Storage temperature during the early stage of ripening determines whether or not the peel of banana (*Musa* spp. AAA, Cavendish subgroup) degreens

Maratree PLAINSIRICHAI[1], David W. TURNER[2]*

[1] Dep. Agric. Technol., Fac. Technol., Mahasarakham Univ., Mahasarakham 44000, Thailand

[2] Sch. Plant Biol. (M084), Fac. Nat. Agric. Sci., Univ. West. Aust., 35 Stirling Highway, Crawley WA 6009, Australia David.Turner@uwa.edu.au

* Correspondence and reprints

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Abstract –– Introduction. The peel of bananas that ripen at ≥ 24 °C does not turn fully yellow, but retains a green colour, reducing the commercial value of the fruit. Historically, in experimental studies of this problem, fruit are ripened at constant temperatures; for example, either 20 °C or 30 °C. In our experiments, we systematically varied the temperature of banana storage during ripening to investigate early events in ripening that may determine whether or not the peel remained green. **Materials and methods**. Fruits were exposed to temperatures of 20 °C or 30 °C for short periods before then storing at 30 °C or 20 °C to complete ripening. The $L^* a^* b^*$ colour system was used to measure change in green colour in the banana peel. **Results**. If the temperature of the fruit stored was 20 °C for the first few hours (≥ 14 h), then the peel became fully yellow, even in fruit subsequently ripened at 30 °C. Conversely, if the temperature of the bananas stored was 30 °C for the first few hours of ripening, then full yellow colour was not obtained even if fruits were subsequently ripened at 20 °C. **Conclusion**. The temperature of the first few hours of banana ripening determines whether or not the peel reaches full yellow colour.

Australia / *Musa* (bananas) / storage / ripening / temperature / colour / peel / degreening / chlorophylls

La température de stockage pendant les premiers stades de maturation détermine si la peau de la banane (*Musa* spp. AAA, sous-groupe Cavendish) va déverdir ou non.

Résumé –– Introduction. La peau des bananes qui mûrissent à ≥ 24 °C ne vire pas entièrement au jaune, mais maintient une couleur verte qui diminue la valeur commerciale du fruit. Historiquement, dans des études expérimentales de ce problème, les fruits étaient mis à mûrir à des températures constantes, à 20 °C ou 30 °C par exemple. Dans nos expérimentations, nous avons systématiquement fait varier la température pendant la maturation pour étudier les événements précoces de la maturation qui pouvaient influencer le déverdissement de la peau. **Matériel et méthodes**. Des bananes ont été exposées aux températures de 20 °C ou de 30 °C pendant de courtes périodes avant d'être stockées à 30 °C ou à 20 °C pour compléter la maturation. Le système de couleur de $L^* a^* b^*$ a été utilisé pour mesurer le changement de la couleur verte de la peau des fruits traités. **Résultats**. Lorsque la température de stockage du fruit a été de 20 °C pendant les premières heures (≥ 14 h), la peau est devenue entièrement jaune, même si le fruit a été mis ensuite à mûrir à 30 °C. Inversement, si la température a été de 30 °C pendant les premières heures de maturation, alors la couleur entièrement jaune n'a pu être obtenue même si les fruits étaient mis ensuite à mûrir à 20 °C. **Conclusion**. La température de stockage pendant les premières heures de maturation de la banane détermine si la peau atteindra ou non la pleine couleur jaune.

Australie / *Musa* (bananes) / stockage / mûrissage / température / couleur / pelure / déverdissage / chlorophylle

