Enzyme activity, sugar composition, microbial growth and texture of fresh Barhi dates as affected by modified atmosphere packaging

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Abstract The effects of modified atmosphere packaging (MAP) on various physicochemical quality attributes of fresh Barhi dates at the Khalal (yellow) stage were studied. Polypropylene (PP) and polyethylene (PE) plastic with varied perforation sizes (0, 50, 100 and 150 µm) were evaluated for storage of date fruits at temperatures of 1, 5, 15 and 25 °C up to 45 days. MAP showed significant effects on fruit quality. PP date packages perforated with 150-µm perforations and stored at 5 °C had the lowest acidity and microbial load. Dates packed in PE exhibited higher pectin methylesterase (PME) and polyphenol oxidase (PPO) activities than dates packed in PP. Dates stored in nonperforated packages had the highest PME, PPO and invertase activities. The maximum fructose and glucose contents were observed in PP-packaged dates stored at 15 °C. Analysis of the interactions of several MAP variables revealed temperature as the major variable controlling the quality of dates. The findings showed that MAP can be effectively employed to preserve the quality and improve the shelf life of Barhi dates using PP with 150-µm perforations as the packaging material during storage for 2 weeks at 5 °C.

Keywords Barhi dates · Enzyme activity · Microbiological characteristics · Modified atmosphere packaging · Physical properties · Sugar composition

Introduction

The popularity of minimally processed food products with fresh-like qualities has increased due to ease of handling, greater value, distinctive sensory traits, and health benefits (Mehyar et al. 2014). Agricultural products such as fruits and vegetables continue to undergo metabolic changes after harvest that significantly influence their quality attributes. The increase in demand for fresh fruits and vegetables requires new preservation techniques for maintaining the quality and safety and extending the shelf life of such products (Lee et al. 1995). Modified atmosphere packaging (MAP) involves modifying the concentrations of gases (O₂ and CO₂) inside a package to preserve product quality by retaining moisture, lowering the rate of metabolic activity and retarding microbial deterioration (Caleb et al. 2012). Other factors such as film permeability, storage temperature, humidity, and a fruit's physiological state also influence the respiration rate and overall quality of a product (García et al. 2006). In addition, fruit enzymes play a significant role in fruit ripening and control various quality characteristics of fruits such as color and sugar composition (Fachin et al. 2003). Ben-Yehoshua (1985) reported that packaging of fruits in low-density polyethylene (PE) bags delays ripening and softening and hence improves marketability. Perforated films are commonly used for MAP of fresh fruits and vegetables to control respiratory activity and the transfer of gases through the polymeric matrix (Lucera et al. 2011). Temperature is generally the most common variable that affects stored

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fruits and vegetables and, along with other climatic factors, causes various metabolic changes in fruits after harvest. This generalized effect of temperature is the same even in fruits stored using MAP (Ochoa-Velasco and Guerrero-Beltrán 2016).

Date palm (*Phoenix dactylifera* L.) is regarded as an essential subsistence crop in several arid and semiarid regions of the world because it is one of the most successful fruiting tree species in such regions. The date fruit is climacteric, and during the course of maturation, physiological changes occur that are attributed to the ethylene hormone (Chao and Krueger 2007). The Barhi date is a yellow variety of date that undergoes four physiological stages that are commonly described using Arabic terms [Kimri (hard, green, and immature), Khalal (hard, yellow, and edible), Rutab (soft, brown, and semi-ripe), and Tamar (soft, dark brown and fully ripe)]; these phases of maturity are associated with distinctive texture, sensory, and chemical attributes (Mehyar et al. 2014). Fruits are consumed in the Khalal, Rutab, and Tamar stages, depending on factors such as the amount of soluble tannins in the cultivars, market demand, and climate conditions (Glasner et al. 1999). The Khalal stage of Barhi dates, although short, is increasing in popularity and economic importance due to its desirable taste, health, and quality attributes (Botes and Zaid 2002). Fresh dates are subject to biological processes such as respiration, ripening, and senescence, which can rapidly influence their quality characteristics (Mehyar et al. 2014). Microbial spoilage of the fruit can increase through the transfer of skin microflora to fruit flesh, where microorganisms grow rapidly (Bracket 1992). The MAP technique can be employed to minimize such postharvest losses and maintain the quality of fresh fruits and vegetables (Mphahlele et al. 2016; Ochoa-Velasco and Guerrero-Beltrán 2016; Anurag et al. 2016; Pinela et al. 2016). MAP has already been applied to preserve certain quality attributes of ‘Khalas’ (Mortazavi et al. 2010) and ‘Khenaziy’ (Aleid and Saikhan 2017) date fruits under refrigerated storage. In addition, preharvest spraying with certain growth regulators has been used to retain most of the hydrolytic enzyme activities of Barhi dates at harvest and during storage (Mohamed et al. 2014). However, there is a lack of application of innovative postharvest techniques to preserve Barhi dates’ fresh-like characteristic qualities for a prolonged duration (Botes and Zaid 2002). The objective of the current study was to monitor the effects of MAP on Barhi date quality at the Khalal stage by controlling quality-influencing attributes such as enzyme activity, sugar composition, microbiological spoilage, and texture. The current study also aimed to evaluate the effects of MAP packaging material, perforation size, storage temperature, and storage period on various physicochemical quality attributes of fresh Barhi dates.

