Ripening of Ethylene-pretreated Bananas Is Retarded Using Modified Atmosphere and Vacuum Packaging

Edna Pesis,¹ Rosa Ben Arie, Oleg Feygenberg, Fanny Villamizar²

Department of Postharvest Science of Fresh Produce, A.R.O., the Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

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Abstract. Bananas have a short shelf life after ethylene treatment and there is a high commercial demand to increase the storage life of individual clusters at the retail stage. To extend the shelf life of ethylene-pretreated banana, two different forms of modified atmosphere packaging (MAP) were used. In the first, individual clusters of ethylene-pretreated bananas were stored in a range of microperforated polyethylene (PE) bags (25 μm) creating various MAPs. Storage in PE bags with low microperforation (PE8) that created an atmosphere with 11% CO_2 and 12% O_2 was the most effective treatment for delaying banana ripening. The banana clusters kept firmer with nice peel color after 1 week at 20 °C, but the humidity inside the bags caused some decay development on the crown cut. In the second type of MAP individual clusters of ethylene-pretreated bananas were stored in air-evacuated PE bags (80 μm) under light vacuum (550 mm Hg) for short periods of 24 to 48 hours followed by storage in the same PE bags after releasing the vacuum. Storing bananas in air-evacuated bags for 24 to 48 hours reduced O_2 levels to 1% and increased the production of CO_2 up to 30%, but perforating the bags dramatically reduced the CO_2 level to around 9% and increased the O_2 level to 12%. Storing ethylene-pretreated banana clusters under vacuum for a limited time (24 to 48 hours), did not cause any damage, although the levels of acetaldehyde (AA) and ethanol increased dramatically. The AA and ethanol levels of 150 and 300 $\mu\text{L}\cdot\text{L}^{-1}$, respectively, that accumulated in the PE bags did not cause any off-flavors; on the contrary, the taste panelists preferred these bananas. Adding ethylene absorbents (EAs) to the air-evacuated PE bags reduced the ethylene levels as well as the AA and ethanol levels in the bags, which indicate that EAs also absorbed the AA and ethanol volatiles. Storing ethylene-pretreated banana clusters under vacuum for 24 to 48 hours was the most effective treatment for delaying ripening and senescence in yellow bananas (stage 3 to 4).

Banana (*Musa sapientum*) is a classical climacteric fruit that has high metabolic activity. It produces large quantities of CO_2 and ethylene during the climacteric phase (Burg and Burg, 1965). The use of modified atmosphere packaging (MAP) for preclimacteric bananas wrapped in polyethylene (PE) was reported as long ago as the 1960s (Scott and Roberts, 1966), and it was soon found that the addition of ethylene absorbents to the PE bags was effective in delaying ripening of preclimacteric bananas (Liu, 1970; Scott et al., 1970). Storage of preclimacteric bananas under hypobaric conditions, at 80 to 150 mm Hg, showed that it is possible to keep them for up to 120 d (Apelbaum et al., 1977). Storing preclimacteric bananas in modified or controlled atmospheres with low oxygen levels was shown to be effective for delaying banana ripening, keeping their green condition for long time, and enabling transportation over long distances (Kader, 1994). Also, treatment with reduced oxygen for short periods (1 to 3 d) before storage retarded the preclimacteric

ripening of bananas by delaying the climacteric peak (Pesis et al., 2001; Wills et al., 1982). Application of ethanol vapor to preclimacteric bananas did not delay ripening, but application of acetaldehyde (AA) succeeded in doing so (Hewage et al., 1995).

All commercial bananas are pretreated with ethylene to enhance their ripening before marketing. However, ethylene-pretreated fruit have a short shelf life: 3 to 5 d at 20 °C. Storage of ethylene-pretreated banana sealed in PE caused dramatic softening of the fruit (Fuchs and Gorodeiski, 1971), but Liu (1976) demonstrated that it is possible to hold ethylene-pretreated banana for 28 d in a 1% O_2 atmosphere or under a pressure of 1/10 atmospheric at 14 °C. Senescence was also effectively delayed by MAP storage of ethylene-pretreated banana in perforated PE bags, in which the ripening process continues normally (Bai et al., 1990). Recently, it was shown that PVC packaging for ethylene-pretreated banana was effective in extending shelf life (Rompophak et al., 2004).

