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SUPPRESSION OF AVOCADO RIPENING WITH NEW PALLADIUM-PROMOTED ETHYLENE SCAVENGER

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The control of ethylene in stored environments plays a key role in prolonging the life of many fresh produce types. However, there has been a paucity of research in recent years on developing novel and more effective ethylene scavenging materials. In this study a palladium (Pd)-promoted powdered material that has significant ethylene adsorption capacity (4162 µl g^{-1} material) at 20°C and approx. 100% RH was identified and was shown to be far superior to $KMnO_4$ when used in low amounts and in conditions of high relative humidity (RH).

Initial screening was carried out in a plug flow reactor with 200 µl l^{-1} ethylene, 10% (v/v) O_2 balanced with He at approx. 100% RH. Further work demonstrated that the Pd-promoted material at 0.03 g l^{-1} effectively scavenged both exogenously administered (100 µl l^{-1}) and/or endogenously produced ethylene by avocado, respectively, to sub-µl l^{-1} concentrations within a 24h period. Optimum ethylene adsorption capacity was calculated as approx. 10000 µl g^{-1}. Accordingly, corresponding inhibition of ethylene-induced ripening was observed. When removed, Pd-material did not disrupt subsequent ripening. The results from this study demonstrate that Pd-promoted material has commercial potential.

Keywords: ethylene adsorption capacity

SUPRESIÓN DE LA MADURACIÓN EN AGUACATES CON UN NUEVO DEPURADOR DE ETILENO PROVOCADO POR PALADIO

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El control de etileno en ambientes de almacenamiento juega un papel principal en la prolongación de la vida de muchos productos frescos. Sin embargo, durante los últimos años la investigación de materiales nuevos y más eficaces para la depuración de etileno ha sido escasa. En este estudio, se identificó un compuesto en polvo provocado por paladio (Pd), el cual presenta una significativa capacidad de adsorción de etileno (4162 µl g^{-1} de material) a 20ºC y

aproximadamente 100% HR que demostró ser muy superior a $KMnO_4$ cuando se utiliza en pequeñas cantidades y bajo condiciones de humedad relativa (HR) alta. Se realizaron estudios preliminares en un reactor de flujo de pistón con 200 µl l^{-1} de etileno y 10% (v/v) de O_2 compensado con He, a aproximadamente 100% HR. Investigaciones posteriores demostraron que el compuesto de Pd, a concentraciones de 0,03 g l^{-1}, adsorbe en forma eficaz tanto etileno exógenamente suministrado (100 µl l^{-1}), como etileno endógenamente producido por el mismo aguacate, hasta concentraciones de sub- µl l^{-1} dentro de un período de 24 horas. La capacidad de adsorción óptima fue calculada aproximadamente como 10000 µl g^{-1}. Por consiguiente, se observó inhibición de la maduración inducida por etileno. Cuando se removió el material con Pd, no provocó alteraciones en la consecuente maduración del fruto. Los resultados de este estudio demuestran que dicho material con Pd tiene un potencial comercial.

1. Introduction

The removal of ethylene and/or inhibition of the effect of ethylene in stored environments are fundamental to maintaining postharvest quality of climacteric produce (Saltveit, 1999). In recent years, however, there has been a paucity of research on developing new and more efficacious ethylene scrubbing materials. In contrast, there has been an exponential increase in research using the ethylene binding inhibitor 1-methylcyclopropene (1-MCP; Watkins, 2006). Thus, research activity has drifted away from ethylene removal to preventing the actions of ethylene through using 1-MCP.

Despite various ethylene scrubbing technologies being available (e.g. high temperature catalytic degradation, activated carbon etc.) most commercial ethylene control systems rely on both adequate ventilation (often periodic) and oxidation of ethylene using potassium permanganate ($KMnO_4$)-based mechanisms. Ventilation, however, is not appropriate in sealed environments (e.g. controlled atmosphere or some packaging formats) or where precise ethylene control is required. $KMnO_4$ supported on activated alumina spheres can have limited long-term efficacy in environments with high relative humidity (e.g. cold stores). The present study details the efficacy of a new palladium (Pd)-promoted material to remove ethylene and hence control ethylene-induced ripening and senescence for avocado fruit (Terry *et al.*, 2007).

