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Storage at elevated ambient temperature and reduced ethylene delays degreening of Persian limes

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Summary

Introduction – **Persian (Tahitian) limes (*Citrus latifolia*) are widely grown in tropical and sub-tropical regions. They are harvested when the skin is green and the major postharvest storage challenge is the color retention as yellow fruit is of considerably lower commercial value. The recommended storage temperature for limes to retain a green peel color is 10 °C but in developing countries refrigeration is often not available or is expensive. This paper examines the potential for storage at elevated ambient temperatures and low ethylene concentrations to delay peel degreening.** *Materials and methods* – **Limes were stored at 30, 20 and 10 °C in an atmosphere containing ethylene at < 0.001, 0.01, 0.1 and 1.0 µL L^{-1}. Green peel color was visually assessed daily and a range of internal quality factors measured at the end of storage life (designated as green life).** *Results and discussion* – **Storage of limes at 30 °C resulted in a longer retention of green skin color than fruit held at the currently recommended temperature of 10 °C, whilst fruits stored at 20 °C had the shortest green life. Limes stored at all temperatures degreened more slowly as the ethylene level in the atmosphere was reduced. Internal quality, as measured by total soluble solids, titratable acidity and antioxidant activity, was not adversely affected by storage at the higher temperatures.** *Conclusion* – **Storage at a high ambient temperature and with exogenous ethylene at < 0.1 µL L^{-1} could have considerable economic benefits for limes grown in tropical climates in developing countries and possibly also in sub-tropical developed countries.**

Keywords
Australia, citrus, lime, *Citrus latifolia*, fruit color, fruit senescence, postharvest quality management

Résumé

Le stockage à température ambiante élevée sous faible teneur en éthylène repousse le déverdissement du citron vert.

Introduction – **Les limes de Perse (ou de Tahiti) (*Citrus latifolia* Tan.) sont largement cultivées dans les régions tropicales et sous-tropicales. Elles sont récoltées lorsque la peau est verte et le principal problème en cours de conservation post-récolte est le changement de couleur car les fruits jaunes présentent une valeur commerciale nettement moindre. La température de stockage recommandée pour que les limes conservent une peau verte est de 10 °C, mais dans les pays en développement, la réfrigération est souvent indisponible ou coûte cher. Cet article examine le potentiel de stockage à des températures ambiantes élevées et à de faibles concentrations d'éthylène pour retarder le déverdissement des citrons verts.** *Matériel et méthodes* – **Les limes ont été stockées à 30, 20 et 10 °C dans une atmosphère contenant de l'éthylène à < 0,001, 0,01, 0,1 et 1,0 µL de L^{-1}. La couleur verte de la peau a été évaluée visuellement chaque jour et plusieurs critères de qualité interne ont été mesurés à la fin du stockage (désignée comme la durée de vie verte).** *Résultats et discussion* – **Le stockage des limes à 30 °C s'est accompagné d'un allongement de la durée de la couleur verte de la peau des fruits en comparaison de ceux conservés à la température recommandée de 10 °C, tandis que les fruits conservés à 20 °C ont présenté la plus longue durée de vie verte. Les limes stockées à toutes les températures ont dégénéré plus lentement avec un niveau d'éthylène dans l'atmosphère plus réduit. La qualité interne, mesurée par la matière soluble totale, l'acidité titrable et l'activité anti-oxydante, n'a pas été affectée par le stockage aux températures les plus élevées.** *Conclusion* – **Le stockage à température ambiante élevée sous l'éthylène exogène à < 0,1 µL L^{-1} pourrait avoir des avantages économiques considérables pour les limes cultivées sous climat tropical dans les pays en développement et éventuellement dans les pays développés subtropicaux.**

Significance of this study

What is already known on this subject?

- Market demand is for green limes with a current recommended storage temperature of 10 °C to maximise retention of a green skin.

What are the new findings?

- Limes stored at 30 °C with exogenous ethylene at < 0.1 µL L^{-1} gave a longer retention of green color than fruit held at 10 °C.

What is the expected impact on horticulture?

- Storage at ambient temperature and low ethylene has potential economic benefit for limes grown in tropical and sub-tropical countries.