Since Sisler et al. (1995) found that application of 1-methylcyclopropene, (1-MCP) the ethylene inhibitor, to preclimacteric banana prevented their ripening, many studies have addressed the inhibition of banana ripening with 1-MCP. The effect of 1-MCP in delaying ripen-

ing was shown on preclimacteric bananas and also on propylene-treated bananas (Golding et al., 1998). Later, it was shown that application of 1-MCP to preclimacteric banana packed in PE bags extended their postharvest life (Jiang et al., 1999). However, it was recently shown that the effectiveness of 1-MCP was inconsistent when applied to ethylene-pretreated bananas (Pelayo et al., 2003). Also, storage of ethylene-pretreated bananas under nitrogen did not extend their shelf life compared with that of air-stored banana; moreover, it induced skin browning (Klieber et al., 2002). Also, application of ethanol to ethylene-pretreated bananas by vacuum infiltration did not delay ripening (Bagnato et al., 2003).

In the present study we examined the effects of packing ethylene-pretreated bananas in microperforated PE bags or under vacuum in PE bags, each of which created various modified atmosphere packagings (MAPs), in order to extend their shelf life at 20 °C.

Material and Methods

Green, unripe, three-quarters-full banana hands (*Musa* spp. AAA group 'Ziv') were harvested and treated with 100 ppm ethylene for 48 h at 17 °C. The ethylene-pretreated bananas were cut into clusters (1000 g/cluster) and chlorine (0.2%) was sprayed on the cut crown. Each cluster was packed in an individual polyethylene (PE) bag, and two different packing technologies were used.

1) Banana clusters (stage 3 to 4) were sealed in PE (25 μm thick) bags with several different microperforation patterns, which created various modified atmosphere (MA) conditions. The treatments included: control = unpacked; unperforated PE (PE); low microperforation = 8 holes (PE8); medium microperforation = 12 holes (PE12) and high microperforation = 16 holes (PE16). Each hole diameter (ϕ) was 0.7 mm.

2) Banana clusters (stage 3 to 4) were sealed under vacuum with a Multivac instrument (Multivac, Germany) (550 mm Hg for 5 s) (Pesis et al., 1986). The PE film was 0.08 mm thick (CO_2 transmission 27,000 $\text{mL}\cdot\text{m}^{-2}\cdot\text{atm}^{-1}$ per 24 h at 25 °C; O_2 transmission 8,900 $\text{mL}\cdot\text{m}^{-2}\cdot\text{atm}^{-1}$ per 24 h at 25 °C). A thicker PE was used because the PE of 25 μm (used in the first experiment) could not stand the vacuum and the PE film was worn out. After 0, 24 and 48 h the air-evacuated PE bags were microperforated with a needle (8 holes, 0.7 mm ϕ /hole) and the vacuum was released. In addition, one ethylene absorbent (EA) sachet based on KMnO_4 (11 g/bag) (Conserver 21, Spain) was placed in some of the PE bags before air evacuation. The treatments included Vac0 = vacuum released immediately; Vac24 = vacuum released after 24 h; Vac48 = vacuum released after 48 h; Vac24+EA = as Vac24 plus EA sachet; Vac48+EA = as Vac48 plus EA sachet.

The gases that accumulated in the packages were determined by gas chromatography (GC). A silicone septum was glued onto each bag, and headspace samples were taken with a 10-mL syringe. The GC for CO_2 and O_2 detection,

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¹Corresponding author; email epesis@agri.gov.il.

²Dept. of Civil and Agricultural Engineering, University National of Colombia, Bogotá, Colombia.

Table 1. Effect of ethylene absorbent (EA) sachets on the levels of acetaldehyde (AA) and ethanol gases during 60 min at room temperature. The gases were injected into 500-mL jars (without fruit) via a latex tube at zero time. Data are means of three jars.

