7.0) containing 0.2 g insoluble polyvinylpyrrolidone at 4 °C. After filtering the homogenate through cheesecloth, the filtrate was centrifuged for 20 min at 19,000 ×*g* and 4 °C. The supernatant was used for the PPO and POD activities assay. The PPO activity was assayed by measuring the oxidation of 4-methylcatechol as substrate at 410 nm, according to the method described by Jiang *et al.* (2005). POD activity was determined using guaiacol as substrate as the method described by Zhang *et al.* (2005). One unit of enzyme activity was defined as the amount of the enzyme which caused a change of 0.001 in absorbance per minute. Results were expressed as enzymatic unit per gram of fresh date pulp (U min^{-1} g^{-1} FW).

Extraction and evaluation of total phenolic content

The extraction of soluble fraction of phenols was performed using a solvent composed of acetone, water and acetic acid (70:29.5:0.5, v/v/v) (Hong *et al.*, 2006). A quantity of 1 g of date powder was extracted in 10 mL of extraction solvent. The mixture was agitated for 1 hour in the dark and centrifuged at 5 °C, 5,000 ×*g* for 5 min. The supernatant was used to determine total phenolic content using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Results were expressed as mass of gallic acid equivalents per fresh weight mass of date pulp (mg GAE 100 g^{-1} FW).

Determination of ascorbic acid content

Ascorbic acid content (ASA) was determined as described by Kampfenkel *et al.* (1995), scaled down for micro-plates (PowerWave HT, BioTek). One g of frozen dates was homogenized in 1 mL of 6% (w/v) trichloroacetic acid (TCA) and then centrifuged at 15,000 ×*g* at 4 °C for 10 min. The supernatant was used for total ascorbate (TAA) and ASA determination as dehydroascorbate (DHA) is reduced to ascorbic acid with dithiothreitol (DTT). Twenty µL of supernatant were mixed with 20 µL of 10 mmol L^{-1} DTT or 200 mmol L^{-1} phosphate buffer (pH 7.4) for TAA and ASA assay, respectively. The plate was incubated at 42 °C for 15 min. Then, 10 µL of 1% *N*-ethyl maleimide (NEM) or distilled water for total ascorbate and ASA assay, respectively, were added and mixed. After the addition of 150 µL of a specific reagent containing the 2.2-bipyridyl and ferric chloride ($FeCl_3$), the plates were stored for 30 min at 42 °C and the absorbance was read at 525 nm. Commercial L-ascorbic acid was used for calibration. Results were expressed on a fresh weight basis in mg per 100 g of date pulp (mg 100 g^{-1} FW).

Statistical analysis

Each analysis was performed in triplicate on four samples, one fruit per sample. The differences between mean values were determined using one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered to be significant at $P < 0.05$.

Results and discussion

Gas content

The measurement of gas content in the LDPE pouches indicated that gas change was only during the first days of storage. Ambient air was achieved after 6 days (Figure 1) in all conditions. The O_2 content reached 20% after 6 days in T_I and T_{II}, and the CO_2 content reached 0% after 2–3 days in T_I and 1 day in T_{II}. The gas changed quickly and ambient air was obtained in all LDPE pouches after 6 days. This rapid return to the ambient air in pouches indicated high gas transfer through the LDPE film because of high gas partial pressure difference at such temperature (35 °C). Indeed, temperature sensitivity of film permeability to gas is known to follow an Arrhenius law.