Materials and methods

Materials

Barhi dates were obtained from a local farm in Riyadh, Saudi Arabia, by selecting only healthy and undamaged fruits at the Khalal stage directly from plants; the fruits were then approved by horticultural experts. The dates were immediately transported to the laboratory under refrigeration for further treatment and storage studies. The chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich Corporation, USA. Round polypropylene (PP) and polyethylene (PE) plastic containers with a wall thickness of 20 μm were obtained from National Plastic Company, Riyadh, Saudi Arabia. Fruits were evaluated every 2 weeks (days 0, 15, 30 and 45). The control sample was fresh Barhi dates on day zero, at the start of various experiments.

Packing under modified atmosphere

A 500 g sample of Barhi dates was kept in each type of plastic container (5 replicates). The initial concentrations of O_2 ($21.3 \pm 0.1\%$) and CO_2 ($0.003 \pm 0.0\%$) were the same in all the containers, which were perforated on the top lid with 20 holes of different diameters, i.e., 50, 100 or 150 μm , yielding a total perforated area of 0.01, 0.02 and 0.03%, respectively, per container. The containers were then stored at 1, 5, 15, or 25 $^{\circ}\text{C}$ for up to 45 days.

Gas composition, acidity and pH analysis

During storage, gas composition (O_2 and CO_2) in packages was measured using a gas analyzer (PBI Dansensor, Check Point, Denmark). Acidity was estimated using reference method 942.15 (AOAC 2005). The pH was recorded according to method 981.12 (AOAC 2005) using a pH meter (HI4521, Hanna Instruments, USA).

Microbial evaluation

Total bacterial, mold, and yeast counts were recorded according to the recommended procedures (APHA 2001). Nutrient agar (Oxoid, Basingstoke, Hampshire, UK) was used for the total bacterial count. Inverted plates were incubated at 37 $^{\circ}\text{C}$ for 24 h. Potato dextrose agar (Oxoid) was utilized for molds and yeasts, and plates were incubated at room temperature (25 $^{\circ}\text{C}$) for 3–5 days. The results were reported as log colony-forming units per gram fresh weight (log cfu/g).

Sugar determination

Glucose, fructose, and sucrose were analyzed in date samples according to method 925.35 (AOAC 2005). Briefly, 5 g of ground Barhi date sample was mixed with 100 mL of distilled water and boiled for 10 min. Neutral lead (CH_3COO) (2 mL) was added to the mixture, which was then diluted to 200 mL and filtered, followed by addition of 1 g of dry potassium oxalate to precipitate excess lead used in clarification, mixing, and filtration. An aliquot of 25 mL of the clarified solution was filtered through a 0.45- μm microfilter and analyzed using an HPLC system (Shimadzu LC-10 AD, Shimadzu, Kyoto, Japan) (AOAC 2005) equipped with a $250 \times 4.6\text{-mm}$ column packed with 5- μm Supelcosil LC-NH₂ (Supelco, Bellefonte, PA, USA) and connected to a refractive index detector (RID-6A, Shimadzu Kyoto, Japan). The mobile phase (20% water and 80% acetonitrile, HPLC grade) was introduced by a delivery pump, model LC-10 AD (Shimadzu), at a flow rate of 2.5 mL/min. Determination of total sugars was carried out according to the Lane–Eynon method (AOAC 2005).

Enzyme activity

Enzymes (PME, PG, and PPO) were extracted by mixing 5 g of the fruit sample with 15 mL of extraction solution (100 mM sodium chloride and 50 mM sodium acetate buffer, pH 5.0) for 2 min at 4 °C, centrifugation at $15,000 \times g$ for 15 min at 4 °C, and removal of the liquid supernatant, which was then adjusted to pH 7.5 with 0.05 N NaOH. The enzyme extract was stored at -40 °C prior to estimation of enzyme activity.

Pectin methylesterase (PME)

The PME activity assay was based on a spectrophotometric procedure (Hagerman and Austin 1986). Briefly, 2.0 mL of 5 g/L pectin (Sigma, from citrus fruit) and 0.15 mL of 0.1 g/L bromothymol blue, which was prepared in 3 mmol/L potassium phosphate buffer (pH 7.5), were mixed with 0.85 mL of distilled water. The reaction was started by adding 100 μL of enzyme extract. The mixture was incubated at 25 °C for 3 min, and changes in absorbance were monitored at 620 nm. The blank sample included all components, with 0.85 mL of 1 mol/L NaCl added in place of the enzyme extract. PME activity is expressed as μmol galacturonic acid/g/min.

Polygalacturonase (PG)

PG activity was assessed by spectrophotometric measurement of the hydrolytic generation of new reducing ends

from the polygalacturonic acid (PGA) substrate (Sila et al. 2008). The reaction mixture was prepared by adding 50 μL of enzyme extract to 350 μL of a 0.2% PGA solution containing 0.04 M sodium acetate buffer (pH 4.4), followed by incubation at 35 °C in a water bath. Aliquots of 200 μL were taken after 0, 10, and 20 min intervals, and the reaction was stopped by adding 2 mL of 0.1 M sodium borate (pH 9.0), followed by addition of 400 μL of 1% solution of 2-cyano-acetamide reagent and boiling the mixture at 100 °C for 10 min. Once the mixture was cooled in an ice-water bath, the absorbance was measured at 276 nm. A standard curve of galacturonic acid (GalA) in 0.04 M Na acetate buffer (pH 4.4) in the range of 0–1.5 mM was also prepared. The enzyme activity was calculated as the release of reducing groups per unit time and per unit fresh weight ($\mu\text{mol/g/min}$).