Jars	Gas	AA or ethanol ($\mu\text{L}\cdot\text{L}^{-1}$)					
		Time (min)					
		0	5	15	30	45	60
Empty	AA	230.3 a ²	230.3 a	217.6 a	202.7 a	201.4 a	196.7 a
1 EA sachet	AA	230.3 a	139.7 b	39.9 b	13.6 b	7.0 b	3.7 b
2 EA sachets	AA	230.3 a	30.3 c	12.3 c	5.3 c	3.5 c	1.9 b
Empty	Ethanol	76.7 a	76.7 a	66.8 a	59.1 a	58.1 a	58.2 a
1 EA sachet	Ethanol	76.7 a	36.1 b	6.6 b	4.1 b	2.9 b	2.4 b
2 EA sachets	Ethanol	76.7 a	14.0 c	5.7 b	3.0 b	2.2 b	1.7 b

²Different letters within a column, in each gas type, indicate that means are significantly different at the 5% level.

was fitted with a thermal conductivity detector with a double-column CTR-1 (Altech, 6 ft $\times$ 1/4 inch), carrier gas helium, oven temp 30 °C (Packard 839). Ethylene was determined with a flame ionization detector (FID) using packed alumina column (4 ft $\times$ 1/8 inch), carrier gas helium, oven temperature 80 °C, injection temperature 100 °C, detector temperature 150 °C (Varian 3300). Acetaldehyde and ethanol were determined with FID using packed 10% Carbowax 20M column (6 ft $\times$ 1/8 inch), carrier gas helium, oven temperature 80 °C, injection temperature 110 °C, detector temperature 180 °C (Varian 3300).

All the banana clusters were stored at 20 °C, and the bags were opened after 1 week. Storage at 20 °C is the regular temperature for shelf life of ethylene-pretreated banana as also chose in other works (Bomphophak et al., 2004; Pelayo et al., 2003). Fruit quality was checked according to banana color, crown cut color, decay, firmness and taste. Fruit firmness was determined on two pared sides of each fruit (10 measurements per treatment) using an electronic penetrometer (Chatillon, New York) with a 0.6-cm conical tip and the data was expressed in Newtons.

Fruit color was determined visually and by using a chromameter (Minolta, Japan), that expresses the color as hue angle. A decreasing hue angle indicates increasing browning of the peel (yellow peel = 90°). The visual appearance was expressed as an index on an eight-grade scale: 1 = green; 3 = breaker; 5 = yellow, green tip; 7 = yellow, flecked with brown; 8 = overripe. The color of the crown cut was evaluated according to a visual index on a five-grade scale: 0 = no darkening, 4 = high darkening. The decay percentage of a hand was calculated according to the number of fingers with decay out of the total number of fingers.

To see if the ethylene absorbent (EA) sachets absorb also other volatiles besides ethylene, we conducted a preliminary experiment. None, one or two sachets were placed in empty 500-mL glass jars and AA and ethanol gases were injected via latex tubes at the same concentrations to all jars. Immediately the gas atmosphere was detected by GC during 60 min at room temperature. The presence of the EA sachets caused the oxidation of AA and ethanol in minutes, and using two EA sachets was more effective in absorbing AA and ethanol than one sachet (Table 1). In addition, the producing company of Conserver21 mentions that the

Conserver21 absorbs other volatiles including aldehydes and alcohols (www.iespana.es/conserver21).

Total soluble solids (TSS) were checked in the peel and pulp of the banana by freezing the tissue for several days at -20 °C. Thawing the frozen tissue enabled to measure the TSS in the outcome juice using a refractometer (Atago 0% to 32%, Japan). This method was adopted from tomato, where it was shown that TSS and other fruit constituents remained relatively constant during the frozen storage and were similar to the values found in fruit before freezing (Buret et al. 1983). The values of the TSS measured by this method were similar to the values determined with the standard method of checking TSS in banana tissue homogenized with water (Dadzie and Orchard, 1996) (data not shown).

A taste panel of 20 people checked unpacked bananas compared to bananas stored in the air-evacuated PE bags after 7 d at 20 °C, and the panelists were asked to indicate which sample they preferred according to a sensory evaluation test (Dadzie and Orchard, 1996).

The data were analyzed using the Statistical Analysis System (SAS, 1999). Analysis of variance (PROC ANOVA), or regression analysis (PROC REG) were used to determine effect of treatment on quality factors or gas evolutions. Means were separated by Duncan's multiple range test at 5% level. The results of the gas analyses are means of five samples $\pm$ SE.