The low O_2 consumption also revealed the very low respiration rate of 'Deglet Nour' dates that could be due to the frozen storage of dates during six months. Previous study have also underlined that the respiration rate of dates was very low: <1 mg kg^{-1} h^{-1} at *tamar* stage according to Yahia and Kader (2011). Abdel-Latif (1988), working with three date cultivars ('Zahdi', 'Derey' and 'Sultani') reported an increase in respiration at the *rutab* stage of date development, preceding the *tamar* one, what refers to the climacteric peak, followed by a decline at the end of this stage. Nevertheless, it is still unclear whether date is a climacteric fruit or not (Thompson, 2003). In this context, Serrano *et al.* (2001) concluded that date should be considered a climacteric fruit since a respiratory peak and a climacteric peak in ethylene production were detected during the ripening of the dates cv. Negros. These authors added that the maximum rate of produced ethylene was very low when compared with kiwi, apricot and tomato fruits. In parallel, Abdel-Latif (1988) reported very high rates of ethylene production for the three above mentioned cultivars, while Abbas and Ibrahim (1996) recorded lower but important values with the cv. Hillawi.

Monitoring of weight loss and water content

The weight of all samples gradually decreased during storage (Table 1). In all analyses, the decrease in mass of the control was the largest in comparison with the two gaseous pretreatments. Moreover, the weight loss in T_I and T_{II} were not significantly different.

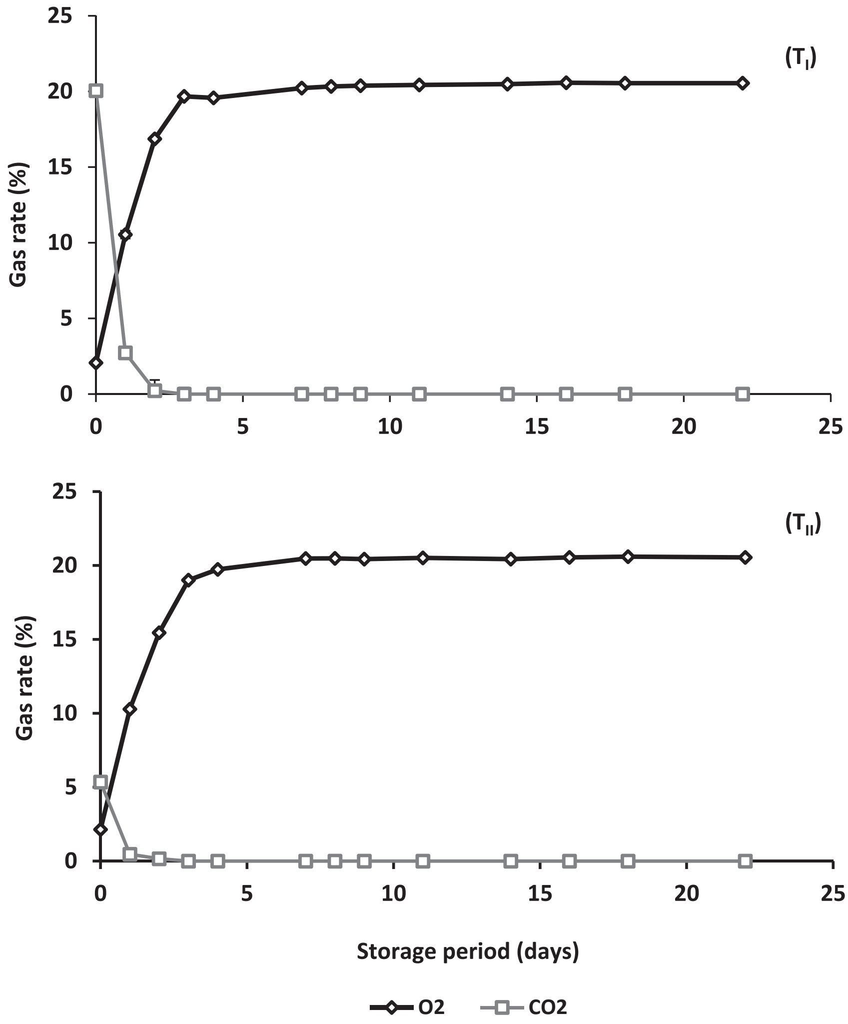

FIGURE 1. Evolution of gas (O_2, CO_2) content in LDPE pouches of the gaseous pretreatments T_I and T_{II} during storage of 'Deglet Nour' fruit at 35 °C for 28 days.