2. Materials and methods

2.1. Preliminary experiments

Initial screening of candidate samples was conducted to determine the ethylene adsorption capacity of the newly discovered Pd-promoted material. The chosen material consisted of a Pd-impregnated zeolite giving finely dispersed Pd particles. Metal loading was 2.5% Pd (m/m). Measurements were carried out using a synthetic gas mixture at room temperature (21°C) in a plug flow reactor using 0.1 g of active material in a gas composition of 200 µl l^{-1} ethylene, 10% (v/v) O_2 balanced with He at approx. 100% relative humidity at a flow rate of 50 ml min^{-1}. Humidity was introduced by passing the reactant gas (minus ethylene component) through a water saturator at 21°C. The material was evaluated against other ethylene scavengers reported in the literature including $KMnO_4$ supported on a high surface area alumina (5% m/m; $KMnO_4/Al_2O_3$). Reactor outlet gas concentrations were analysed using a Thermo Onix ProLab mass spectrometer (Thermo Onix, TX) using mass/charge ratios of 26 and 44 for ethylene and CO_2, respectively. Ethylene uptake capacity was measured using a simple breakthrough measurement in which the total integrated ethylene removal was determined after the outlet ethylene concentration from the reactor had reached the inlet ethylene concentration showing that the scavenger was saturated with ethylene. Further experiments were carried out at room temperature in an unstirred batch reactor (0.86 l) with 0.1 g active material and an initial gas composition of 550 µl l^{-1} ethylene, 40% (v/v) air balanced with Ar. Selected gas concentrations were measured at hourly intervals with a Varian CP-4900 Micro GC (Varian Inc., CA). Gas samples (40 ms duration) were taken via an automated recirculting sampling system. Column and injector temperatures were set at 60 and 70°C, respectively. The 0.15 mm diameter, 10 m long column was packed with PoraPLOT Q. Ethylene and CO_2 were calibrated against 10 µl l^{-1} ethylene balanced with air and 5% (v/v) CO_2 balanced with Ar (Air Products Europe, Surrey, UK). A thermal conductivity detector was used with He carrier gas at 276 kPa inlet pressure. Peak integration was carried out within the Varian STAR software.

2.2. Plant material and experimental set-up

Three experiments were carried out. In the first experiment, early season pre-climacteric avocado (*Persea americana* Mill.) cv. Hass fruit (n = 72), originating from Navobani Boerdery and imported through Westfalia Marketing (Tzaneen, LP, South Africa), were supplied by a tropical fruit supplier (Minor, Weir and Willis Ltd., Birmingham, UK) and stored overnight at 12°C. Fruit were packed on 4 May 2006 and received in the UK on 31 May 2006. On arrival at the laboratory, fruit were 32-days-old, which is not unusual for avocado fruit being imported into the UK. Fruit were not pre-treated with 1-MCP. Fruit (n = 2) were held in the dark at 12 °C within 3 l sealed jars (n = 36) for 3 days, which were initially treated with or without ethylene (100 µl l^{-1}), and contained 0, 100 or 1000 mg of powdered Pd-promoted material (2.5% Pd (m/m)) or powdered Ethysorb® ($KMnO_4$ supported on activated alumina spheres), respectively. An additional treatment was also used, whereby fruit were treated with or without ethylene as before, but then after one day 1000 mg Pd-promoted material was put into jars. Fruit were removed after 3 days to avoid CO_2 poisoning and kept on open trays at 12 °C for a further 7 days. Effect of treatments (n = 12) on ethylene, and CO_2 concentrations was recorded over 3 days. Objective colour change of fruit and firmness was recorded at regular intervals.