Results and Discussion

Ethylene-pretreated bananas stored in MAP. Storing ethylene-pretreated banana clusters in PE (25- μm) bags without perforation (PE) or with different

levels of microperforation (PE8, PE12, PE16) created various MA conditions. During storage at 20 °C the CO₂ levels in all perforated bags were around 15%, dropping to 10%, while in unperforated PE bags the CO₂ levels rose to 25% and fell to 17% after 7 d at 20 °C (Fig. 1). The accumulation of CO₂ on the second and third days in bananas stored in unperforated PE bags represents a climacteric peak that follows the ethylene treatment; similar to the CO₂ peak that was observed in Cavendish bananas or Sucrier bananas that were sealed after ethylene treatment and stored at 18 to 20 °C (Fuchs and Gorodeiski, 1971; Romphophak et al., 2004). The O₂ levels were significantly lowest in the unperforated PE bags, and increased as the number of microperforations increased (Fig. 1). There was a high linear correlation between the levels of O₂ and the time in all treatments (PE— $R^2=0.954$; PE8— $R^2=0.905$; PE12— $R^2=0.938$; PE16— $R^2=0.83$). Perforating the PE with different numbers of holes (0, 8, 12, and 16) caused a greater permeation of O₂ molecules via the thin PE as the numbers of holes increased. In PE16 bags O₂ levels reached 12% while in unperforated PE bags they reached just 4% O₂ after 7 d at 20 °C (Fig. 1). The levels of ethylene increased in all the MAPs because the bananas continued to ripen and to produce more ethylene after they had been removed from the ethylene treatment (Fig. 2). The lowest ethylene levels were found in the PE and the PE16 treatments (Fig. 2); in the PE treatment, probably because of inhibition by the high CO₂ in the bag (Fig. 1), while in PE16, the ethylene probably permeated via the holes. Inhibition of ethylene by high CO₂ levels was shown in many works done on storage of banana in MAP (Kubo et al., 1993; Liu, 1970;

Fig. 1. Effects of various microperforation levels in PE bags on gas composition (CO₂, O₂) of ethylene-pretreated bananas during 7 d at 20 °C. Each bag contained one cluster. PE = no perforation; PE8 = 8 holes; PE12 = 12 holes; PE16 = 16 holes. Results are means of five clusters $\pm$ SE.

Fig. 2. Effects of various microperforation levels in PE bags on volatiles accumulation [acetaldehyde (AA), ethanol, and ethylene] of ethylene-pretreated bananas during 7 d at 20 °C. Each bag contained one cluster. PE = no perforation; PE8 = 8 holes; PE12 = 12 holes; PE16 = 16 holes. Results are means of five clusters $\pm$ SE.

greener (Fuchs and Gorodeiski, 1971; Liu, 1976). Under low O_2 there is accumulation of AA and ethanol which was shown to retain the green color in fruit including banana (Pesis et al., 2001) and tomato (Beaulieu et al., 1997). The accumulation of AA and ethanol volatiles in the unperforated PE bags occurred probably because of the PE film (25 μ m) that was less permeable to these molecules than to CO_2 or O_2 (Fig. 2 vs. Fig. 1).

All the banana clusters became yellow at the end of 1 week at 20 °C, except for those in the unperforated PE bag, which remained greener although they were softer (Table 2). The banana that was stored in unperforated PE suffered from higher decay level, than those stored in perforated PE bags, probably because of accelerated softening (Table 2). Also Bai et al. (1990) and Romphopphak et al. (2004), showed that using unperforated PE bags caused injuries to the ethylene-pretreated bananas stored at 20 °C.

The color grade indicated by the hue angle expressed mainly the spots on the peel. The control unpacked fruit were already overripe, with black spots that led to a lower hue angle ($H^\circ = 78.3$); all the other treatments had higher hue values, which decreased as the number of perforations and the O_2 levels inside the bag increased (Table 2, Fig. 1). The ripening index revealed that bananas in unperforated PE bags reached only color stage 5, and that the fruit became more ripened as the perforation rate increased (Table 2). Storing ethylene-treated banana in perforated PE bags was efficient in delaying ripening (Table 2). The highest level of O_2 was found in the high micro perforation (PE16)

treatment, and this made the fruit yellow but soft. The lowest microperforation (PE8) treatment had around 5% to 7% O_2 and 12% CO_2 , and was the best treatment among the MAPs; it maintained fruit firmness, generated a nice yellow color ($H^\circ = 88.8$), had low sugar in the peel (indicating that the peel was not overripe) and high TSS in the pulp, representing normal ripening (Dadzie and Orchard, 1996; Marriott et al., 1981) (Table 2). The normal ripening of the bananas continued in all the perforated PE bags, as can be seen from the continuous accumulation of ethylene in the bags, whereas in the unperforated bags the ethylene levels remained lower (Fig. 2). Bai et al. (1990) obtained similar results, which showed that storing ethylene-pretreated bananas in perforated PE bags enabled normal ripening and normal volatile production at 20 °C.