TABLE 1. Changes in fruit quality (weight (ΔW), water (WC) and total soluble solid (TSS) contents, pH and titratable acidity (TA)) of 'Deglet Nour' samples as related to gaseous pretreatment (Air, T_I, T_{II}) before storage at 35 °C for 28 days. Each value in the table is the mean ± standard deviation (n=4).

Storage period (days)	Pretreatments	ΔW(%)	WC (%)	TSS (°Brix)	pH	TA (%)
0		-	28.09 ± 0.50	21.85 ± 1.70	5.73 ± 0.21	0.25 ± 0.02
7	Control	7.01	22.91 ± 1.76a	24.82 ± 1.01a	5.57 ± 0.08	0.28 ± 0.02a
	T_I	2.82	26.66 ± 2.32b	22.42 ± 0.79b	5.66 ± 0.08	0.27 ± 0.03a
	T_{II}	2.45	24.78 ± 0.89b	23.47 ± 0.35ab	5.60 ± 0.09	0.28 ± 0.02a
14	Control	12.94	18.12 ± 0.57a	24.90 ± 0.60a	5.49 ± 0.07	0.34 ± 0.03a
	T_I	4.89	22.03 ± 0.51b	23.55 ± 0.36a	5.48 ± 0.04	0.33 ± 0.03b
	T_{II}	4.60	22.39 ± 2.50b	23.65 ± 0.05a	5.45 ± 0.05	0.33 ± 0.03b
21	Control	16.74	14.05 ± 1.10a	26.10 ± 0.63a	5.60 ± 0.07	0.35 ± 0.02a
	T_I	7.09	21.99 ± 0.63b	24.32 ± 0.48b	5.47 ± 0.05	0.33 ± 0.02a
	T_{II}	6.71	21.45 ± 1.09b	23.90 ± 0.79b	5.50 ± 0.06	0.34 ± 0.04a
28	Control	17.58	12.06 ± 0.52a	26.62 ± 0.59a	5.51 ± 0.05	0.41 ± 0.02a
	T_I	8.94	18.41 ± 1.40b	24.92 ± 0.46b	5.43 ± 0.09	0.35 ± 0.00b
	T_{II}	9.85	17.56 ± 1.85b	25.00 ± 0.92b	5.35 ± 0.11	0.38 ± 0.03a

Values in the same column and same storage period followed by different letters are significantly different (P<0.05).

Similarly, the fruit water content decreased in the three treatments especially in the control (Table 1). At D_0 the fruit weight decreased from 28.09 to 12.06, 18.41 and 17.56% for the control, T_I and T_{II}, respectively after one month. The water loss in both T_I and T_{II} packaging was significantly lower compared to the control. It was better maintained in all gaseous pretreatments conditions than in control samples. This can be mainly due to the good water barrier of low-density polyethylene pouches as confirmed by Cooksey (2007). Kader and Hussein (2009) have also noted that packaging in plastic bags or use of plastic liner in the box helps in reducing water loss of date fruit.

Total soluble solids, pH and titratable acidity

The total soluble solid content (TSS) logically increased during storage (Table 1). The highest value was recorded for the control samples, but significant difference was only measured after 21 days of storage. The increase in TSS was proportional to the decrease of the water content. Usually sugars are the soluble solids that are in largest quantity in the date fruit. Noting that, measuring the soluble material in samples of the juice can give a reliable measure of its sugar content (Thompson, 2003). In addition to the loss of water, the enzymatic conversion of large polysaccharides into small sugars is also responsible for this increase. In fact, the date was found to be rich in hydrolysable polysaccharides such as hemicelluloses (xylan, glucomannan) and pectin. El Arem *et al.* (2012) reported that 'Deglet Nour' contains at *tamar* stage around 63.16% total sugars. The low respiration rate of the fruit during storage cannot explain any consumption of the fruit's reducing sugars, which are the substrates of respiration. TSS values of the control were higher than those of the two treatments, probably because water loss was greater and respiration lower, since an increase in fruit breathing rates has been observed with higher moisture content (Yahia and Kader, 2011). It was reported that 'Deglet Nour' fruit with 20–22% moisture produced 0.4 mg kg^{-1} h^{-1} CO_2 at 24 °C and 2 mg kg^{-1} h^{-1} CO_2 when its moisture was 27% (Rygg, 1975). Therefore, it is clear that the two gaseous treatments have maintained the nutritional quality of 'Deglet Nour' date through their TSS content.