Polyphenol oxidase (PPO)

PPO activity was measured with catechol as a substrate according to a spectrophotometric procedure (Plaza et al. 2003). The enzyme extract (0.1 mL) was immediately added to 2.9 mL of 0.11 M catechol solution prepared in 0.05 M sodium phosphate buffer (pH 6.5) at 25 °C, and the absorbance was recorded at 420 nm. One unit of enzyme activity was defined as the amount of the enzyme that causes a change in absorbance per min per gram of sample and is expressed as (OD/g/min).

Invertase

The invertase activity was measured using a previously described method (Rastegar et al. 2012). Approximately 15 g of deseeded dates cut into small pieces was blended for 2 min in a Waring blender with 4% NaCl solution (100 mL) containing polyvinylpyrrolidone (1 g) to prevent the formation of protein-tannin complexes. The homogenate was centrifuged at $20,000 \times g$ for 30 min at 4 °C, and the supernatant was decanted and retained. The residue was subjected to a further extraction using 4% NaCl (100 mL) as described above. A portion (30 mL) of the combined supernatant extract was dialyzed against several changes of water at 2 °C until sugars were no longer detectable. This dialysate was described as soluble invertase. The residue after centrifuging was washed with water to remove all remaining sugars, and the sugar-free residue was designated insoluble invertase.

The assay mixture included 1 mL of 0.5 M acetate buffer (pH 4.5), 1 mL of 1.5 M sucrose and enzyme extract (1 mL) and 2 mL of distilled water, for a total volume of 5 mL. This mixture was incubated for 20 min at 30 °C; then, a 1 mL sample was withdrawn every 5 min over a period of 20 min, followed by addition of 1 mL of the

reagent 3,5-dinitrosalicylic acid (in control tubes, the substrate was added after the heat treatment) and heating in a boiling water bath for 5 min. The absorbance was measured at 550 nm, using glucose at concentrations of 0.01–0.1 μmol as a standard. The amount of reducing sugar released was calculated from the calibration graph. One unit of invertase activity was defined as the amount of enzyme that hydrolyzed 0.5 μmol of sucrose per min under the above conditions.

Texture profile analysis (TPA)

A texture profile analysis was carried out using a texture analyzer (TA-HDi, Model HD3128, Stable Micro Systems, Surrey, England). Fruit samples were compressed with a rod velocity of 1.5 mm/s to a depth of 5 mm. Samples were compressed twice to yield two complete texture profile curves. The values for hardness (the maximum force required to compress the sample), resilience (the ability of the sample to recover its original form after deformation) and chewiness (the work required to make a solid food swallowable, which is numerically formulated as the product of gumminess and springiness) were measured. Cohesiveness and elasticity were also measured. Data were processed using Texture Expert Exceed, version 2.05 (Stable Micro Systems).

Statistical analysis

The current study consisted of a completely randomized factorial design for four variables (packaging materials, perforation size, storage time and temperature) and was analyzed according to the following model:

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \alpha\beta_{ij} + \alpha\gamma_{ik} + \alpha\delta_{il} + \beta\gamma_{jk} + \beta\delta_{jl} + \gamma\delta_{kl} + \alpha\beta\gamma_{ijk} + \alpha\beta\delta_{ijl} + \alpha\gamma\delta_{ikl} + \beta\gamma\delta_{jkl} + \alpha\beta\gamma\delta_{ijkl} + \epsilon_{ijklm} \quad (1)$$

The model consisted of overall mean and main effects for the four factors, two-factor interactions, three-factor interactions, four-factor interactions and the error (Granato et al. 2014). Analysis of variance (ANOVA) for triplicate determinations of analytical measurement was carried out using SAS 9.2 (SAS Institute, Cary, NC, USA), and significance was accepted at $p < 0.05$. Multivariate statistical analysis and principal component analysis (PCA) of the data were also performed using HJ-biplot methods via the free MULTBIPLLOT software (Vicente-Villardón 2010).

Results and discussion

This study was carried out to investigate the effects of two types of packaging materials, polypropylene (PP) and polyethylene (PE), as well as the effects of four sizes of perforations [0 (H0), 50 μm (H3), 100 μm (H6), and 150 μm (H9)] of the materials on various characteristics of fresh Barhi dates stored for up to 45 days at 1 to 25 °C. Fresh Barhi dates at the zero time point were used as a control to compare the effects of different storage variables on various physicochemical characteristics at storage temperatures of 1 °C (C1), 5 °C (C5), 15 °C (C15) and 25 °C (C25) after 0 (T0), 15 (T15), 30 (T30) and 45 (T45) days of storage using the model presented in Eq. 1.

Microbiological characteristics, pH, total acidity, and gas composition

The effects of plastics types on total acidity, pH, and O_2 and CO_2 concentration for Barhi dates are presented in Table 1, while the effects on microbiological characteristics are shown in Fig. 1. The plastic type was observed to have significant ($p \leq 0.05$) effects on acidity, pH, and the total plate count (TPC), while there were no significant effects on the yeast count, mold count, or O_2 or CO_2 concentration. Dates kept in PE packages had higher acidity, lower pH, and a higher TPC. The increase in TPC count may have contributed to the production of more acids in the dates. The gas compositions of PP or PE packages containing Barhi dates are presented in Table 1, showing that there were no significant differences in the percentages of O_2 and CO_2 between the two types of packages. When fruits are packed using the modified atmosphere packaging (MAP) technique, respiration of the packed product consumes O_2 and generates CO_2 . Generally, a lower (6%) O_2 concentration and a higher (10%) CO_2 concentration can improve microbial control and extend the shelf life of fresh fruits (Oliveira et al. 2015).

