THE USE OF IT'SFRESH! ETHYLENE REMOVER TECHNOLOGY WITH e+® ACTIVE AS A PRACTICAL MEANS FOR PRESERVING POSTHARVEST FRUIT QUALITY

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ABSTRACT

The presence of ethylene in a storage environment can undermine both quality and postharvest life, often generating significant waste and associated economic losses. A demand for discovering alternative technologies capable of scavenging ethylene has led to the development of a new material, e+® active, which has significant ethylene adsorption capacity. The material has been shown to remove ethylene to below physiologically active levels during fruit storage at 0-20°C and consequently extend postharvest life for a variety of fresh produce types. Different formats have been evaluated. For example, It'sFresh! sheets containing e+® active were shown to be a highly efficacious format for suppressing ethylene and extending storage life of imported avocado and pluot plums in a series of trials. Indeed, It'sFresh! technology has also been shown to have profound effects on non-climacteric fruit types such as strawberry where quality-related attributes were affected. The mode of action of It'sFresh! technology with e+® active and the implications of scavenging ethylene in both non-climacteric and climacteric systems are discussed.

INTRODUCTION

Ethylene is involved in nearly every phase of plant development. In fruit, quality parameters associated with taste and aromas have been shown to be regulated by ethylene. That said, while ethylene is crucial for optimum taste and quality of fresh produce, it can incur detrimental effects on the postharvest life of climacteric fruits. As such, it is widely recognised that high levels of ethylene in fruit environments are concomitant with rapid softening and reduced storage life of climacteric fruits.

The use of technologies capable of inhibiting, or those capable of removing, ethylene can reduce ethylene production in the fruit environment and therefore help to maintain quality of climacteric fruits. The e+ ® active has been reported to have significant ethylene adsorption capacity (Terry et al., 2007; Smith et al., 2009). The e+® active reduced exogenous and endogenous ethylene from avocados during storage under high %RH and low temperature (Meyer et al., 2010); under the same conditions, potassium permanganate ($KMnO_4$) treatment was found to be far less effective in comparison (Terry et al., 2007). Successful results during laboratory trials motivated the production of different formats of the material (Elmi et al., 2011). Formats now in commercial use include sheets of different sizes coated with the active, suited to transit cases, retail packs and point-of-sale displays.

The involvement of ethylene in the ripening and development of climacteric fruits is widely reported in the literature. In contrast, less consideration has been given to understanding the role of ethylene in non-climacteric fruits. Strawberries (*Fragaria* x *ananassa*) are non-climacteric fruits and are highly perishable. Recent studies have considered the effect of ethylene on the flavour, texture and colour development of strawberries (Tian et al., 1997; Bower et al., 2003 and Trainotti et al., 2005). Some research has suggested that the deterioration of strawberries can be exacerbated by the presence of ethylene during storage (Jiang et al., 2001 and Bower et al., 2003). Wills and Kim (1995) reported an ethylene concentration of 0.36 µl L^{-1} in strawberry punnets; however significantly lower concentrations of 0.1 µl L^{-1} were reported to reduce storage life of strawberries.

The objectives of this work were: (a) to test the feasibility and effectiveness of It'sFresh! sheets in the real world supply chain and (b) determine the benefits of applying the treatment (at the packing house) on pluot plums (cv. Flavor King) packed with or without the treatment. Additionally, the role of ethylene on the postharvest quality of strawberries; a non-climacteric fruit, was investigated. Ethylene production was measured using a highly sensitive ethylene detector; capable of measuring ethylene at very low concentrations (0.3 nl L^{-1}), to determine the effects of It'sFresh! sheets.

MATERIALS AND METHODS

Experiment 1: Pluots (*Prunus salicina* L. *x Prunus armenica* L. cv. 'Flavor King')

Pluots were sourced from South Africa and supplied by Univeg UK. Fruits were harvested and transported to the packing house, where fruits were commercially packed in crates (double layer) and treated with or without It'sFresh! sheets (19 cm x 12.5 cm). The It'sFresh! sheets (5 sheets) were placed face up in each layer on top of the fruits. Fruits were held at 1-3^{o}C during transit. Following a 4 week transit period, the fruits were stored at 2^{o}C in their original crates inside 320L chambers. For each treatment, there were 3 chambers and each chamber contained 2 crates, hence 6 crates were sampled per treatment. The chambers were not sealed; instead the lids were left slightly ajar.