The pH of date fruit slightly decreased during storage without significant difference between conditions. Titratable acidity (TA) increased during all the storage period with lowest value for T_I (0.35%) after 28 days against 0.38 and 0.41% for T_{II} and the control, respectively ($P<0.05$). These results are in good agreement with those of Baloch *et al.* (2006), who observed a consistent rise in titratable acidity and decay in pH value during the storage of Dhakki dates in controlled atmosphere. Yahia and Kader (2011) also reported an increase of TA and skin darkening during storage under air or oxygen atmosphere. In this study, the transient changes of gas allowed to maintain the initial date quality parameters.

Color parameters and browning index

The values of the color parameter a*, assessed at d 7, 14, 21 and 28, increased in all three types of storage compared to D_0 (Figure 2). The a* values of the control always remained superior to those of the two gaseous pretreatment up to 28 days, but significant differences were only observed at D_7 with the lowest value for T_I and the highest for the control. The decrease of L* values was observed for all types of pretreatment during the 28 days of storage (Figure 2). Values were significantly different at D_{14} with lower decrease in T_{II} compared with the control and T_I.

After 7 days, the absorption at 430 nm, increased in the control and T_I samples, while in the T_{II} samples, there was no change compared to the first day (Figure 2). At D_{14} significant differences were observed between the samples with higher values for the control, intermediate for T_I and lower ones for T_{II}. The decrease in L* values and the increase in a* values indicated the occurring of fruit browning and even darkening, especially in the case of the control. The measurement of absorption at 430 nm confirmed the reduction of darkening in the treated samples compared with that of the control: significant differences were observed for T_{II} samples at D_7 and D_{14} and for T_I at D_{14}. These results were consistent with those of Baloch *et al.* (2006), who reported that 'Dhakki' dates stored in controlled atmosphere were browning according to the gaseous composition. Browning was inhibited at low oxygen rates (Mutlak and Mann, 1984; Yahia and Kader, 2011)