An increasing perforation size significantly ($p \leq 0.05$) decreased acidity, and the package containing no perforation had the highest acidity. The effects on pH, TPC, yeast, and mold were consistent with the acidity, i.e., the sample (H0) with the highest acidity showed the lowest pH and highest TPC and yeast and mold counts. Increasing the perforation size significantly ($p \leq 0.05$) decreased the percentage of CO_2 in the package, whereas that of O_2 was significantly increased. Perforated films are commonly used for MAP of fruits and vegetables to control respiratory activity and the transfer of gases through the polymeric matrix (Lucera et al. 2011). Various studies have reported the effects of perforations on the transport of water vapor and gases to achieve the desired modified

Table 1 Changes in titratable acidity (TTA), pH, CO₂ and O₂ of Barhi dates in relation to packaging material, perforation levels, storage temperature and time

Treatment	Sample	TTA (% malic acid)	pH	CO ₂ (%)	O ₂ (%)
Plastic type	Control	0.099 ± 0.01 ^C	6.46 ± 0.12 ^A	0.003 ± 0.01 ^B	20.94 ± 0.08 ^A
	PP	0.178 ± 0.01 ^B	5.82 ± 0.04 ^B	11.21 ± 0.21 ^A	12.85 ± 0.51 ^B
	PE	0.186 ± 0.04 ^A	5.74 ± 0.08 ^C	11.01 ± 0.14 ^A	12.87 ± 0.60 ^B
Holes	Control	0.099 ± 0.01 ^D	6.46 ± 0.12 ^A	0.003 ± 0.01 ^E	20.94 ± 0.08 ^A
	H0(0 µm)	0.206 ± 0.02 ^A	5.62 ± 0.06 ^D	14.29 ± 0.31 ^A	11.11 ± 0.82 ^E
	H3(50 µm)	0.179 ± 0.01 ^B	5.80 ± 0.05 ^C	12.16 ± 0.77 ^B	12.01 ± 0.34 ^D
	H6 (100 µm)	0.176 ± 0.01 ^B	5.84 ± 0.09 ^C	9.54 ± 0.90 ^C	13.72 ± 0.54 ^C
	H9 (150 µm)	0.164 ± 0.01 ^C	5.94 ± 0.05 ^B	8.46 ± 0.53 ^D	14.60 ± 0.20 ^B
	Control	0.099 ± 0.01 ^E	6.46 ± 0.12 ^A	0.003 ± 0.01 ^D	20.94 ± 0.08 ^A
Temperature	C1	0.128 ± 0.03 ^D	6.18 ± 0.26 ^B	8.23 ± 0.24 ^C	10.53 ± 0.62 ^E
	C5	0.135 ± 0.04 ^C	6.01 ± 0.21 ^C	9.74 ± 0.42 ^B	11.03 ± 0.33 ^D
	C15	0.185 ± 0.07 ^B	5.60 ± 0.94 ^D	10.84 ± 0.94 ^A	11.49 ± 0.52 ^C
	C25	0.295 ± 0.05 ^A	5.11 ± 0.04 ^E	11.28 ± 0.55 ^A	13.37 ± 0.95 ^B
	Control	0.099 ± 0.01 ^D	6.46 ± 0.12 ^A	0.003 ± 0.01 ^D	20.94 ± 0.08 ^A
Storage time	T15	0.116 ± 0.02 ^C	6.23 ± 0.23 ^B	12.32 ± 0.87 ^C	10.85 ± 1.23 ^B
	T30	0.196 ± 0.08 ^B	5.71 ± 0.76 ^C	18.83 ± 0.17 ^B	8.18 ± 0.85 ^C
	T45	0.259 ± 0.03 ^A	5.24 ± 1.08 ^D	19.86 ± 0.21 ^A	7.23 ± 1.63 ^D

Values represent mean ± standard deviation. Means not sharing the same superscript(s) (A–E) in a column for each treatment are significantly different at $p \leq 0.05$. Control: fresh Barhi dates; packaging materials: [PP polypropylene, PE polyethylene]; perforation levels: [0 (H0), 50 µm (H3), 100 µm (H6) and 150 µm (H9)]; storage temperature: [1 °C (C1), 5 °C (C5), 15 °C (C15) and 25 °C (C25)]; storage time: [0 (T0), 15 days (T15), 30 days (T30) and 45 days (T45)]

atmosphere within a package of fresh horticultural produce (González et al. 2008).

Optimization of MAP is important to avoid extremely low levels of O₂ and/or high levels of CO₂, which could result in anaerobic metabolism with possible off-flavor generation and an increased risk of anaerobic microorganism proliferation (Beaudry 2000). Hence, one of the general objectives of perforation in MAP is to create an equilibrium for gas transfer. The gas composition also depends on various other factors such as maturity, storage temperature, time of storage, and permeability of packaging material. For instance, a recent study described a close dependence of gas composition on prickly pear storage temperature. That study reported that one of the objectives of MAP is to decrease the O₂ concentration to limit respiration of the packed fruit (Ochoa-Velasco and Guerrero-Beltrán 2016). A study investigating MAP of Barhi dates at the Khalal stage in Iran (Mortazavi et al. 2010) revealed that a CO₂ concentration of 30% resulted in conversion of 50% of dates to the low-quality Rutab stage. The best quality and longest shelf life of Khalal dates could be achieved when the CO₂ concentration was 5%. Another recent study also revealed a close association between CO₂ and quality of Deglet Nour dates at the Tamar stage when preserved using the MAP technique (Jemni et al. 2016).