Experiment 2: Strawberries (*Fragaria* x *ananassa.* cvs. 'Elsanta' and 'Jubilee')

Strawberry plants (cv. 'Elsanta' n=72, Exp 2.1) were grown in a glasshouse (at Cranfield University) during 2011 (April and July). Fruits were harvested at optimum ripeness. After harvest, fruits of similar size and weight were treated with or without e+® and stored in 13L boxes (at 5^{o}C) with controlled continuous air exchange every 5 mins. After a subsequent storage period (0, 2, 4, 7 days), fruits were removed from cold storage and the weight, objective colour and ethylene production determined. In a separate experiment, strawberry fruits (cv. 'Jubilee', Exp 2.2) purchased from a local grower (H & H Duncalfe Cambs., UK) were treated with or without e+® as described in Exp 2.1 and fruit colour, weight and ethylene production measured following 0, 1, 2, 4 and 7 days of cold storage. The e+® treatment was repeated in another trial (Exp 2.3) on cv. 'Jubilee' fruits purchased from the same grower and measurements conducted as described above.

Respiration and Ethylene production

Experiment 1: Pluots

Real time ethylene production was monitored with a newly developed photo acoustic laser based ethylene detector (ETD-300; Sensor Sense B.V., Nijmegen, The Netherlands) incorporated with a gas handling system. There are two types of gas measurements which can be performed using this machine: *Stop and Flow* and *Continuous flow*. In this trial, *Continuous flow* was performed to measure real time ethylene production. This guarantees continuous gas refreshment and stable conditions for the samples.

There were a total of six jars connected to the ethylene detector each containing a single fruit. Fruits (control *n*=2 and e+® *n*=2) were removed from cold storage following 0, 7, 14 days. Fruits were weighed and each placed inside 1L glass jars at 18^{o}C and sealed tightly with a rubber septum ready for ethylene measurements. Fruits receiving the e+® treatment were placed inside jars containing e+®. For each treatment, one jar was labelled 'Long (L)' and fruits in these jars were kept at ambient temperature for a period of 4 days. The other jar (*n*=1) per treatment was labelled 'Short (S)'. Fruits in these jars (S) were replaced daily with new fruits after 24h at 18^{o}C where ethylene production was measured continuously. Two control jars were included consisting of a sealed empty jar (Blank) and a jar containing e+$^{®}$ alone.

Experiment 2: Strawberries

Ethylene production was measured using the ETD-300 ethylene detector described above (for Exp 2.1, 2.2, 2.3). Fruits treated with (*n*=12) or without (*n*=12) e+$^{®}$ were separated into 1L jars (6 jars, 3 for each treatment) (4 fruit in each jar) with gas tight lids and rubber septums. Fruits initially treated with e+$^{®}$ were placed inside jars containing e+$^{®}$.

Colour and firmness measurements

The objective colour; lightness (L*), chroma (C*) and hue angle (H^{o}) were determined for each fruit (Terry et al., 2007). Flesh firmness (Exp 1) was measured using a uniaxial testing machine (model 5542, Instron, Norwood, MA) programmed to Bluehill 2, version 2.11, Instron (Terry et al., 2007).

Biochemical measurements

Experiment 2.1: Strawberries (*Fragaria* x *ananassa.* cv. 'Elsanta')

Sugars (sucrose, fructose and glucose) were determined by HPLC as described previously (Giné Bordonaba and Terry, 2010).

Statistical analysis

All statistical analyses were carried out using Genstat for Windows version 11 (VSN International Ltd., Herts., U.K.). Data obtained were subjected to analysis of variance (ANOVA). Least significant difference values (LSD; P<0.05) were calculated for mean separation using critical values of *t* for two-tailed tests.