However, the ethylene-treated banana clusters that were packed in the various MAPs suffered from mycelium development on the crown cut, which indicates that chlorine treatment alone is not enough to prevent crown rot (Table 2). The unpacked control suffered from more general decay than the banana clusters packed in the various MAPs (Table 1). The ethylene-pretreated banana that were stored in unperforated PE bags had the highest CO_2 levels, which led to reduced crown rot development, but the peel remained greener and softer (Table 2). Packing preclimacteric bananas in PE bags increased rot development on the crown cut (Scott et al., 1971) and on the bunch main stalk (Wade et al., 1993). In the present study, in order to see how MAPs influence decay control, we used chlorine alone, which was not enough to prevent mycelial development on the crown cut (Table 2). The use of chemical fungicides on the crown cut has been previously suggested as a means of preventing decay development (Scott et al., 1971; Wade et al., 1993).

Ethylene-pretreated banana stored in MAP under vacuum. Packing the ethylene-pretreated bananas in PE bags under vacuum caused dramatic changes in the gas atmospheres inside the bag. In the bags in which the vacuum was released immediately (Vac0), the level of CO_2 remained constant, around 14%, and the level of O_2 around 6% to 7% (Fig. 3). When the bananas were under vacuum for 24 or 48 h the levels of O_2 were significantly low, around 1%, and the CO_2 accumulated to a higher level than 30% (Fig. 3). The levels of CO_2 accumulation in the bags (Fig. 3) were similar to those found previously in air-evacuated PE bags containing persimmon fruit (870 g) dur-

Kader, 1994; Scott and Roberts, 1966; Scott et al., 1970, 1971; Thompson, 1998).

In addition, there was accumulation of the anaerobic metabolites, AA and ethanol, in the unperforated PE bags, whereas their levels were significantly lower in all the perforated PE bags (Fig. 2). The levels of ethanol were around 20 times higher than those of AA and both of these metabolites kept accumulating during 7 d at 20 °C (Fig. 2). The higher ethanol levels can be attributed to the fact that ethanol, which is an end product of the anaerobic respiration, accumulates to higher levels than AA, which is an intermediate metabolite (Pesis et al., 1986; 2001). The present findings about accumulation of AA and ethanol in unperforated PE bags are similar to those of Bai et al. (1990) and Romphopphak et al. (2004) who found increased fermentation flavor in ethylene-pretreated bananas sealed in PE bags at 20 °C.

The higher accumulations of CO_2 , AA and ethanol in the unperforated PE bags compared with those in the perforated PE bags (Figs. 1 and 2) probably caused the greener peel. It was shown previously that high CO_2 or low O_2 kept the ethylene-pretreated banana much

Table 2. Effects of various MAP conditions on ethylene-pretreated banana quality after 8 d at 20 °C. Control = unpacked; PE = unperforated PE; PE8 = microperforation with 8 holes; PE12 = microperforation with 12 holes; PE16 = microperforation with 16 holes. Results are means of five clusters.

Treatment	Firmness (N)	Peel color (H°)	Ripening index (1–8)	TSS peel	TSS pulp	Mycelium crown cut index (0–4)	Decay finger (%)
Control	6.2 c ^a	78.3 b	7.5 a	7.5 a	20.6 a	3.00 a	9.05 a
PE	6.8 c	89.4 a	5.0 c	5.7 b	19.8 b	0.50 c	1.91 b
PE8	10.2 a	88.8 a	6.0 b	5.9 b	21.2 a	1.33 b	0.48 c
PE12	8.4 b	87.4 a	7.0 a	6.4 a	21.3 a	1.33 b	0.48 c
PE16	7.2 bc	85.1 ab	7.2 a	6.3 a	20.5 a	1.75 b	0.00 c