In this study, the effects of temperature on the total acidity, pH, O₂ and CO₂ concentration (Table 1), and

microbiological growth (Fig. 1) of dates stored in MAP showed that the storage temperature significantly ($p \leq 0.05$) affected most of these attributes. Acidity significantly ($p \leq 0.05$) increased, whereas pH was significantly ($p \leq 0.05$) reduced with increasing storage temperature up to 25 °C. TPC and the yeast/mold count increased with increasing storage temperature, with the highest values observed at 25 °C. This increase in temperature was favorable for the growth of microorganisms, similar to the results obtained in studies carried out with prickly pear fruit (Ochoa-Velasco and Guerrero-Beltrán 2016). Cold storage is preferable for fruits and vegetables packed in MAP, as the senescence process could otherwise progress rapidly (García et al. 2006). Storage temperature had significant ($p \leq 0.05$) effects on gas composition. Among the treatments (excluding the control), CO₂ and O₂ values were the highest at a storage temperature of 25 °C, whereas the lowest values were observed in date packages stored at 5 °C. Another study (Ochoa-Velasco and Guerrero-Beltrán 2016) also reported a rapid decrease in CO₂ and an increase in the O₂ concentration in fruit stored under cold conditions. However, a standard gas composition must be maintained to prevent the senescence process.

The biological properties of fresh fruits and vegetables continue to change during storage as a result of ongoing metabolic activities, including respiration and ripening, which are influenced not only by storage time but

Fig. 1 Effects of packaging materials, perforation levels, storage temperature and time on total plate count (log cfu/g) and yeast and mold count (log cfu/g) of Barhi date. Error bars represent the standard deviation. Bars bearing different letters are significantly different ($p \leq 0.05$) between treatments. Control: fresh Barhi dates; packaging

materials: [PP polypropylene, PE polyethylene]; Perforation levels: [0 (H0), 50 μm (H3), 100 μm (H6) and 150 μm (H9)]; storage temperature: [1 $^{\circ}\text{C}$ (C1), 5 $^{\circ}\text{C}$ (C5), 15 $^{\circ}\text{C}$ (C15) and 25 $^{\circ}\text{C}$ (C25)]; storage time: [0 (T0), 15 days (T15), 30 days (T30) and 45 days (T45)]

also by factors such as temperature and packaging materials (Mphahlele et al. 2016). Barhi dates were stored upto 45 days. The effects of the storage period were monitored at 15-day intervals, and changes in microbial load (Fig. 1), pH, acidity, and CO_2 and O_2 composition (Table 1) revealed that storage had significant ($p \leq 0.05$) effect on all these characteristics. The microbial count was highest in dates stored for 45 days, which also showed the lowest pH and highest acidity values of the date fruit samples. Increasing storage time increased the percentage of CO_2 and decreased that of O_2 . The two gases might be in equilibrium before the 15th day of storage. A similar trend involving a decrease in O_2 and an increase in CO_2 was reported by others (Mphahlele et al. 2016).

Enzyme activities and sugar composition

The effects of packaging materials on the enzyme activities of Barhi dates are shown in Fig. 2 (A,C,E,G). The activities of pectin methylesterase (PME), polyphenol oxidase (PPO), and polygalacturonase (PG) were significantly ($p \leq 0.05$) affected by the choice of packaging material, whereas there were no significant differences in the activity of invertase. The activities of PME and PG were higher in dates packed in PE packages, whereas PPO and invertase activities were higher in PP-packaged dates. Changes in the sugar composition (fructose, glucose, and sucrose) of dates from different packaging materials are also shown in Fig. 3 (A,C,E). The fructose and glucose contents of dates in PP packages were significantly ($p \leq 0.05$) higher than those of dates in PE packages, while the sucrose content of PE dates was significantly ($p \leq 0.05$) higher than that of PP-packaged dates. The sugar analysis was carried out on a dry-

Fig. 2 Effects of packaging materials, perforation levels, storage temperature and time on changes in sugar levels of Barhi date. Error bars represent the standard deviation. Bars bearing different letters are significantly different ($p \leq 0.05$) between treatments. Control: fresh Barhi dates; packaging materials: [PP polypropylene, PE polyethylene]; perforation levels: [0 (H0), 50 μm (H3), 100 μm (H6)

and 150 μm (H9)]; storage temperature: [1 °C (C1), 5 °C (C5), 15 °C (C15) and 25 °C (C25)]; storage time: [0 (T0), 15 days (T15), 30 days (T30) and 45 days (T45)]. PME: Pectin methyl esterase (μmol g⁻¹ min⁻¹); PPO polyphenol oxidase (OD/g/min); PG polygalacturonase (μmol g⁻¹ min⁻¹); Invertase (U/g f.w.)