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Mots-clés
Australie, agrume, limettier, *Citrus latifolia*, coloration du fruit, sénescence du fruit, gestion de la qualité post-récolte

Introduction

Persian (Tahitian) limes (*Citrus latifolia* Tan.) are harvested when the fruit is mature and the skin is green. The major postharvest handling problem for maintenance of fruit quality is retention of chlorophyll in the skin, as yellow fruit is of considerably lower value to consumers. Limes thus differ from most other citrus fruits where the desirable color is derived from yellow-orange carotenoid pigments and is generally achieved after degradation of the masking green chlorophyll. The recommended storage temperature for limes to retain a green peel color is 10 °C, which is the threshold temperature below which the fruit is susceptible to chilling injury (Arpaia and Kader, 2013; Burns, 2016). However, the relationship between temperature and loss of green color of citrus fruit is not simple. Natural loss of chlorophyll for other citrus fruits requires some exposure to temperatures below about 15 °C during development (Manera *et al*., 2012; Porras *et al*., 2014). This contrasts with the commercial postharvest degreening process of oranges and mandarins where green fruits are exposed to relatively high temperatures of 25–30 °C in the presence of a relatively high ethylene of about 5 µL L^{-1} (Porat, 2008; Ritenour *et al*., 2015). The effectiveness of the degreening technique is due to the requirement for ethylene to induce chlorophyll degradation with the high temperature accelerating chlorophyll loss (Apelbaum *et al*., 1976; Purvis and Barmore, 1981). Thus, the degradation of chlorophyll is a result of an interaction between ethylene and temperature. However, there have been few systematic studies on horticultural produce to quantify the relationship between temperature and ethylene at the lower concentrations likely to be encountered during marketing of ≤ 1 µL L^{-1} (Cantwell and Reid, 1993; Wills and Kim, 1996; Wills *et al*., 2000). Wills *et al.* (2014) reported the time to ripen banana fruit held at all combinations of temperature from 15 to 25 °C and ethylene from 0.001 to 1.0 µL L^{-1}, but no similar study has been reported for the lime fruit. This report examined the interaction of storage temperatures above the chilling threshold and continuous atmospheric concentrations of ethylene from 0.001 to 1.0 µL L^{-1} on the loss of green peel color of limes during storage. Internal fruit quality factors of total soluble solids (TSS), titratable acidity (TA), and antioxidant activity (as a measure of plant stress) were also assessed when fruit reached the end of green life.

Materials and methods

Limes of commercial maturity were obtained from two growers on the Central Coast region of New South Wales, Australia on two occasions that were one month apart. Each batch of fruit was sorted to select those with similar size (about 30 g), uniform intensity of green color and no visible defects. For each batch, 390 fruits were randomly distributed into 39 treatment units each with 10 fruits per unit. Thirty-six units were placed into different 4-L plastic containers that were fitted with inlet and outlet airflow ports. Twelve containers were each placed into three temperature controlled cabinets held at 30, 20 or 10 °C. Within each temperature cabinet, three containers (replicates) of each treatment were ventilated with humidified air (100 mL min^{-1}) containing ethylene at either < 0.001, 0.01, 0.1 or 1 µL L^{-1}. The desired concentrations of ethylene were obtained by mixing ethylene from a gas cylinder (BOC Gases, Sydney) with compressed air that was made "ethylene-free" by passing through a jar containing potassium permanganate pellets, and humidified to approximately 90% RH by bubbling through water. Ethylene concentration was monitored daily by GC-FID (Wills *et al*., 2014). The "ethylene-free" air was designated as < 0.001 µL L^{-1} ethylene as this was the limit of detection of the analytical method. The temperature and RH were monitored at hourly intervals with calibrated Tiny-Tag View 2 data loggers.

The standard for assessment of loss of fruit rind green color was examined in a preliminary experiment. This showed that the fruits used in this study had an initial dark green rind color with a hue angle (h°) of about 120 as assessed by a colorimeter (Minolta CR-300, Osaka). The loss of intensity of green rind was visually assessed over time and considered to become unacceptable for high quality fruit at a pale yellow-green color which was when fruit had a hue angle of about 110. A photograph was taken of fruit at this color stage and used to maintain consistency of the subjective assessment of rind color during the main experiments. In these experiments, each fruit was visually examined daily and the time for each fruit to reach this pale yellow-green color was noted. The mean time for all ten fruit in a treatment unit to show the pale yellow-green color was assigned as the green life of that unit.