^aDifferent letters within a column indicate that means are significantly different at the 5% level

Vac (h)	-EA	+EA
0	○	
24	□	■
48	△	▲

Fig. 3. Effects of vacuum duration and the addition of ethylene absorbent (EA) sachet on gas composition (CO_2 and O_2) formed by ethylene-pretreated bananas sealed in air-evacuated PE bags for 6 d at 20 °C. Each bag contained one cluster. Results are means of five clusters $\pm$ SE.

ing the first 2 d storage at 20 °C (Pesis et al., 1986). However, punching the air-evacuated bags with a needle (eight holes), immediately released the vacuum and the O_2 levels rose to around 14% and the CO_2 dropped to 7% in all vacuum-treated bananas (Fig. 3).

The ethylene levels increased linearly and gradually only in the Vac0 treatment ($R^2 = 0.999$), which had the highest level of ethylene in the first day after enclosure (Fig. 4), whereas in the banana clusters under vacuum the levels of ethylene were significantly low, and releasing the vacuum increased the ethylene level in Vac24 to $2 \mu\text{L}\cdot\text{L}^{-1}$ on the second day and that in Vac48 to $4.5 \mu\text{L}\cdot\text{L}^{-1}$ on the third day (Fig. 4).

The addition of ethylene absorbent (EA) sachets to the PE bags reduced the ethylene levels significantly in both the Vac24+EA and the Vac48+EA treatments (Fig. 4). Many other works showed the effectiveness of the EA sachets (from various manufacturing companies)

to reduce the levels of ethylene by oxidizing the ethylene (Bai et al., 1990; Fuchs and Gorodeiski, 1971; Liu, 1970; Scott et al., 1970). The highest AA and ethanol production during 6 d at 20 °C was found in the Vac48 treatment, and the second high levels of AA and ethanol were found in the Vac48+abs treatment (Fig. 4). AA and ethanol accumulated because the evacuation of the air led to low O_2 levels inside the fruit, which, in turn, induced the anaerobic metabolism (Fig. 4). Also in persimmon fruit, closing persimmon in PE bags under vacuum induced high levels of AA and ethanol during storage at 20 °C (Pesis et al., 1986). The vacuum treatments were applied only for short periods—24 or 48 h—to minimize the accumulation of anaerobic metabolites, since the longer the period under vacuum, the more was the production of AA and ethanol (Fig. 4). In ethylene-pretreated bananas cv. Sucrier (AA

Vac (h)	-EA	+EA
0	○	
24	□	■
48	△	▲

Fig. 4. Effects of vacuum duration and the addition of ethylene absorbent (EA) sachet on volatile production [acetaldehyde (AA), ethanol, and ethylene] by ethylene-pretreated bananas sealed in air-evacuated PE bags for 6 d at 20 °C. Each bag contained one cluster. Results are means of five clusters $\pm$ SE.

group), fruit in PVC film and PE bags exhibited less senescence spotting than the controls, but those sealed in PE bags exhibited fermentation flavor (Romphopha et al., 2004). Also, Klieber et al. (2002) have shown that holding ethylene-pretreated bananas under a nitrogen atmosphere at 22 °C for 6 to 24 h increased the discoloration of the peel. In the present study, application of vacuum for 24 to 48 h did not cause any problem of discoloration, and the fruit ripened normally and developed a nice yellow color (Fig. 5).

The reduction of AA and ethanol levels in the Vac24+EA and Vac48+EA probably occurred because the EA also oxidized other compounds such as AA and ethanol in addition to ethylene. In the preliminary experi-

Table 3. Effects of various conditions in air-evacuated PE bags on ethylene-pretreated banana quality after 1 week at 20 °C. Control = unpacked; Vac0 = vacuum released immediately; Vac24 or Vac48 = vacuum for 24 or 48 h; Vac24+EA or Vac48+EA = vacuum for 24 or 48 h plus EA sachet. After the vacuum was released the banana clusters remained in the PE bags for up to 6 d at 20 °C. Results are means of five clusters.