Fig. 3 Effects of packaging materials, perforation levels, storage temperature and time on changes in sugar levels of Barhi date. Error bars represent the standard deviation. Bars bearing different letters are significantly different ($p \leq 0.05$) between treatments. Control: fresh Barhi dates; packaging materials: [PP polypropylene, PE

polyethylene]; perforation levels: [0 (H0), 50 μ m (H3), 100 μ m (H6) and 150 μ m (H9)]; storage temperature: [1 $^{\circ}$ C (C1), 5 $^{\circ}$ C (C50), 15 $^{\circ}$ C (C15) and 25 $^{\circ}$ C (C25)]; storage time: [0 (T0), 15 days (T15), 30 days (T30) and 45 days (T45)]

weight basis for fresh fruit. Enzymes play important roles in fruit ripening and in controlling various quality characteristics of fruits such as color and sugar composition. PME deesterifies pectin in fruits, and this reaction leads to the

production of methanol and free carboxyl moieties. PG depolymerizes the demethoxylated pectin, hydrolyzing α -1,4-glycosidic bonds of the polygalacturonic acid chain, resulting in the formation of low-molecular-weight

polygalacturonic acids and free galacturonic acid monomers. These two enzymes, when acting synergistically, lead to the depolymerization of cell wall pectin chains and a marked drop in viscosity (Fachin et al. 2003). Hence, decreased activity of these enzymes may prolong the period during which date fruits are of high quality. PPO mostly acts on *o*-quinones and produces various types of colored pigments, which cause enzymatic browning or color changes in fruits (Ghafoor and Choi 2012). Both PE and perforations in packaging materials decreased the activity of PPO.

The highest PME, invertase, and PPO activities (Fig. 2 (A,C,E,G)) were observed in the nonperforated package; however, the observed activity of PG was similar among H0-H6. For the H9 treatment, which had the largest perforations, the enzyme activity was the lowest among the packaged dates and was more similar to the fresh control sample. Despite significant ($p \leq 0.05$) differences in the fructose and glucose compositions of dates in relation to perforation, it cannot be concluded whether increasing the perforation size or number can increase the sugar content of dates (Fig. 3A, C, E). The fructose level was higher in the H0 treatment than in the other treatments, whereas the glucose level was highest in the H9 treatment. Sucrose contents were highest in the control and decreased in both types of packaging material. The hydrolysis of sucrose may be due to the increase in the activity of invertase with increasing perforation size (Fils-Lycaon et al. 2011).

As shown in Fig. 2 (B,D,F,H), the highest PME activity was observed at a storage temperature of 15 °C, whereas the lowest activity was observed in the dates stored at 25 °C. PPO and invertase activities were highest at 25 °C, whereas the activity of PG was highest at 15 °C. Enzyme activity usually increases with temperature, as well as in response to other factors. Ambient temperatures are usually favorable for the activity of different enzymes in fruits (Juan et al. 2011). Our findings regarding the effect of temperature on enzymes in date fruit are in agreement with those of others (Ali et al. 2004) who observed that increasing the storage temperature resulted in an increase in wall-softening enzymes in carambola fruit stored using the MAP technique. Increases in pectinesterase, β -galactosidase, polygalacturonase, and α -(1,4)-glucanase activities were observed at a higher storage temperature (28 °C) compared with those observed at 5 and 10 °C, even when the fruit was packed in MA packages. Higher fructose and glucose contents were observed in dates stored at 15 °C, whereas sucrose contents were higher at 5 °C (Fig. 3B, D, F). This discrepancy was due to the action of invertase, which decreases the sucrose content by hydrolyzing it into fructose and glucose and is consistent with increased activity of invertase.

Figures 2B, D, F, H and 3B, D, F show the effects of different storage periods on the enzyme activities and sugar composition of the date fruits, respectively. These characteristics showed significant ($p \leq 0.05$) differences between treatments. PME and PG showed initial increases in activity until the 30th day of storage but then decreased as the storage time was prolonged to 45 days. The PPO and invertase activities increased until the 15th day of storage and then gradually decreased. The invertase activity may be correlated with sugar content, particularly that of glucose, which increased until the 30th day of storage and then decreased as the storage time was prolonged to 45 days. The dates showed a gradual and significant ($p \leq 0.05$) decrease in sucrose but an increasing fructose content. Sucrose was entirely broken down to fructose and glucose by invertase by the 45th day of storage. Prolonged storage is generally detrimental to the quality of fruit, but strategies such as low-temperature storage in proper packaging with suitable perforations (MAP) may effectively preserve quality characteristics, such as the contents of sugars and other nutrients (Mphahlele et al. 2016).

Textural properties

The data showing the effects of the two types of packaging material on the textural properties of Barhi dates indicate that the hardness, elasticity, chewiness, and resilience remained largely unaffected by PE or PP plastic. The cohesiveness of the dates kept in PP packages was higher than that of dates kept in PE package (Table 2). The effects of perforation levels in the packaging material on the textural properties of the dates showed that textural properties varied ($p \leq 0.05$) between different treatments. Generally, H9 treatment produced textural properties relatively similar to those of fresh dates, indicating that the dates were fresher and less affected by cell wall-degrading enzymes and that the O₂ and CO₂ levels might have been appropriate for keeping respiration and ripening under control, as supported by data in Table 1.

Temperature is generally the most common variable that influences stored fruits and vegetables and, along with other climatic factors, initiates various metabolic changes in fruits after harvest. This generalized effect of temperature is the same even for fruits stored using the MAP technique (Ochoa-Velasco and Guerrero-Beltrán 2016). It was also observed that increasing storage temperature significantly worsened various textural parameters of the dates, including hardness, cohesiveness, elasticity, chewiness, and resilience. A loss of texture at elevated temperatures occurred because enzymes at high temperatures act on cell walls and break down the cellular structure, making fruits soft, requiring less force for fracturing and chewing and decreasing the ability of the fruit to retain its original

Table 2 Textural properties of Barhi dates in relation to packaging material, perforation levels, storage temperature and time