1. Introduction

Bananas (*Musa* spp., AAA) of the Cavendish subgroup are fruit of tropical origin [1] in which the peel remains green if ripened at ≥ 24 °C [2–11]. Degreening is the result of chlorophyll catabolism, which occurs in a sequence [12, 13], and loss of green colour in ripening bananas takes several days. It speeds up as the temperature rises from (15 to 22) °C and then it slows as the temperature increases further up to 35 °C or more [5, 14]. This is not a characteristic of all bananas since, in cultivars of the other AAA subgroups, and AAB and ABB groups, green colour is lost more rapidly as temperature increases [6]. On the available evidence, Drury *et al.* [8] concluded that the effect of high temperature on chlorophyll catabolism was unlikely to act through a general suppression of the process of ripening. They suggested that the part of the process that was most likely to be sensitive to temperature was the release of the chlorophyll molecules from the thylakoid membranes, the step that supplies the substrate for chlorophyll catabolism. Without release of the chlorophyll from the membranes, chlorophyllase does not gain access to its substrate. Little is known about this step [10, 13]. Release of chlorophyll could occur throughout the ripening process or it may be restricted to a small window in the ripening process when temperature affects the release of chlorophyll. If the latter is the case, then starting ripening at high temperature (> 24 °C) should retain the green colour in the peel and transfer to a cooler temperature (< 24 °C) would not reveal the yellow colour. Likewise, starting ripening at a cool temperature should release chlorophyll from the membranes and promote full development of yellow colour, even if the fruit was subsequently ripened at a higher temperature.

In our experiments, fruits were exposed to temperatures of 20 °C or 30 °C either alternately or for various periods to investigate the effects of temperature on the appearance of yellow colour. If the temperature of the first few hours of ripening sets the pattern for subsequent loss of green colour in the peel, then this is consistent with the idea that only a short time is available after the initiation of ripening for the chlorophyll to be released from membranes.

2. Materials and methods

Four experiments were conducted with banana fruits. In the first experiment, fruit began ripening at either 20 °C or 30 °C and were then switched daily between the two temperatures during ripening. In the other experiments, the fruit was exposed to 20 °C or 30 °C for different lengths of time before completing ripening at one temperature or the other. Controls were fruit ripened at either 20 °C or 30 °C continuously. For each experiment, green banana fruits were harvested at the commercial 'full' stage at the University of Western Australia, Crawley, WA (lat. 32° S). The hard green fruits received ethylene at a concentration of 100 $\mu L \cdot L^{-1}$ for 1 day at 20 °C. In each treatment, the fruit were placed in a paper box within a plastic bag. In all four experiments, the control treatments were stored continuously at either 20 °C or 30 °C. Peel colour was measured non–destructively and daily, over 4 days (5 days in experiment 1) and at the same position on each fruit using a chromameter (Konica Minolta® CR –300, Osaka, Japan) with the *L* a* b** colour system. More negative values of *a** indicate more greenness.

2.1. Effect of alternating temperatures (experiment 1)

In the first experiment, the effect of alternating temperatures on changes in peel colour was studied. Twenty green fruits of banana cv. Hsien Jen Chiao (*Musa* spp. AAA, Cavendish subgroup) were used. After receiving ethylene, the fruits were divided into five replicate fruits per treatment. In the control treatments, fruits were stored either at 20 °C or at 30 °C continuously. One set of fruits was stored at 20 °C for 24 h and then alternated between 30 °C, 20 °C, 30 °C and 20 °C after spending 24 h at each temperature. Another set of fruits was stored at 30 °C for 24 h and then they were alternated between 20 °C, 30 °C, 20 °C and 30 °C after spending 24 h at each temperature. In this experiment, the fruit that was transferred from one temperature to another ripened under a similar average temperature, although starting at different temperatures.

2.2. Transfer from 20 °C to 30 °C at 12 h or 24 h (experiment 2)

The number of hours at 20 °C that was needed to induce yellow colour in banana, subsequently ripening at 30 °C was studied. Twenty green fruits of cv. Hsien Jen Chiao were used. After receiving ethylene, the fruits were allocated to five fruits per treatment. One set of fruits was stored at 20 °C for 12 h and then transferred to 30 °C. Another set of fruits was stored at 20 °C for 24 h and then transferred to 30 °C.