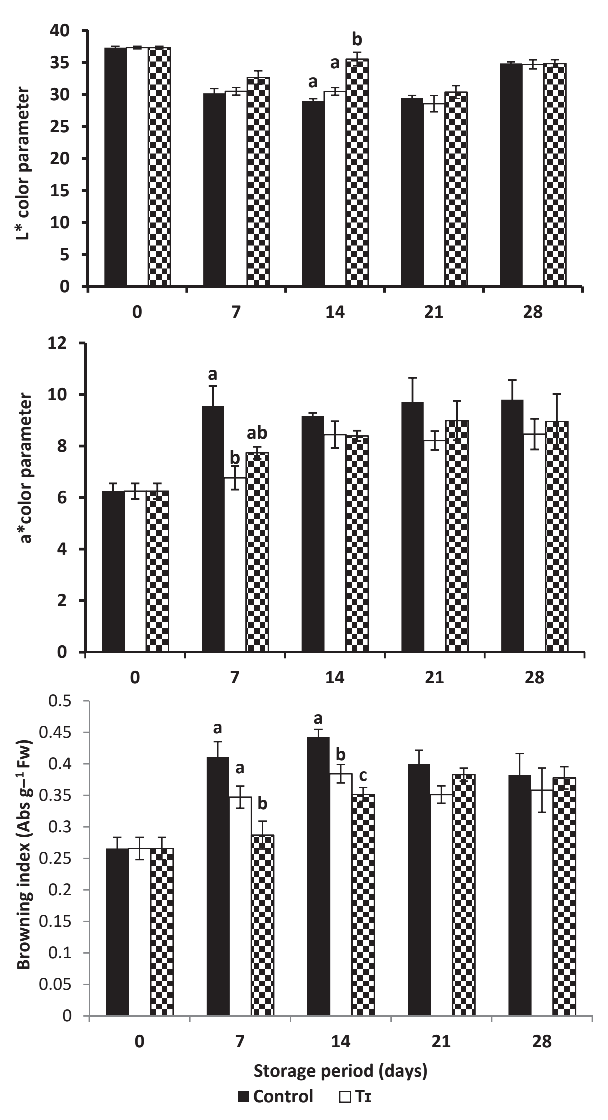

FIGURE 2. Evolution in external color (a* and L*) and index browning of 'Deglet Nour' fruit during storage at 35 °C for 28 days after different gaseous pretreatments: Air (control), T_I, and T_{II}. Each value is the mean of four replicates. Vertical bars represent standard errors of the means. Different letters indicate a significant difference between the three pretreatments at each storage period.

and at low temperature as well (Kader and Hussein, 2009). This study underlined that a gaseous pretreatment can also reduce date browning, and thus maintain the marketable fruit quality, as the color of dates is the most important attribute affecting fruit acceptability (Ismail *et al.*, 2001). Good quality dates tend to be light brown in color, also confirmed by Dowson (1982) for fresh 'Deglet Nour' dates.

Oxidation enzyme activity and total phenol content

The polyphenoloxidase (PPO) activity of T_I and T_{II} samples significantly decreased during the first 7 days of storage (Figure 3). The PPO activity of control samples remained stable during this time and then decreased until D_{14}. It was significantly higher for T_I than for T_{II} during all the storage period. The peroxidase (POD) activity of T_I and T_{II} samples decreased during the first 7 days and then increased to reach higher values than the control until the end of the storage (Figure 3).

The total phenol contents were higher in T_I and T_{II} samples compared with the control for the whole storage duration with greatest values around 200 mg 100 g^{-1} FW after 14 days of storage (Figure 3). In contrast to the total phenolic contents in T_I and T_{II} samples, in the control it was quite stable (~150 mg 100 g^{-1} FW) throughout the storage period except a slight drop at D_{21}.

Both gaseous pretreatments decreased the PPO and POD activity until 14 days of storage compared to the control sample. The low oxygen value injected into the pouches of LDPE at the beginning of the analysis can explain the decrease of these enzymes activities as oxygen is required to initiate enzymatic browning reaction. The decrease of POD activity may be also related to the decrease in PPO activity. There is a metabolic relation between POD and PPO enzymatic activities; in fact, the possible role of PPO as a promoter of POD activity is suggested since hydrogen peroxide is generated during the PPO catalyzed oxidation of phenolic compounds (Tomas-Barberan and Espin, 2001). In this study, T_{II} was more efficient to reduce PPO activity than T_I, maybe due to the lower initial CO_2 content.

This enzyme activities reduction was well correlated with the decrease of darkening of dates during the first 14 days of storage. Reduced browning associated with reduced PPO and POD activities have already been reported in dates (Pesis *et al.*, 2002; Hershkovitz *et al.*, 2005). Moreover, in our study a higher content of phenols was recorded in the treated samples that could be correlated with the browning decrease. In addition, shifting to higher storage temperature should have enhanced the level of phenolic compounds, as Biglari *et al.* (2009) found that storing dates at 18 °C after six months at 4 °C resulted in an increase of flavonoids and total phenolic compounds in the fruit.