Treatment	Sample	Hardness (N)	Cohesiveness	Elasticity (mm)	Chewiness (J)	Resilience (J)
Plastic type	Control	48.82 ± 1.82 ^A	0.27 ± 0.10 ^A	0.96 ± 0.03 ^A	32.12 ± 0.66 ^A	0.51 ± 0.12 ^A
	PP	40.52 ± 0.23 ^A	0.22 ± 0.04 ^B	0.79 ± 0.15 ^B	26.37 ± 0.96 ^B	0.42 ± 0.12 ^B
	PE	39.31 ± 0.64 ^A	0.22 ± 0.04 ^B	0.78 ± 0.15 ^B	26.04 ± 0.05 ^B	0.41 ± 0.13 ^B
Holes	Control	48.82 ± 1.82 ^A	0.27 ± 0.10 ^A	0.96 ± 0.03 ^A	32.12 ± 0.66 ^A	0.51 ± 0.12 ^A
	H0(0 µm)	40.55 ± 0.76 ^{AB}	0.20 ± 0.05 ^E	0.71 ± 0.18 ^E	23.90 ± 0.61 ^D	0.38 ± 0.13 ^C
	H3(50 µm)	39.12 ± 0.50 ^{AB}	0.23 ± 0.04 ^C	0.81 ± 0.13 ^C	27.2 ± 0.45 ^{BC}	0.43 ± 0.12 ^{BC}
	H6(100 µm)	33.12 ± 0.81 ^B	0.21 ± 0.04 ^D	0.77 ± 0.15 ^D	25.81 ± 0.85 ^C	0.41 ± 0.14 ^C
	H9(150 µm)	46.88 ± 0.11 ^{AB}	0.25 ± 0.03 ^B	0.83 ± 0.12 ^B	27.92 ± 0.30 ^B	0.44 ± 0.11 ^B
Temperature	Control	48.82 ± 1.82 ^A	0.27 ± 0.10 ^A	0.96 ± 0.03 ^A	32.12 ± 0.66 ^A	0.51 ± 0.12 ^A
	C1	46.18 ± 0.24 ^B	0.23 ± 0.03 ^B	0.84 ± 0.11 ^B	28.00 ± 0.09 ^B	0.44 ± 0.13 ^{AB}
	C5	42.36 ± 0.86 ^C	0.23 ± 0.02 ^B	0.82 ± 0.12 ^B	27.58 ± 0.03 ^B	0.44 ± 0.10 ^{AB}
	C15	36.81 ± 0.63 ^D	0.21 ± 0.04 ^C	0.77 ± 0.16 ^C	25.66 ± 0.05 ^{BC}	0.41 ± 0.12 ^B
	C25	34.39 ± 0.34 ^E	0.20 ± 0.05 ^D	0.71 ± 0.19 ^D	23.63 ± 0.87 ^C	0.37 ± 0.14 ^B
Storage time	Control	48.82 ± 1.82 ^A	0.27 ± 0.10 ^A	0.96 ± 0.03 ^A	32.12 ± 0.66 ^A	0.51 ± 0.12 ^A
	T15	43.13 ± 0.23 ^A	0.20 ± 0.03 ^C	0.72 ± 0.12 ^B	24.08 ± 0.25 ^B	0.38 ± 0.11 ^B
	T30	30.94 ± 0.41 ^B	0.20 ± 0.02 ^B	0.73 ± 0.07 ^B	24.38 ± 0.27 ^B	0.39 ± 0.10 ^B
	T45	30.04 ± 0.39 ^B	0.19 ± 0.03 ^D	0.68 ± 0.11 ^C	22.88 ± 0.98 ^B	0.36 ± 0.10 ^B

Values represent mean ± standard deviation. Means not sharing the same superscript(s) (A–E) in a column for each treatment are significantly different at $p \leq 0.05$. Control: Fresh Barhi dates; Packaging materials: [PP polypropylene, PE polyethylene]; perforation levels: [0 (H0), 50 µm (H3), 100 µm (H6) and 150 µm (H9)]; storage temperature: [1 °C (C1), 5 °C (C5), 15 °C (C15) and 25 °C (C25)]; storage time: [0 (T0), 15 days (T15), 30 days (T30) and 45 days (T45)]

structure once compressed (Ali et al. 2004). In addition to disruption of the cell membrane/wall, elevated temperatures may also cause the loss of turgor or strength and thus cause fruit to become rubbery and soft (Lillford 2001). The textural properties of dates remained acceptable up to a storage temperature of 5 °C; this finding indicates that even when produce is packaged using the MAP technique, elevated or ambient temperatures may be detrimental to various quality characteristics of fresh fruits and vegetables (Juan et al. 2011). Indeed, this result is consistent with our observation that a storage temperature of up to 5 °C preserved the quality parameters of dates much more effectively than did storage temperatures of 15 and 25 °C. In another study, MAP storage of carambola fruits was recommended when accompanied by storage at 5–10 °C (Ali et al. 2004).

As shown in Table 2, textural quality was significantly reduced as the storage time was increased. The hardness value was not significantly different between the control and 15-day dates but then gradually decreased over longer storage periods. The values for all other textural properties were significantly ($p \leq 0.05$) lower than those of the fresh dates. With increasing storage time, the PME and PG activities increased, thereby increasing cell wall breakdown and loss of fruit vigor, which is consistent with the data for these enzymes in Fig. 2A, B, E, F. In addition to increased

enzyme activity, the storage time may also trigger various other mechanisms in fruits affecting fruit structure and softening the fruit. These mechanisms may include displacement of the cellulose microfibrils through the amorphous matrix of the cell wall, increased flow of fruit matrix, changes in the concentration of the molecular components (especially cellulose), changes in turgor pressure, and air occlusion in the matrix (Loredo et al. 2014).