2.3. Transfer between 20 °C and 30 °C from 18 h to 48 h (experiment 3)

In the third experiment, fruits were exposed to either 20 °C or 30 °C and then swapped when they had reached a preset colour, on the assumption that peel colour may indicate the 'developmental stage' within the ripening process. Thirty banana fruits of cv. Valery (*Musa* spp. AAA, Cavendish subgroup) were used. After receiving ethylene, the fruits were divided into five fruits per treatment. One set of fruits was stored at 20 °C for either 18 h or 40 h then transferred to 30 °C. Another set of fruits was stored at 30 °C for either 24 h or 48 h and then transferred to 20 °C.

2.4. Transfer between 20 °C and 30 °C from 7 h to 24 h (experiment 4)

The minimum time required at 20 °C to induce yellow colour in the peel of banana was studied in addition to the minimum time needed at 30 °C to remain green. Forty banana fruits of cv. Valery were used. Groups of five fruits were stored at 20 °C for (7, 14 or 19) h then transferred to 30 °C. Groups of five fruits were stored at 30 °C for (7, 14 or 24) h and then transferred to 20 °C.

2.5. Data analyses

In each experiment, the values of a^* (greenness) for each day were subjected to one-way ANOVA (GenStat 10th edition, VSN International, Hemel Hempstead, UK). The significance of differences between treatment means was determined with the F ratio at a probability of 0.05. Data for days 0 and 4 or 5, when the fruit ripened at 20 °C were fully yellow, are presented.

3. Results

In all experiments, significant differences between treatments in the value of a^* usually appeared on day 2 and thereafter (*table I*). There were differences in the measured greenness of unripe fruit on day 0 between experiments but not within experiments (*table II*) and this was related to the cv. available at the time of each experiment. Fruit held at 20 °C throughout ripening was fully yellow after four or five days in all experiments and fruit held at 30 °C retained its green appearance in three of the four experiments. Nonetheless, the a^* values became less negative during ripening at 30 °C, even though the fruit retained a greenish appearance after four or five days (*table II*). Fruit that started ripening at 20 °C and then experienced 30 °C, 20 °C, 30 °C and 20 °C, each for 24 h (experiment 1), were fully yellow after five days, the a^* values not differing significantly ($P = 0.05$) from those of fruit ripening continuously at 20 °C (*table II*). Fruit that started ripening at 30 °C and then experienced 20 °C, 30 °C, 20 °C and 30 °C, each for 24 h, retained its green appearance after five days, with the a^* values not differing from the control fruit ripened continually at 30 °C (*table II*). The separation between these two groups of treatments started two days after ripening had begun.

When fruits were exposed to 20 °C for 24 h before switching to 30 °C (experiment 2) the a^* values did not differ significantly from those fruits stored at 20 °C continuously (*table II*). This confirmed the results of experiment 1. Fruit stored at 20 °C for 12 h before transfer to 30 °C was greener than fruit stored continuously at 20 °C but was less green than those stored continuously at 30 °C ($P = 0.05$) (*table II*).

Table I.
The F probabilities for a one-way ANOVA analysis applied to *a** values (from the *L*, a*, b** colour system) for each day in four experiments where banana fruits were exposed to temperatures of 20 °C or 30 °C, either alternately or for various periods, to investigate the effects of temperature on the appearance of peel yellow colour.

Experiment no.	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
1	Effect of alternating temperatures	0.824	0.462	0.001 *	0.013 *	0.013 *	0.040 *
2	Transfer from 20 °C to 30 °C at 12 h or 24 h	0.306	0.445	0.006 *	0.016 *	0.005 *	–
3	Transfer between 20 °C and 30 °C from 18 h to 48 h	0.736	0.055	< 0.001 *	0.001 *	< 0.001 *	–
4	Transfer between 20 °C and 30 °C from 7 h to 24 h	0.820	0.002 *	< 0.001 *	< 0.001 *	< 0.001 *	–

* Significant differences ($P = 0.05$) among treatments.

Fruits exposed to 30 °C for either 24 h or 48 h before transfer to 20 °C (experiment 3) failed to develop full yellow colour and were not significantly different from those stored continuously at 30 °C (*table II*). Storing fruits at 20 °C for either 18 h or 40 h before transfer to 30 °C caused full yellow colour to develop as these fruit did not differ in greenness from the control fruit stored continuously at 20 °C.