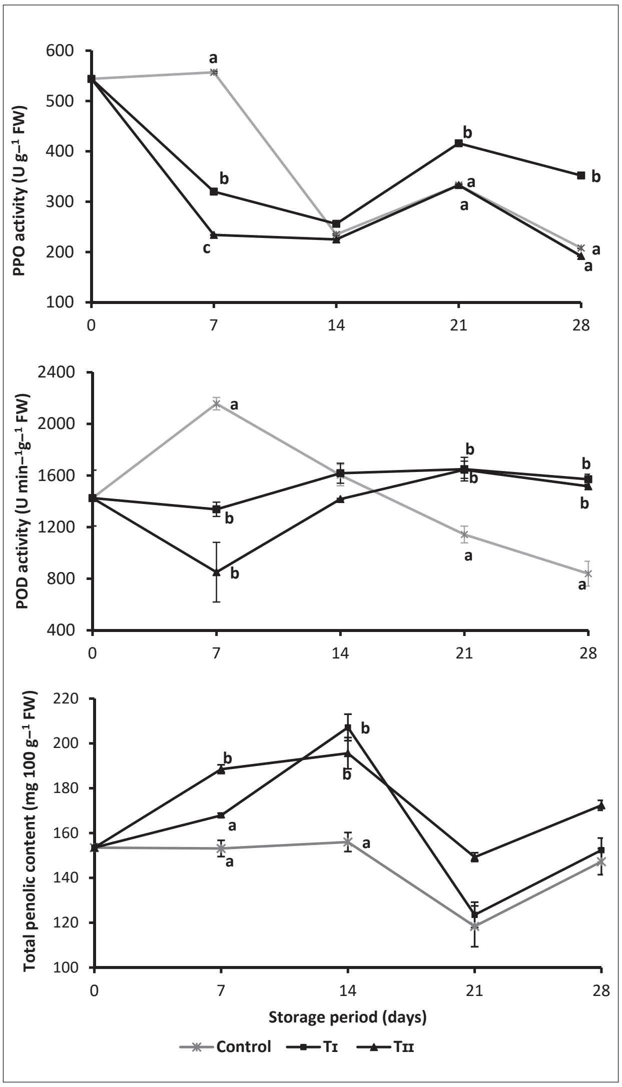

Figure 3. Changes in PPO and POD activities and phenolic contents of 'Deglet Nour' fruit during storage at 35 °C for 28 days after different gaseous pretreatments: Air (control), T_I, and T_{II}. Each value is the mean of four replicates. Vertical bars represent standard errors of the means. Different letters indicate a significant difference between the three pretreatments at each storage period.

Ascorbate content

The total ascorbate content (TAA) decreased after 28 days of storage in all the samples (Table 2) with a maximum value for each one at D_{14}. In the control, TAA was quite stable up to 21 days at 28.34 mg 100 g^{-1} FW and then dropped to 18 mg 100 g^{-1} FW at D_{28}. In the treated samples, TAA was much more variable up to 28 days except great increase at 52.33 mg 100 g^{-1} FW for T_{II}. Similarly, the content of ascorbic acid (ASA), which is the reduced form of ascorbic acid, was quite stable in the control sample up to 21 days and then decreased to 13.93 mg 100 g^{-1} FW at D_{28}. For the treated samples, the evolution profile of ASA followed that of TAA. The content of L-dehydroascorbic acid (DHA), which is the oxidized form of L-ascorbate (Vermerris and Nicolson, 2006), ranged from 20.87 to 28.46% in T_I and from 15.55 to 51.09% in T_{II}. At D_{14} the T_{II} samples showed a large increase of TAA to 52.33 mg 100 g^{-1} FW due to a rise in DHA to 51.09%.