In a second experiment, pre-climacteric avocado cv. Hass fruit (n = 72), originating from Spain, were supplied by a fruit importer (Mack Multiples, Kent, UK) and stored overnight at 12°C. On arrival at laboratory, fruit were 4-days-old. Fruit (n = 2) were, as before, placed into individual sealable 3 l jars and treated with or without Pd-promoted material for 24 h at 12°C by adding 1000 mg of Pd-promoted material (1% Pd (m/m)) into each jar. Fruit were treated with 100 µl l^{-1} or 0 µl l^{-1} ethylene the following day. Fruit were removed after 2 days to avoid

CO_2 poisoning and kept on open trays at 12 °C for a further 7 days. Effect of treatments (*n* = 12) on ethylene, and CO_2 concentrations was recorded over 2 days. Additionally, ethane, a sub-product of ethylene reaction with the Pd-promoted material (Terry *et al*., 2007) was monitored. Objective colour change of fruit and firmness was recorded at regular intervals.

A third experiment was aimed at testing the material under commercial conditions to determine the potential of extending shelf-life of harvested avocados when applied at increasing intervals from harvest. Avocado cv. Fuerte fruit (*n* = 960) were obtained from a commercial grove in the Soutpansberg Mountain region of Levubu in Limpopo Province, South Africa. Fruit were transported to Mataffin Farm within 6h of harvest and kept at 6°C until treatment. Fruit (*n* = 2) were treated at 0, 12, 24, 36, and 48 hours after arrival by sealing them in Versapack foamalite trays (Crown National, South Africa) containing 0, 100 or 500 mg Pd-promoted material. Shelf life and firmness were determined.

2.3. Ethylene and CO_2 quantification

Gas samples from 3 l jars were removed with repeated full withdrawal-injection displacements of a 20 ml plastic syringe (Terry *et al*., 2007). Ethylene concentration was quantified using a GC8340 gas chromatograph (Carlo Erba Instruments, Herts., UK) fitted with an EL 980 FID and DP800 integrator (Thermoquest, Herts., UK). Oven and detector temperatures were set at 100 °C. The 2 m long stainless steel column was packed with Porapak P mesh range 80-100 (Jones Chromatography, Mid Glamorgan, UK). Ethylene was calibrated against 10.6 $\mu l\ l^{-1}$ ethylene balanced in N_2 (British Oxygen Company (BOC) Gases, Surrey, UK). For Experiment 2, oven and detector temperatures were set at 100 °C and 250°C respectively. Ethylene and ethane were calibrated against 1.05 $\mu l\ l^{-1}$ ethylene and 50.3 $\mu l\ l^{-1}$ ethane balanced in N_2 (BOC). CO_2 was quantified using the same GC system with hot wire detection. The hot wire detector was operated at 120 °C and the oven at 80 °C. The GC was calibrated with 10.06% (v/v) CO_2 in N_2 (BOC). Gases were not measured for Experiment 3.

2.4. Colour measurements

The objective colour of each fruit was measured at regular intervals during storage using a Minolta DP-400 colorimeter (Minolta Co. Ltd., Japan) with an 8 mm light path aperture. The instrument was calibrated with a Minolta standard white tile CR-400 (*Y* = 93.5, *x* = 0.3114, *y* = 0.3190) for the first experiment. For the second experiment, objective colour measurement was determined using the same Minolta DP-400 colorimeter but this time using light source D65 (Ashton *et al*., 2006). The mean of three readings at three equidistant points (*n* = 9) around the equatorial axis for avocado was recorded and the lightness (L*), chroma (colour saturation; C*) and hue angle (H°) automatically calculated. Colour was not measured for Experiment 3.