The longer fruit were stored at 30 °C (experiment 4), the greater the retention of green colour (*table II*). When fruits were stored at 30 °C for 7 h before transfer to 20 °C, the *a** values were not significantly different from the banana ripening continuously at 20 °C ($P = 0.05$) but those fruit stored for 24 h retained green colour. Storing the fruits at 20 °C for either (7, 14 or 19) h before changing to 30 °C did not significantly alter the *a** values compared with fruits ripening continuously at 20 °C ($P = 0.05$). At day 4 of ripening, fruits stored at 20 °C for 14 h or 19 h had less green colour than those stored at 30 °C for 14 h or 24 h ($P = 0.05$) (*table II*). In experiment 4, control fruit stored at 30 °C continuously lost all their green colour, in contrast to the results in experiments 1, 2 and 3. Loss of green colour occurred between measurements on the second and third days of ripening (data not shown). This was not a feature of the cv. (Valery) as fruit in experiment 3 retained its green colour, as did those fruit in experiment 4 that were stored at 30 °C for 14 h or 24 h before transfer to 20 °C. However, this observation tempers our interpretations of the data from experiment 4.

4. Discussion

These data support the view that the temperature of the early stages of ripening determines whether or not greenness completely disappears from the peel of banana (*Musa* spp. AAA, Cavendish subgroup) during ripening. If the temperature of the fruit was 20 °C for more than 14 h then full yellow colour was obtained, even in fruit subsequently ripened at 30 °C. Conversely, if the temperature was 30 °C for the first few hours (≥ 14 h) of ripening, then green colour was retained even if fruits were subsequently ripened at 20 °C. Extending the initial time of ripening at 20 °C increased yellow colour in fruits subsequently ripened at 30 °C. Conversely, increasing the initial time at 30 °C increased greenness, even in the fruits subsequently ripening at 20 °C.

Our data support the view of Drury *et al.* [8] that an early event, such as separation of chlorophyll molecules from the thylakoid membrane, prevents the catabolism of chlorophyll in the peel of banana during ripening. The experiments also support the view that there is only a small window of time when separation of chlorophyll from membranes can occur. Fruit that began ripening at 20 °C and was then transferred to 30 °C retained no green colour. Thus, at 20 °C the separation of chlorophyll from the thylakoid membranes could be quite fast. At 30 °C, the picture may be somewhat different. Fruit stored at 30 °C for 7 h turned yellow when subsequently ripened at 20 °C. As the time of exposure to 30 °C increased from 7 h to 24 h, more green colour was retained. Increasing storage at 30 °C from 24 h to 48 h did not have any further effect on the retention of green colour in the peel. At 30 °C, it may be that the amount of separation that can occur decreases the longer the time at 30 °C.

Based on a Q_{10} of 2 we would expect that at 30 °C the overall ripening process would be twice as fast as at 20 °C. In fruit commencing ripening at 20 °C, metabolism could expect to be accelerated on transfer to 30 °C and one may expect the loss of green colour to follow this pattern. However, the loss of green colour did not follow this expected pattern. Temperature at the early stages of ripening determined whether or not greenness was retained during ripening in the peel of banana. It appears that the separation of chlorophyll from the membranes is restricted to the first few hours of ripening, because even though initial storage at 30 °C may prevent the separation, subsequent storage at 20 °C has no effect. In other words, the damage caused by the high initial temperature to the process of chlorophyll catabolism cannot be reversed by subsequent storage at 20 °C.

5. Conclusions

Storing banana fruits at 20 °C even for a few hours, before transfer to 30 °C, induces yellow colour in the peel. Storing the fruits at 30 °C for ≥ 14 h results in the retention of green colour and chlorophyll in banana subsequently ripened at 20 °C. These observations are consistent with high temperature interfering with the early events of chlorophyll catabolism, as suggested by Drury *et al.* [8].

Table II.
The greenness of the peel of ripening banana fruit, expressed as a^* values measured with a chromameter. The more negative the value of a^*, the greener the appearance of the fruit. At day 4 or 5, the fruit ripened continuously at 20 °C were fully yellow in appearance.