Treatment	Firmness (N)	Peel color (H°)	Ripening index (1–8)	TSS peel	TSS pulp	Blackening crown cut index (0–4)	Taste preference (%)
Control	9.58 c ^z	81.2 c	7.0 a	7.6 a	22.8 a	3.00 a	24
Vac0	11.64 b	90.5 b	5.5 b	7.1 a	21.4 b	0.50 b	---
Vac24	11.98 b	90.1 b	5.0 b	6.2 b	21.2 b	0.25 b	35
Vac48h	12.56 ab	90.3 b	5.2 b	6.3 b	21.1 b	0.00 b	48
Vac24+EA	13.48 a	91.3 a	4.2 c	6.0 b	22.3 ab	0.00 b	---
Vac48+EA	13.30 a	91.3 a	4.7 c	6.8 ab	21.7 b	0.50 b	---

^zDifferent letters within a column indicate that means are significantly different at the 5% level.

Fig. 5. Effect of vacuum treatment on ethylene-pretreated bananas after 1 week of storage at 20 °C: Control = unpacked; Vac24 = vacuum for 24 h; Vac24+EA = vacuum for 24 h plus EA sachet. After the vacuum was released the banana clusters remained in the PE bags for up to 6 d at 20 °C.

ment without the fruit we showed that the EA sachets oxidized the AA and ethanol that was injected inside the jar (Table 1). In the website of Conserver21 it is stated that EA sachets, besides ethylene, also absorb other volatiles including aldehydes and alcohols (www.ies-pana.es/conserver21).

In the bags in which the vacuum was released immediately (Vac0), the AA and ethanol levels were the lowest, and they rose slightly with time as ripening progressed (Fig. 4). It is obvious that the vacuum condition enhanced the production of these two metabolites; however, the levels of ethanol were higher than those of AA in all the treatments because ethanol is the end product of the anaerobic process and, therefore, it accumulated to higher levels (Fig. 4).

The fruit that were kept in the air-evacuated bags for 24 or 48 h maintained an excellent quality compared with the control unpacked ones after 1 week at 20 °C (Table 3, Fig. 5). In all bananas stored in PE under vacuum there was no darkening of the crown cut and the weight loss was especially low compared to the control (0.69% vs. 3.55%). The unpacked control had a high ripening grade (7) which means that the banana was already overripe after 1 week at 20 °C, whereas all the bananas that were packed under vacuum had a ripening index grade of 4 (Table 3). Moreover, under vacuum conditions there was no fungal de-

velopment, the crown cut remained clean of mycelium, and there was almost no crown cut browning after 1 week at 20 °C (Table 3). The addition of the EA sachets, which dramatically reduced ethylene production, caused additional delay in ripening, as shown by the ripening index, firmness and the color of the peel, but not by the TSS of the peel and pulp (Table 3, Fig. 5). High TSS levels in the pulp indicate normal ripening accompanied by hydrolysis of starch and accumulation of sugars (Marriott et al., 1981).

After the removal of the fruit from the bags a taste panel graded the bananas for taste preference. The banana clusters that were stored in Vac48 gained the highest scores, 48% of the panelists preferred this treatment, whereas 35% preferred Vac24 and only 24% of them preferred the unpacked bananas (Table 3). The control fruit had highest TSS values in the pulp; however, it was less preferred by the panelists probably because it was already overripe as shown by the lowest firmness (Table 3). This result indicated that storing ethylene-pretreated banana in air-evacuated bags for up to 48 h at 20 °C did not cause any off flavors; on the contrary, this treatment was preferred by the greatest number of the panelists (Table 3). Moreover, application of vacuum for 24 to 48 h did not cause any problem of discoloration, and the fruit ripened normally and developed a nice yellow color (Fig. 5).

Liu (1976) demonstrated that it was possible to hold ethylene-pretreated banana in 1% O₂ or under a pressure of one-tenth of atmospheric pressure for 28 d at 14 °C. However, at 14 °C the accumulation of AA and ethanol is much lower than at 20 °C, and Liu (1976) had exposed the bananas to ethylene for only 8 h, so that it was possible to keep them for a longer time under low O₂. In the present study, the levels of AA and ethanol that developed in Vac24 or Vac48 after 6 d at 20 °C (150 and 300 µL·L⁻¹ AA and ethanol, respectively) did not cause any off-flavors (Table 3). It is known that in banana ripening the AA and ethanol increase as part of the processes of ripening and aroma production (Macku and Jennings, 1987; Bai et al., 1990), and normal AA and ethanol levels can reach 300 and 800 µL·L⁻¹, respectively (Pesis et al., 2001).