2.5. Firmness and shelf-life evaluation

For Experiment 1 and Experiment 2, avocado fruit firmness was measured on opposite sides of peeled fruit using an Instron Universal Testing Machine (Model 1122, Bucks., UK) fitted with a flat-head 8 mm probe. Crosshead speed was set at 20 mm min^{-1}. Firmness was expressed as the maximal force (N) required for tissue failure. In Experiment 3, fruit was pressure-tested using a bench-top Sinclair IQ non-destructive pressure tester (Sinclair International Ltd., Norfolk, UK) and the mean of three measurements per fruit recorded. Shelf life was determined as number of days for fruit to reach an acceptable eating quality as defined by H. L. Hall & Sons Ltd.

2.6. Statistical analysis

All statistical analyses were carried out using Genstat for Windows Version 7.1.0.198 (VSN International Ltd., Herts., UK). Least significant difference values (LSD; $P = 0.05$) were calculated for mean separation using critical values of t for two-tailed tests.

3. Results

3.1. Preliminary experiments with synthetic ethylene gas

After screening a range of different materials on a plug flow reactor, one Pd-promoted material was identified as having considerable ethylene adsorption capacity at room temperature. The Pd-promoted material typically removed all measurable ethylene until breakthrough occurred (Figure 1). The plug flow reactor experiments demonstrated that the Pd-promoted material outperformed $KMnO_4$ by having an approx. six-fold higher ethylene adsorption capacity; typically 4162 µl g^{-1} under approx. 100% RH. This performance was increased to 45600 µl g^{-1} under dry conditions (low %RH), thus, representing a sixty-fold higher activity than $KMnO_4$. All ethylene adsorption capacity was lost when $KMnO_4$ was pre-treated with 100% RH for 72 hours at 21 °C prior to analysis (data not shown). The loss in performance of the $KMnO_4$-based material appeared to be due to conversion of the $KMnO_4$ to manganese oxide judging from the change in colour of the material from purple to brown.

A small amount of Pd-promoted material (0.1 g) was sufficient to totally remove 550 µl l^{-1} ethylene in air mixture within 2h (Figure 2). Some ethane (65 µl l^{-1}) production and a slight rise in CO_2 concentration were also observed (Figure 2), indicating that some of the ethylene was reacting over the Pd-promoted material. However, these products represent only approx. 16% of the total carbon balance present in the container used, suggesting that most of the ethylene was adsorbed on the Pd-promoted material.

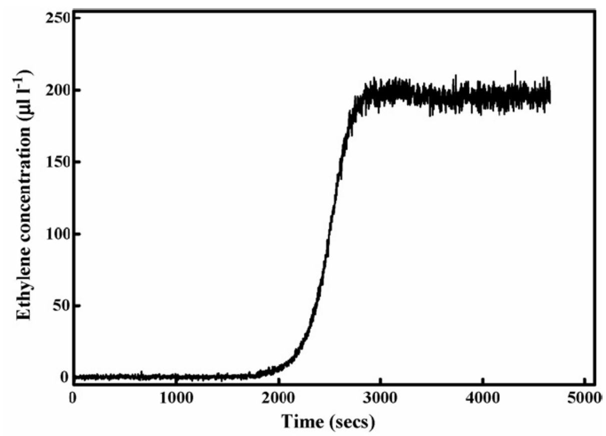


Figure 1. Ethylene breakthrough measurement on Pd-promoted material held in humid conditions.

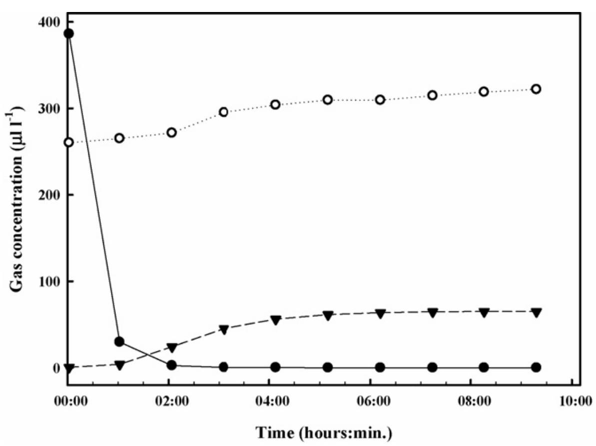


Figure 2. Effect of Pd-promoted material on gaseous composition (carbon dioxide, (○); ethylene, (●); ethane, (▲)) within sealed jars, initially containing 550 µl l^{-1} ethylene in air.