RESULTS AND DISCUSSION

The presence of ethylene in the fruit environment can have undesirable effects on their overall quality and shelf life. The presence of e+$^{®}$ in the storage atmosphere of avocado (cv. 'Hass') has been shown to significantly lower ethylene (Terry et al., 2007; Meyer and Terry, 2010; Elmi et al., 2011). Treatment of pre-climacteric avocados at source resulted in further reduction in ethylene during storage at 5^{o}C for 31 days (Elmi et al., 2011). Pluots (a plum hybrid) like avocados undergo long distant transit whereby ethylene can accumulate in the surrounding atmosphere. Pluot fruits stored at 18^{o}C for 24h (control (S) and (E+ (S)) exhibited a surge of ethylene production, which reached a maximum level and then decreased rapidly (Fig 1). Fruits stored with e+$^{®}$ (E+ (S)) treatment displayed a similar ethylene production pattern as the controls, however, this peak in ethylene was reduced substantially (Fig 1). It is clear that when the fruits were removed from cold storage and placed under ambient conditions (18^{o}C) they exhibited a dramatic rise in ethylene production. Measuring ethylene in real-time using the ETD-300 ethylene detector meant that the ethylene produced by the fruits could be monitored continuously throughout the ripening period, allowing this peak in ethylene production to be observed. Such observations are more difficult with conventional techniques such as gas chromatography. Following 14 days in cold storage, fruits displayed a gradual increase in ethylene production during the ripening period (control (L) and E+ (L)). That said, control fruits produced significantly higher ethylene (Fig 1). High ethylene observed for control fruits infer that these fruits had a higher metabolic rate leading to loss of quality. Results herein are in accordance with results reported previously where the effectiveness of e+$^{®}$ in conditions of low temperature (5^{o}C) and high RH% (Terry et al., 2007; Meyer and Terry, 2010; Elmi et al., 2011) was demonstrated. Results indicate that fruits treated with e+® were overall significantly firmer (13.71 N) than their corresponding control fruits (9.23 N) following a ripening period of 1-6 days (at 18^{o}C). Elmi et al., (2011) also demonstrated the importance of treating fruits at source. The presence of It'sFresh! sheets at source rather than after 5 weeks of transit, when the fruits were more mature, was reported to enhance the efficacy of the treatment (Elmi et al., 2011). In accordance with this, firmness of the pluots on arrival suggests that the presence of e+® during transit resulted in reduction of ethylene during transit, since fruits packed with the treatment were considerably firmer (29.68 N) than control fruits (14.12 N).

Studies have shown that deterioration of strawberries can be exacerbated by the presence of ethylene during storage, hence increasing potential waste (Bower et al., 2003). Results of the present study demonstrated that ethylene influences the postharvest quality of strawberries (cvs. 'Elsanta' and 'Jubilee'). Removal of ethylene using e+® was associated with significantly redder fruit in all three trials (Table 1). An increased concentration of sucrose was also measured in e+® treated fruits (Exp 2.1 and 2.3), which suggests that low sucrose may result as a consequence of enhanced metabolic activity in the control fruits. Villarreal et al., (2010) reported significantly higher content of reducing sugars (glucose and fructose) in ethephon (an ethylene releasing agent) treated strawberries in comparison to 1-methylcyclopropene treated fruits. However, while accumulation of sugars in climacteric fruits has been widely reported, little has been reported about this process in non-climacteric fruits. Moreover, the present study has identified an ethylene production pattern similar to climacteric fruits albeit at a much lower level. The ethylene production of strawberry fruit (Exp. 2.2, cv. 'Jubilee') increased significantly after harvest peaking after 4 days of cold storage (5°C) followed by a rapid decrease to its lowest level (after 7 days at 5^{o}C) as depicted in Fig 2. These results support the study by Ianneta et al., (2006), where strawberries were shown to undergo a respiratory increase and concomitant ethylene production during ripening *in planta*. Intriguingly, studies have found commonality between climacteric and non-climacteric ripening. Trainotti et al., (2005) established that strawberries produced ethylene concurrently with ripening. In addition, Trainotti et al., (2006) observed enhanced expression of *FaACO1* as well as increased ethylene production, similar to climacteric fruits. That said, strawberry fruit is a 'false fruit' being described as an enlarged receptacle. The achenes (seeds) situated on the epidermis of the receptacle are in fact the true fruits (Fait et al., 2008). Many processes associated with strawberry maturity have been shown to be regulated by the presence or absence of the achenes (Given et al., 1988; Manning, 1994). Thus, it is likely that different areas of the fruit (achenes and receptacles) may exhibit different ripening mechanisms. In support of this notion, Ianneta et al., (2006) analysed the ethylene production of dissected fruit. It was demonstrated that the ethylene produced by the achenes account for 50% of the ethylene produced by the fruit, even though they make up a small fraction of weight.

CONCLUSIONS

Treatment of fruits with It'sFresh! sheets at source delayed softening and colour change in avocados 'Hass' fruit (Elmi et al., 2011). In addition, pluots placed into fruit boxes containing the It'sFresh! sheets were of better quality than control fruits, which substantiates previous findings. This study confirms the advantages of e+® in preserving postharvest fruit quality and has also demonstrated with the use of a highly sensitive ETD-300 ethylene detector, that ethylene may have a role in determining the postharvest quality of strawberry fruit.

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TABLES AND FIGURES

Table 1: Effect of treatment on the colour of strawberries (cvs. 'Elsanta' and 'Jubilee') stored for 7 days at 5°C with or without e+®.