Globally, ASA and TAA decreased during the storage period such as previously observed during processing and ripening of many fruits (Lee and Kader, 2000). TAA (L-ascorbate) was reported to be a good reducing agent that prevents oxidation (Padayatty, 2003; Vermerris and Nicholson, 2006). Ascorbic acid is a highly effective inhibitor of enzymatic browning in many tissues, primarily because it is able to reduce quinones to phenolic compounds, thus preventing the synthesis of the brown pigments (Walker, 1995). At high concentration, ASA could inhibit PPO activity by decreasing the cytosol pH (Vámos-Vigyázó and Haard, 1981; Degl'Innocenti *et al.*, 2007). Hence, the large increase in TAA observed in T_{II} samples after 14 days of storage is consistent with a sig-

Table 2. Changes in the ASA and DHA contents of 'Deglet Nour' date as related to gaseous pretreatment (Air, T_I, T_{II}) before storage at 35 °C for 28 days. Each value in the table is the mean ± standard deviation ($n = 4$).

Storage period (days)	Pretreatments	TAA (mg 100 g^{-1} FW)	ASA (mg 100 g^{-1} FW)	% DHA to TAA
0	-	27.45 ± 3.30	19.41 ± 1.88	29.28
7	Control	24.30 ± 1.38a	16.30 ± 0.61	32.92
	T_I	16.79 ± 4.87ab	12.01 ± 4.43	28.46
	T_{II}	25.81 ± 0.87ac	18.04 ± 0.76	30.10
14	Control	29.17 ± 1.97a	20.25 ± 0.49a	30.57
	T_I	22.84 ± 1.94a	16.25 ± 1.11b	28.85
	T_{II}	52.33 ± 5.22b	25.59 ± 0.65c	51.09
21	Control	28.34 ± 1.69a	19.90 ± 1.04a	29.78
	T_I	14.52 ± 0.95b	11.15 ± 2.45b	23.20
	T_{II}	14.14 ± 0.42b	11.94 ± 0.78b	15.55
28	Control	18.50 ± 0.55a	13.93 ± 1.34a	24.70
	T_I	17.10 ± 0.16a	13.53 ± 0.80a	20.87
	T_{II}	18.58 ± 0.30a	12.39 ± 0.12a	33.31

Values in the same column and same storage period followed by different letters are significantly different ($P < 0.05$).

nificant lower browning index observed at the same storage time, the conversion of ASA into DHA allowing to slow down the browning reaction. In this study, the transient changes in initial gaseous composition could be considered as an abiotic stress that induces the synthesis of plant defense metabolites such as ascorbate for 14 days and phenolics as well during the whole storage duration.

With an equal level of O_2, T_{II} with 5% CO_2 was more efficient than T_I with 20% CO_2 in slowing down the browning of 'Deglet Nour' dates during storage. The higher content of CO_2 initially injected in T_I could explain the difference. The effects of CO_2 during storage is very variable according to fruit or vegetable species: Gadalla (1997) indicated that CO_2 around 10% damaged onions (*Allium cepa*), resulting in internal browning; Thompson (2010) reported that high CO_2 levels could cause surface scald browning, pitting and excessive decay in eggplant (*Solanum melongena*). In this study, when comparing with the control, a high initial content of CO_2 (20%) in T_I did not alter the nutritional quality of the date, whereas a low initial CO_2 (5%) was effective to reduce browning. Together with Navarro *et al.* (2001) we suggest that the tolerance of dates to high CO_2 could be exploited to develop biologically safe alternatives to fumigation treatments to control storage pests.

Conclusion

Exposure of date samples to initial low levels of O_2 increased the phenolic content and avoided fruit browning. The decrease of PPO and POD activities was associated with the maintenance of high total phenol content, what may account for delaying the pericarp browning. The two gaseous pretreatments maintained the fruit quality, considering the slight decrease in pH, preservation of titratable acidity, TSS, ASA and phenols as well as the noticeable slowdown of fruit darkening. This better understanding of the postharvest behavior of 'Deglet Nour' fruit after cold storage is useful to find solutions for long conservation of dates.

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