At the start of storage, the three remaining units that were not placed into treatment containers were assessed for internal quality factors (TSS, TA and antioxidant activity). Internal quality was also assessed on each treatment unit when it reached the end of green life. The 10 fruits in each unit were manually juiced and passed through two layers of cheese cloth. The TSS in the juice was determined with a digital refractometer (PR-32 Atago, Japan) and expressed as °Brix. TA was determined by titrating juice (1 mL) with 0.1 N NaOH to pH 8.2 with an automatic titrator (Mettler Toledo, Switzerland) and data were expressed as citric acid equivalents (g L^{-1}). Antioxidant activity was measured as 2,2-diphenyl-1-picrylhydrazyl derivatives (DPPH) using the method described by Thaipong *et al.* (2006) with fresh juice (0.2 mL) mixed with methanol (5 mL) and 0.15 mL of this solution mixed with DPPH working solution (2.85 mL) and the absorbance at 515 nM converted to µM Trolox equivalents (TE) per g of juice. Statistical procedures were performed using SPSS for Microsoft version 18.0 software package (SPSS Chicago, IL). A two-way analysis of variance was conducted using a 3 × 4 factorial treatment structure that was repeated on each batch of fruit. The least significant difference (LSD) at $P = 0.05$ for means were calculated to determine significant differences between treatments.

Results and discussion

There was a significant interaction of storage temperature and ethylene ($P < 0.001$) on the retention of green color (green life) of limes. The green life of limes stored at 30 °C was significantly greater than for fruits stored at the current recommended temperature of 10 °C (Table 1), with the shortest green life being with limes held at 20 °C. While there was an increase in green life with decrease in ethylene concentration around the fruits held at all temperatures, the sensitivity to reduced ethylene differed between temperatures. Fruits stored at 30 °C attained maximum green life at < 0.1 µL L^{-1} ethylene while limes at 10 and 20 °C did not

Table 1. Effect of storage temperature and ethylene concentration on the green life of limes. Each value is the mean of 60 fruits (3 units each of 10 fruits × 2 batches of fruit).

Ethylene (µL L^{-1}) during storage at	Green life (days)		
	30 °C	20 °C	10 °C
1.0	15.3	7.1	14.7
0.1	26.1	18.4	20.2
0.01	26.9	20.7	24.3
<0.001	27.1	20.6	24.2
LSD[y]		*2.1*	

[y] Least significant difference between values at $P = 0.05$.

attain maximum green life until ethylene had decreased to 0.01 µL L^{-1}. Reducing the ethylene concentration from 0.01 to < 0.001 µL L^{-1} did not significantly increase the green life of limes at any temperature.

The current recommendation of storing lime fruit at 10 °C to minimise loss of green color was mostly developed in research laboratories in sub-tropical regions where 20 °C is often used as the ambient temperature. The finding in this study that the longest green life of limes was attained at 30 °C is consistent with market observations in tropical countries for various commodities, such as banana and orange, which retain a green skin even when the flesh has ripened. While not fully understood, the retention of chlorophyll at elevated temperatures has been associated with the partial retention of thylakoid membranes (Blackbourn *et al.*, 1990) and upstream genetic factors regulating chlorophyll degradation (Yang *et al.*, 2009). Regardless of the mechanism involved in green color retention, the use of a high ambient storage temperature would seem to have application for limes grown in tropical regions, particularly in developing countries where refrigeration is either not available or too expensive to utilize. Even in developed tropical regions, maintaining a high temperature should be less energy intensive and therefore more economical than to refrigerate to 10 °C. An added advantage of using 30 °C is the greater tolerance to ethylene before it causes loss of green life, as this would require less management of the accumulation of ethylene whether from limes or cross-contamination by other produce or industrial sources to be ≤0.1 µL L^{-1}. It is acknowledged that these findings are for Persian lime grown in only one country and the use of high temperature needs to be evaluated in other growing environments. In addition, 30 °C may not be the optimum temperature to extend green life and a more detailed examination of temperatures in the range of 25–35 °C is warranted.

For lime grown sub-tropical regions, depending on the ambient temperature, it may be economically feasible to heat storage chambers to 30 °C rather than refrigerating to 10 °C. Any reduction in energy usage would also assist the horticultural industry signify to the community that it is acting responsibly from an environmental perspective to reduce greenhouse gas emissions (East, 2010). The maintenance of a low-ethylene atmosphere in the storage chamber would also make storage at temperatures around 20 °C a more viable marketing option. The economics would depend on the cost of maintaining a low ethylene concentration around fruit being less than the energy savings. Various technologies are available for ethylene management and range from ventilation with ambient air, to oxidation of ethylene with a chemical or physical reactant, and inhibition of ethylene action.