3.2. Ethylene and CO_2 concentrations

For Experiment 1 and Experiment 2, exogenous and endogenous ethylene concentration was reduced significantly (P <0.001) with increasing amount of Pd-promoted material (Table 1). Pd-promoted material (100 mg) was approx. 50-fold more effective at reducing exogenous ethylene than Ethysorb (100 mg) (Terry *et al.*, 2007). In the presence of Pd-promoted material, ethylene was reduced to sub-physiologically active levels as demonstrated by maintenance of firmness (P = 0.004) after 7 days. Firmness of fruit previously treated with ethylene (100 µl l^{-1}) and held in the presence of 100 or 1000 mg Pd-promoted material was 114.4 and 106.7 N vs. 13.0 and 12.0 N for control and Ethysorb (100 mg)-treated fruit, respectively (data not shown). However, there was no significant effect (P >0.05) caused by the presence of Pd-promoted material on CO_2 concentration over time (data not shown) and thus, the reduction in ethylene caused by the presence of Pd-promoted material had no effect on respiration for the first 3 days. CO_2 concentrations rose from 5.10 to 13.23 % between days 1 and 3, respectively (data not shown). Internal fruit coloration was not affected by presence/absence of ethylene (data not shown).

When fruit were treated with ethylene and then subsequently held in the presence of Pd-promoted material (1000 mg) after day 1, ethylene was reduced to sub-physiologically active levels (Table 1). Despite the climacteric being initiated for these fruit, subsequent total removal of ethylene resulted in better maintenance of fruit firmness than controls (data not shown) and thus, disruption of the normal and expected climacteric respiratory rise. Therefore, an ethylene scavenger has been shown to be capable of extending shelf-life even when the climacteric respiratory rise has been initiated (Terry *et al.*, 2007).

Table 1. Ethylene concentration (µl l^{-1}) within 3 l sealed jars (n = 36) containing pre-climacteric avocado cv. Hass fruit (n = 2) held in presence of Pd-promoted material (0, 100, and 1000 mg, 2.5% Pd (m/m)) or Ethysorb® (100 and 1000 mg). Jars were initially treated with or (without) 100 µl l^{-1} ethylene. *Pd-promoted material (1000 mg) was put in jars after day 1 after fruit had been treated with or without 100 µl l^{-1} ethylene. LSD (P = 0.05) = 4.918