The high burst of ethylene accumulation after the vacuum was released (Fig. 4) probably occurred because of the penetration of O₂ into the bag that enabled the production of great quantities of ethylene from the substrate ACC. This result is similar to the ethylene burst that Liu (1976) found after the removal of ethylene-pretreated bananas from low-O₂ treatment, or to the effect of 1-MCP treatment on propylene-treated bananas, which delayed the ethylene peak, but significantly enhanced the production rates (Golding et al., 1998). In the present study, in all the air-evacuated PE bags that were punched after 24 or 48 h the levels of ethylene were lower than in the regular MAPs during 6 d at 20 °C (Fig. 4 vs. Fig. 2), and were correlated with firmer and greener bananas (Table 3 vs. Table 2). The reduction of ethylene in the PE bags by addition of EA sachets based on KMnO₄ was previously shown to be an effective method to reduce ethylene and to delay ripening (Bai et al., 1990; Fuchs and Gorodeiski, 1971; Liu, 1970; Scott et al., 1970). In the present study we found an additional effect, i.e., that the EA sachets can absorb other compounds such as AA and ethanol, in addition to ethylene.

The major difference in AA accumulation between the two methods applied in the present study is that, following exposure to vacuum the levels of AA were 10 times higher than those developed in regular perforated PE during 7 d at 20 °C (Fig. 4 vs. Fig. 2). It is possible that the induced production of AA and ethanol in the vacuum-treated fruit delayed ripening, as was previously shown in preclimacteric bananas (Pesis et al., 2001) and in ethylene-treated banana stored under low O₂ (Imahori et al., 1998; Liu, 1976). It was shown that, application of ethanol to ethylene-treated banana was not effective to extend their shelf life (Bagnato et al., 2003; Hewage et al., 1995). On the other hand, Hewage et al. (1975) found AA to be effective in delaying ripening in ethylene-pretreated banana. It is most likely that AA and not ethanol is the main agent in delaying ripening (Beaulieu et al., 1997). Our present results are in agreement with those of Imahori et al. (1998), who showed that increased AA and ethanol contents were found in bananas after exposure to ethylene, and that during subsequent continuous exposure to low O₂,

the fruit accumulated more ethanol, and ripening ceased.

Under vacuum conditions the fungal development was delayed, and the crown cut remained clean of mycelium compared to storage under regular MAP (Table 3 vs. Table 2). This is consistent with the previous findings that spore germination, mycelial growth and sporulation of fungi were inhibited under hypobaric pressure at 23 °C (Apelbaum and Barkai-Golan, 1977). In the present study, the vacuum was released after 24 or 48 h, and although the banana clusters remained in the perforated PE bags for up to 6 d at 20 °C, no decay developed on the crown cut (Fig. 5). It is possible that the accumulation of AA levels in the PE bags after the vacuum treatment led to reduction in decay development (Avissar et al., 1990).

Conclusions

There is a strong commercial requirement to extend the shelf life of ethylene-pretreated bananas at 20 °C. In the present study we compared two different forms of MAP.

1) Packing ethylene-pretreated banana clusters in unperforated PE bags (25 µm) did not show positive results: the bananas remained greener and were softer, probably because of the high CO₂ levels that accumulated in this treatment. On the other hand, packing in low microperforation PE bags (PE8) was effective in delaying banana ripening and senescence. However, the bananas exhibited increased rot development on the crown cut.

2) Packing ethylene-pretreated banana in PE bags (80 µm) under partial vacuum (550 mm Hg) was the most effective treatment for delaying ripening. The observed inhibition of ripening in air-evacuated PE bags can be attributed to the reduction of the O₂ partial pressure inside the plant cells, which reduced ethylene production, but induced that of AA and ethanol. Adding EA sachets to the PE bags further delayed the ripening processes, but the inhibition by the vacuum treatment alone (without EA sachets) was sufficient. Although ripening was markedly retarded under vacuum conditions, all the fruit ripened normally after the vacuum in the PE bag had been released. This indicates that up to 48 h no damage was caused to the fruit tissue by the treatment, and that the fruit retained its ability to undergo normal ripening processes and to develop the proper color, texture and taste, without any decay development.

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