The assessment of internal quality at the end of green life for each treatment was conducted to determine if there had been a differential effect of temperature and ethylene on parameters of juice quality. It needs to be recognised that the assessments were made at storage times ranging from 7 days for fruit held in 1 µL L^{-1} ethylene at 20 °C to 30 days for fruit in low ethylene at 30 °C. The results presented in Table 2 show that TSS was statistically ($P < 0.05$) affected by the interaction of storage temperature and ethylene. However, the relatively low magnitude of the differences in TSS between treatments (<0.6 °Brix) would not translate into a discernible effect on taste. It is noted that all treatments showed an increase in TSS during storage from the initial 8.2 °Brix present at harvest. This was probably due to an increase in sugars during storage as the TA level in limes did not significantly change during of storage while changes in the TSS/TA ratio reflected those found for TSS.

The antioxidant activity (as measured by DPPH) in limes also did not change from the original level of 0.57 µM TE during storage with the range in all treatments being 0.56–0.58 µM TE at the end of green life; thus the oxidative status of fruit was maintained during storage under all conditions (Table 3). These results indicate that storage at 30 °C did not adversely affect internal lime quality compared to fruit held at 10 °C.

However, two issues that arose during storage around 30 °C were the propensity for the calyx to abscise and development of mould on the calyx region. The calyxes on most fruit abscised after about 3 days and 7 days at 30 °C, respectively, for the two batches of limes used in this study. Mould growth also occurred on the surface around the calyx on most fruit after about 10 and 20 days at 30 and 20 °C, respectively, but did not penetrate into the underlying tissue. The importance of having an attached calyx varies in different markets while the mould was only a mild superficial de-

Table 2. Total soluble solids (TSS), titratable acidity (TA) and TSS/TA ration in lime juice at the end of green life. Each value is the mean of 60 fruits (3 units each of 10 fruits × 2 batches of fruit).

Ethylene (µL L^{-1}) during storage at	30 °C	20 °C	10 °C
TSS (°Brix)			
At harvest		8.2	
1.0	8.7	8.9	8.8
0.1	9.1	9.3	8.8
< 0.001	9.1	9.0	8.8
LSD[y]		*0.18*	
TA (g L^{-1})			
At harvest		69	
1.0	69	71	70
0.1	68	70	69
< 0.001	69	71	68
TSS/TA			
At harvest		0.119	
1.0	0.126	0.125	0.126
0.1	0.131	0.133	0.127
< 0.001	0.132	0.127	0.129
LSD[y]		*0.004*	

[y] Least significant difference between TSS and TSS/TA values at $P = 0.05$. Data for TA were not significant at $P = 0.05$.

TABLE 3. Antioxidant activity, as measured by Trolox equivalents (TE) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) derivatives, in lime juice at the end of green life. Each value is the mean of 60 fruits (3 units each of 10 fruits × 2 batches of fruit).

Ethylene (µL L^{-1}) during storage at	Antioxidant activity[z] (TE g^{-1}) 30 °C	20 °C	10 °C
At harvest		0.57	
0.0	0.56	0.59	0.58
0.1	0.58	0.57	0.59
1.0	0.57	0.56	0.58

[z] Data were not significant at $P = 0.05$.

fect. If preventative action was required, it would seem both conditions could be avoided by application of the synthetic auxin, 2,4-dinitrophenoxyacetic acid (2,4-D) (Dekazov, 1983; Gates, 1949; Jideani and Jideani, 2012) which is an approved treatment in some countries. In addition, prolonged storage at 30 °C would benefit from maintenance of high humidity in the storage container to minimise water loss. Many tropical countries have high ambient humidity but this could be augmented by wrapping a plastic sheet around the fruit or around stacks of produce.

Conclusion

Storage of limes at 30 °C resulted in a longer retention of green peel color than fruits held at the current recommended temperature of 10 °C without any adverse effect on internal fruit quality. Maximum green life at 30 °C was attained when ethylene in the storage environment was maintained at ≤ 0.1 µL L^{-1}. Storage at a high ambient temperature could have considerable benefits for lime from tropical climates where refrigeration is either not available or too expensive. It could also have application in sub-tropical climates with a lower energy requirement and hence cost to maintain the required storage temperature. Managing the exogenous ethylene concentration to be < 0.1 µL L^{-1} will ensure that maximum green life is achieved at ambient temperatures.

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