	Hue angle (H°)		
Experiment	**Control**	**E+**	**LSD** ***(P<0.05)***
1 (cv. Elsanta)	31.67	33.52	0.709
2 (cv. Jubilee)	34.77	35.70	0.862
3 (cv. Jubilee)	34.28	35.19	0.827

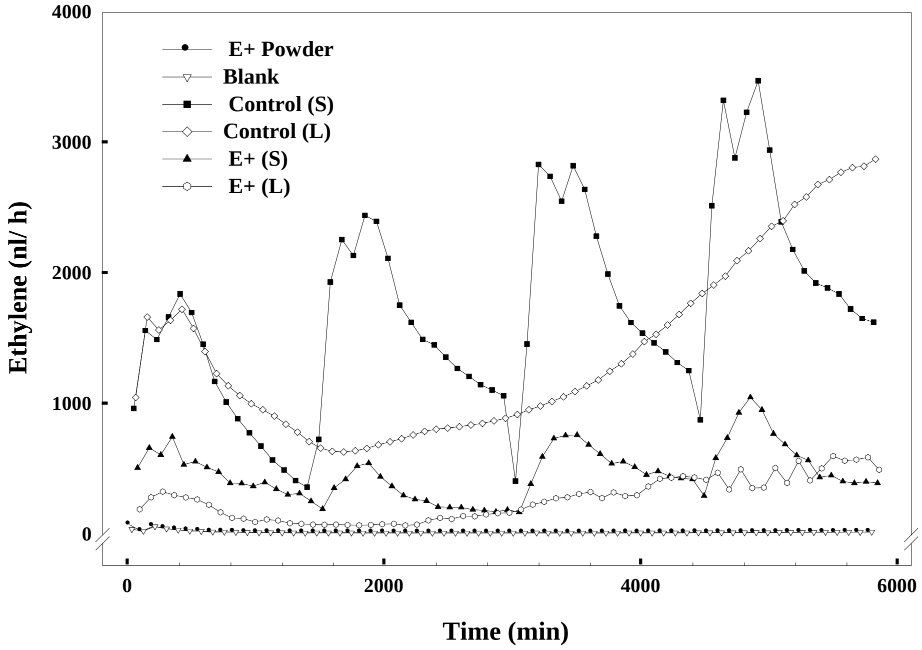

Figure 1. Ethylene production (nl/ h) of pluots (cv. 'Flavor King') during ripening after storage at 18°C, of fruits that were removed from cold storage after 7 days at 2°C. Long (L) stored fruits were stored at 18°C for the ripening period of 4 days. Short (S) stored fruits were stored at 18°C. Ethylene production was measured using ETD-300 detector for 4 days.

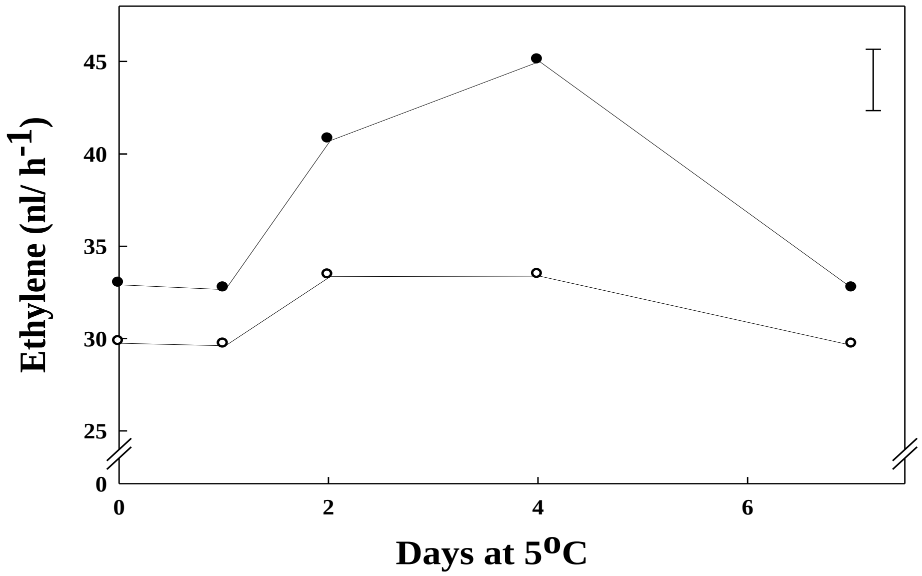

Figure 2. Ethylene production (nl/ h) of strawberry fruits (cv. 'Jubilee' Exp 2.2) following storage at 5°C in 13L boxes with (○, E+) or without (●, control). Ethylene production was measured at 18°C inside 1L capacity jars (n=3 per treatment) connected to ETD-300. LSD bar ($P<0.05$).