Treatment	a^*	
Experiment 1	Day 0	Day 5
20 °C continuous	– 19.39 a	– 2.15 a
30 °C continuous	– 19.89 a	– 5.65 b
(20 / 30 / 20 / 30 / 20) °C changed every 24 h	– 18.98 a	– 1.76 a
(30 / 20 / 30 / 20 / 30) °C changed every 24 h	– 19.19 a	– 6.37 b
LSD, *P* = 0.05	1.02	2.98
Experiment 2	Day 0	Day 4
20 °C continuous	– 18.54 a	– 1.48 a
30 °C continuous	– 19.53 a	– 9.64 b
20 °C for 12 h then 30 °C	– 19.20 a	– 5.24 c
20 °C for 24 h then 30 °C	– 19.53 a	– 3.93 ac
LSD, *P* = 0.05	1.16	3.72
Experiment 3	Day 0	Day 4
20 °C continuous	– 16.38 a	– 1.92 a
30 °C continuous	– 16.34 a	– 5.47 b
20 °C for 18 h then 30 °C	– 15.81 a	– 2.59 a
20 °C for 40 h then 30 °C	– 15.84 a	– 1.07 a
30 °C for 24 h then 20 °C	– 15.92 a	– 5.48 b
30 °C for 48 h then 20 °C	– 16.16 a	– 6.77 b
LSD, *P* = 0.05	1.26	2.33
Experiment 4	Day 0	Day 4
20 °C continuous	– 15.71 a	– 1.35 a
30 °C continuous	– 15.44 a	– 2.01 ab
20 °C for 7 h then 30 °C	– 15.38 a	– 2.50 ab
20 °C for 14 h then 30 °C	– 15.72 a	– 2.66 ab
20 °C for 19 h then 30 °C	– 15.40 a	– 1.82 ab
30 °C for 7 h then 20 °C	– 15.80 a	– 2.88 b
30 °C for 14 h then 20 °C	– 15.46 a	– 5.04 c
30 °C for 24 h then 20 °C	– 15.28 a	– 6.61 d
LSD, *P* = 0.05	0.74	1.51

Means followed by the same letter within an experiment and column are not significantly different at *P* = 0.05.

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La temperatura de almacenamiento durante los primeros estados de maduración determina si la cáscara del banano (*Musa* spp. AAA, sub-grupo Cavendish) perderá o no su color verde.

Resumen –– **Introducción**. La cáscara de los bananos que maduran a ≥ 24 °C no se vuelve del todo amarilla, sin embargo mantiene un color verde que disminuye el valor comercial del fruto. Históricamente, en el estudio experimental de este problema, se ponían a madurar los frutos a temperaturas constantes, a 20 °C o a 30 °C, por ejemplo. En nuestros experimentos variamos sistemáticamente la temperatura durante la maduración para estudiar así los acontecimientos precoces de la maduración, que podían influenciar la pérdida del color verde de la cáscara. **Material y métodos**. Se expusieron bananos a las temperaturas de 20 °C o de 30 °C durante cortos periodos antes de almacenarse consecuentemente a 30 °C o a 20 °C para completar la maduración. El sistema de color de *L* a* b** se empleó para medir el cambio del color verde de la cáscara de los frutos tratados. **Resultados**. Cuando la temperatura de almacenamiento del fruto fue de 20 °C durante las primeras horas (≥ 14 h), la cáscara se volvió completamente amarilla, incluso si se colocaba a madurar el fruto a 30 °C. Por el contrario, con una temperatura de 30 °C durante las primeras horas de maduración, no se logró obtener así el color completamente amarillo, incluso colocando después los frutos a madurar a 20 °C. **Conclusión**. La temperatura de almacenamiento durante las primeras horas de maduración del banano determina si la cáscara alcanzará o no el color completamente amarillo.

Australia / *Musa* (bananos) / almacenamiento / maduramiento / temperatura / color / piel (vegetal) / desverdización / clorofilas