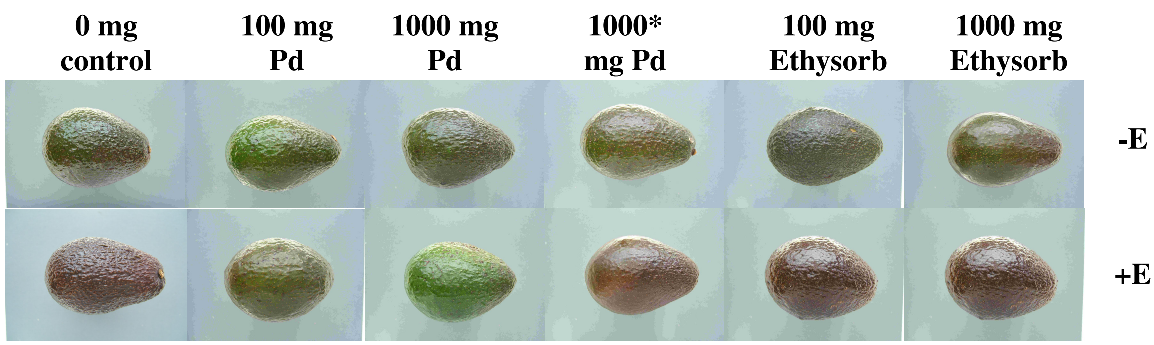
Material (mg)	Ethylene concentration (µl l^{-1})			
	Day 0	Day 1	Day 2	Day 3
Control	98.44 (0.00)	82.06 (0.69)	80.35 (1.57)	78.41 (3.74)
100 Pd-promoted	93.87 (0.00)	0.00 (0.07)	0.00 (0.06)	0.00 (0.04)
1000 Pd-promoted	88.56 (0.00)	0.00 (0.00)	0.00 (0.01)	0.00 (0.00)
1000*Pd-promoted	98.34 (0.00)	83.11 (0.24)	0.00 (0.14)	0.00 (0.12)
100 Ethysorb®	97.07 (0.00)	47.38 (0.18)	47.53 (0.21)	43.72 (0.10)
1000 Ethysorb®	94.19 (0.00)	0.07 (0.54)	0.21 (0.24)	0.02 (0.10)

For Experiment 2, both exogenously administered (100 µl l^{-1}) and/or endogenously produced ethylene were reduced significantly (P = 0.05) with 1000 mg of Pd-promoted material. In the presence of the Pd-promoted material, *ca.* 10% of exogenously administered ethylene (100 µl l^{-1}) was scavenged within seconds. Ethylene was reduced to sub-µl l^{-1} concentrations within 24 hours. On day 1, ethylene concentration in jars containing Pd-promoted material and treated with and without 100 µl l^{-1} ethylene was 0.00 µl l^{-1} and 0.92 µl l^{-1}, respectively, as compared against 109.39 µl l^{-1} and 23.32 µl l^{-1} for control jars treated with and without 100 µl l^{-1} ethylene, respectively. As for Experiment 1, there was no significant effect (P >0.05) caused by the presence of Pd-promoted material on CO_2 concentration over time (data not shown).

3.3. <u>Colour change</u>

The overall visual colour change of avocado cv. Hass fruit was also affected by ethylene and thus presence/absence of Pd-promoted material. In the first experiment, fruit held in the presence of 100 or 1000 mg for 3 days were generally greener, and thus less ripe, than control fruit after 7 – 10 days (Plate 1). The colour of 10 day-old fruit previously treated with ethylene (100 µl l^{-1}) and held in the presence of 100 mg Pd-promoted material was 90.8 H° as compared against 53.1 and 60.2 H° for control and Ethysorb (100 mg)-treated fruit, respectively. In Experiment 2, the hue angle value of 9 day-old fruit previously treated without ethylene and held in presence of 1000 mg Pd-promoted material was 74.37 H° as compared against 48.85 H° and 48.11 H° for control (100 µl l^{-1}ethylene) and control (0 µl l^{-1}ethylene), respectively (Figure 3).

Plate 1. Colour of 7-day-old avocado cv. Hass fruit (*n* = 2) previously held for 3 days at 12°C in 3 l sealed jars (*n* = 36) in presence of Pd-promoted material (0, 100, 1000 mg) or Ethysorb (100, 1000 mg) and initially treated with (+E) or without (-E) 100 µl l^{-1} ethylene when at pre-climacteric stage (i.e. green) at day 0. * = Pd-promoted material (1000 mg) was put in jars after day 1 after fruit had been treated with or without 100 µl l^{-1} ethylene.