Multivariate analysis

Multivariate analysis (HJ-Biplot) was carried out to definitively determine the interactive effects of the various treatments (plastic type, perforation, temperature, and time) on different quality attributes of Barhi dates (Fig. 4). Principal component analysis (PCA) demonstrated an excellent contribution of the axes (PC1 and PC2) to the plotted components due to a high percentage (> 68%) of the total variability of the data. Hierarchical clustering analysis clearly indicated the interaction between the treatments as three distinct groups clustered in the figure. The first group (upper right quadrant of the graph, circles) contains the treatments of PE and PP with 0-, 50-, 100-, and 150-µm holes stored at 25 °C for 15 and 30 days (P6, P8, E8, P9, P17, E18, P24, P26, E26, P27, E27, P30, P31, P32, E33, E35, E36). These treatments mostly affected the total

plate count (TPC), yeast count, titratable acidity (TA), and invertase (IN) and polyphenol oxidase (PPO) activities. The second group (lower right quadrant of the graph, square symbols) is composed of the treatments of PE and PP with 0-, 50-, 100-, and 150- μ m holes stored at 15 °C for 15 and 30 days (P7, E7, P16, E16, P24, E24, P25, E25, P33, E33, P34, E35). These treatments strongly contributed

to the attributes polygalacturonase activity (PG), fructose (FR), glucose (GL), and CO₂ as well as to the microbial loads, TA, IN, and PPO. The last group (center and left half of the graph, triangle symbols) contains the treatments of PE and PP with 0-, 50-, 100-, and 150- μ m holes stored at 1 and 5 °C for 15, 30, and 45 days (P1–P5, E1–E5, P10–P15, E10–E15, P19–P23, E19–E23, P28–P32, E28–E32). These

Fig. 4 The HJ-biplot based on principal component analysis for Microbial evaluation, Sugars determination, Enzyme activity, Texture profile analysis (TPA), Gas composition, acidity and pH analysis of modified atmospheric packaging treated Barhi dates. Titratable acidity (TA); PH; Total Plate count (TPC); Yeast and mold (YEST); O₂; CO₂; Pectin methyl esterase (PME); Polyphenol oxidase (PPO); Polygalacturonase (PG); Invertase(IN); Fructose (FR); Glucose (GL); Sucrose (SU); Hardness (HAR); Cohesiveness (COH); Elasticity (ELA); Chewiness (CHE); Resilience (RES). [P polypropylene; E polyethylene; (1–3) 0 hole, 1 °C, 15, 30, and 45 days;(4 and 5) 0 hole,

5 °C, 15 and 30 days; (6 and 7) 0 hole, 15 °C, 15 and 30 days; (8 and 9) 0 hole, 25 °C, 15 and 30 days; (10–12) 50 hole, 1 °C, 15, 30, and 45 days; (13 and 14) 50 hole, 5 °C, 15 and 30 days; (15 and 16) 50 hole, 15 °C, 15 and 30 days; (17 and 18) 50 hole, 25 °C, 15 and 30 days; (19–21) 100 hole, 1 °C, 15, 30 and 45 days; (22 and 23) 100 hole, 5 °C, 15, and 30 days; (24 and 25) 100 hole, 15 °C, 15 and 30 days; (26 and 27) 100 hole, 25 °C, 15 and 30 days; (28–30) 150 hole, 1 °C, 15, 30 and 45 days; (31 and 32) 150 hole, 5 °C, 15 and 30 days; (33 and 34) 150 hole, 15 °C, 15 and 30 days; (35 and 36) 150 hole, 25 °C, 15 and 30 days]

treatments mainly effected the sucrose content, pH, O₂, pectin methyl esterase (PME), and textural properties (hardness, cohesiveness, elasticity, chewiness, resilience). Regardless of plastic type and perforation size, storage temperature was the major factor that exhibited the greatest effect on the microbial growth, enzyme activities, sugar composition, and texture of Barhi dates. High storage temperature (25 °C) contributed positively to microbial growth, acidity, and invertase and polyphenol oxidase activities, with negative effects on pH, sucrose, O₂ and textural properties. A moderate storage temperature (15 °C) positively enhanced the enzyme activities and reducing sugars (fructose and glucose) in addition to acidity and microbial growth, while it negatively affected the texture, pH, oxygen, and sucrose content of the dates. However, the low storage temperatures (1 and 5 °C) contributed positively to the texture, oxygen, sucrose and pH and negatively to the enzyme activities, reducing sugars, acidity, and microbial growth during storage of Barhi dates.

Conclusion

This study was carried out to evaluate the effects of four variables, namely, plastic type, package perforation size, temperature, and storage time, on various quality attributes of fresh Barhi dates. All four factors showed significant effects on various physicochemical and microbial characteristics. Generally, PP could be considered a better packaging material. Treatments involving 150-µm perforations generally showed desirable effects on the quality of the dates. Storage at 5 °C for up to 2 weeks is preferable to ensure that Barhi dates packed and stored using the MAP technique have fresh-like properties. Changes in various quality attributes of yellow Barhi dates, such as enzyme activity, sugar composition, and texture, were closely interrelated under various MAP conditions. Extension of shelf life was proven possible with proper application of suitable MAP conditions for fresh dates. This study also highlights the importance of using the MAP technique for fresh dates, which currently are primarily processed and packed using conventional methods. The storage temperature was found to be a principal MAP variable, and lower temperatures had positive effects on multiple quality attributes of the Barhi dates.

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