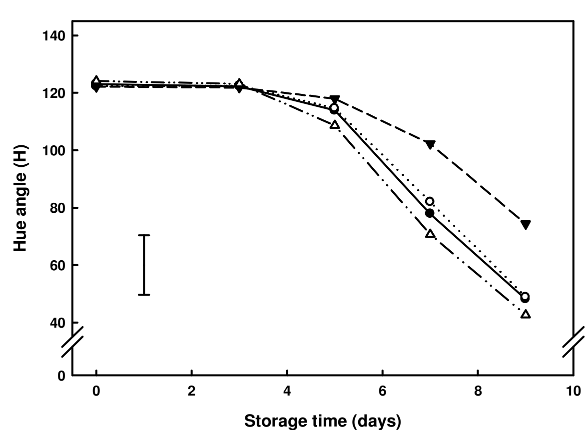


Figure 3. Effect of Pd-promoted material on hue angle (H°) of avocado cv. Hass fruit (*n* = 2) held within 3 l jars (*n* = 36) at 12°C for 3 days and containing Pd-promoted material (1000 mg). Jars were initially treated with 0 or 100 μl l^{-1} ethylene when at pre-climacteric stage (i.e. green) at day 0 (control - 0 μl l^{-1} ethylene, •; control - 100 μl l^{-1} ethylene,○; Pd - 0 μl l^{-1} ethylene,▼; Pd - 100 μl l^{-1} ethylene, Δ). LSD (P = 0.05) = 20.80.
N.B. A high H° value means greener fruit.

3.4. Shelf life extension and fruit quality

In Experiment 3, there was an increase in shelf life of approx. 10 days for avocado cv. Fuerte fruit held in presence of Pd-promoted material as compared against controls. The efficacy of Pd-promoted material (500 mg) to extend shelf life increased as the time interval of treatment after harvest was reduced (data not shown).

Firmness of fruit treated with either 100 or 500 mg Pd-promoted material at 6 h after harvest were significantly firmer as compared to control. Taste and incidence of physiological disorders were unaffected by any of the treatments.

4. Discussion

For effective long-distance transport and subsequent storage many climacteric fruit need to be held in a low (sub 1.0 - 0.01 μl l^{-1}) ethylene environment. Control of the effects of ethylene in stored environments has tended in recent times to have drifted away from ethylene removal to preventing the actions of ethylene using 1-MCP. The reasons for this are varied, but probably have resulted from not only 1-MCP being highly effective at blocking ethylene perception, but also there being a lack of commercially available ethylene scavengers that are sufficiently efficacious at removing ethylene for extended periods in environments with high RH% (e.g. holding chambers, packaging etc.). The Pd-promoted material, like 1-MCP, can be used to control ripening; however, the mechanism by which this is achieved is fundamentality different. Rather than blocking the perception of ethylene, the Pd-promoted material effectively removes ethylene rapidly from an environment, and thus there is less risk that unwanted side-effects occur. This said, for ethylene scavengers to work effectively there is a requirement for them to be in the presence of the commodity.

For avocado cv. Hass fruit, complete ethylene removal using Pd-promoted material was generally more effective as compared to control and Ethysorb in slowing down ripening. Presence of Pd-promoted material delayed ethylene-induced softening for both cv. Hass and Fuerte fruit and, from a commercial perspective, there was a meaningful extension of shelf-life for cv. Fuerte fruit treated with Pd-promoted material. The capacity to delay ripening using Pd-promoted material (1000 mg) after ethylene (100 μl l^{-1}) treatment was also demonstrated in the present study.

5. Conclusion

The current study has demonstrated that presence of a Pd-promoted material was effective at reducing ethylene to sub-physiological active levels (ethylene adsorption capacity approx. 10000 µl g^{-1}) when used for only 3 days on pre-climacteric green avocado cv. Hass fruit. The efficacy of Pd-promoted material was far superior to $KMnO_4$ when used in low amounts and especially at high RH%.

The results from this study demonstrate that Pd-promoted material has potential to be used commercially. Future research will further elucidate the optimum concentrations, timing of application and format to extend postharvest life of avocado and other climacteric fresh produce types. If optimised there remains the possibility of the Pd-promoted material being used to extend shelf-life of climacteric fruit even when the climacteric respiratory rise has been just initiated.

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