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PRO-LONG 涂膜处理对后熟香蕉果实乙烯形成以及其它有关生理变化的影响

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摘要 香蕉(*Musa acuminata* Colla cv. Dwarf Cavendish)果实采后以商业上推荐使用的1.5% Pro-long溶液处理，贮藏于20℃和75%相对湿度下，分别测定果实的ACC含量、MACC含量、EFE酶活性、乙烯释放、叶绿素含量的变化和果实的硬度变化。结果表明，PRO-LONG处理延缓了香蕉果实果皮的叶绿素降解、硬度的下降以及乙烯释放的增加，在后熟过程中，处理果实的ACC含量发生积累，ACC含量的高峰在乙烯释放高峰和EFE酶活性高峰之前出现，与对照比较，处理果实的ACC含量和EFE酶活性的高峰延迟了5d出现，在后熟过程中，以Pro-long处理果肉圆片，其EFE酶活性受部分抑制(抑制率为19.45%至40.51%)。果实MACC含量在贮藏起初处于一个较显著水平，随着后熟的发展而逐步增加，但与ACC含量的明显增加相比变化是微小的，我们的研究进一步阐明了PRO-LONG涂膜对香蕉果实后熟的影响主要是通过减少氧的供给，部分地抑制了EFE酶活性，延缓了乙烯的形成和释放，从而延长了后熟过程。

关键词 Pro-long涂膜；氨基环丙烷羧酸(ACC)，丙二酰氨基环丙烷羧酸(MACC)，乙烯形成酶(EFE)；乙烯；香蕉果实

EFFECTS OF PRO-LONG COATING ON ETHYLENE BIOSYNTHESIS AND OTHER RELEVANT PHYSIOLOGICAL CHANGES OF BANANA FRUIT DURING RIPENING

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Abstract Coating banana (*Musa acuminata* Colla cv. Dwarf Cavendish) fruits with 1.5% Pro-long delayed chlorophyll breakdown in peel, the decrease in firmness of the fruit and the increase in ethylene production of the whole fruit. During ripening, the accumulation of ACC content occurred in banana fruit treated with Pro-long. The peak in ACC content

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appeared a short time before that in ethylene production and in EFE activity. The peaks in ACC content and EFE activity of Pro-long treated fruits occurred 5 days later than that of the control. During ripening EFE activity in banana pulp discs treated with Pro-long was partially inhibited (from 19.45% to 40.51%). MACC contents in banana fruit were at significant levels at the beginning, and then increased gradually with the development of ripening, but only slightly as compared with the marked increase in ACC levels. We suggest that effects of Pro-long coating on banana fruit during ripening mainly due to affecting ethylene biosynthesis of the fruit through partially inhibiting EFE activity by reducing the supplement of oxygen, so as to delay the increase in ethylene production and so extend the ripening processes.

Key words Pro-long coating; 1-aminocyclopropane carboxylic acid (ACC); Malonyl-1-aminocyclopropane carboxylic acid (MACC); Ethylene forming enzyme (EFE); Ethylene; Banana fruit

1 Introduction

Ripening of climacteric fruits such as banana shows a great increase in respiration rate and autocatalytic ethylene production. In these fruits ethylene plays an important role in the ripening process of fruits. Ethylene is generally considered to be the hormonal regulator of the ripening of climacteric fruits. Adams and Yang described the pathway of ethylene production in higher plants as follows: Methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4[1]. ACC synthase and ethylene forming enzyme (EFE) are the key enzymes in the pathway[2]. The content of ACC is low during the preclimacteric stage of many fruits, but increases greatly during the climacteric, and then decreases in the postclimacteric stage[3]. In climacteric fruits, regulation of ethylene biosynthesis seems to depend on both ACC and the capability of the tissue to convert ACC to ethylene[4]. The ACC level can be regulated by its rate of synthesis and conversion to ethylene as well as by its conjugation to malonyl-ACC (MACC)[3]. On the other hand, ethylene is capable of regulating the activity of EFE[5], ACC synthase[6] and MACC transferase[7].

Pro-long is a coating material for fruits comprising a mixture of sucrose esters of fatty acids and sodium salt of carboxymethylcellulose. Coating banana fruit with an aqueous solution of Pro-long has been shown to form a physical barrier to gaseous diffusion through the stomata on the fruit surface, which are the principal route for gaseous exchange of the the fruit tissues with the external atmosphere[8]. Application of Pro-long to C_2H_4-treated bananas reduced their rates of respiration and C_2H_4 production, and delayed the decline in skin chlorophyll content, these effects were ascribed to the reduced O_2 levels

found in coated fruits[9]. Application of Pro-long to bananas at different times relative to initiation of ripening reduced their respiration rates, C_2H_4 production and chlorophyll loss[10]. In addition, other studies have been performed on the effects of Pro-long on ripening process of banana, babaco and papaya[11,12]. However, little is known as to how Pro-long coating affects ethylene biosynthesis so as to delay the ripening process of fruits even though some applications of Pro-long to fruits were done[13]. This work was designed to examine the effects of Pro-long coating on biosynthesis of ethylene and other relevant physiological changes in banana fruits during ripening.

2 Materials and Methods

2.1 Plant material and treatments

Banana (*Musa acuminata* Colla cv. Dwarf Cavendish) fruit, green and slightly immature by commerical standards, were obtained from Windward Isles through Banana Section of Geest PLC. The bananas were washed with 1% sodium hypochloride solution, and allowed to dry. Afterwards, the fruits were dipped into 1.5% Pro-long coating solution for several minutes, and then allowed to dry for about 1 hour. The fruits were stored at 20 ℃ with 75% relative humidity.

2.2 Determinations of ethylene production

Fruits were sealed in plastic boxes with septa for 4 hours and then 1ml gas samples were taken through septa and injected into Shimadzu gas chromatograph with a gas-tight syringe. The Shimadzu gas chromatograph was fitted with a flame ionization detector with the following conditions: Al_2O_3 column, oven temperature 90 ℃, injection temperature 120 ℃ and nitrogen carrier gas flow rate 40ml min^{-1}.

2.3 Determination of ACC and MACC contents

Pulp tissue of banana, 10g fresh weight, was homogenized in 10ml of 90% ethanol by means of an Ystral Gmbh D-7801 Dottingen homogenizer. The shaft was washed with additional 10ml of 90% ethanol. The extract and washings were combined and extracted in a water bath at 70 ℃ for 1 hour, and then centrifuged at 4000rpm for 15min. The supernatant was collected, and the residue was extracted twice with 10ml of 90% ethanol and then centrifuged and all supernatants were combined. The supernatant was concentrated under reduced pressure at 45 ℃ by means of CORNING vacuum rotary evaporator to remove all ethanol and the extract was mixed with 3ml of chloroform to remove pigments, and then with 5ml of water and was vigorously shaken. ACC in the water phase was assayed by the method of Lizada and Yang[14]. For measuring MACC content, an 2ml aliquot was hydrolyzed in 3mol L^{-1} HCl at 100 ℃ for 3 hours to liberate ACC. Following

neutralization with 3mol L^{-1} NaOH the resulting hydrolysate was assayed for ACC as described above. MACC content was calculated as differences in ACC content after and before HCl-hydrolysis.

2.4 EFE assays

EFE activity was determined by measuring conversion of administered ACC to ethylene *in vivo*. 2g of pulp discs, about 3mm thick and 6mm in diameter, were sampled with a No.4 core borer and placed in a 22ml flask. Following addition of 0.5ml of 5mmol L^{-1} ACC solution, they were infused in vacuum for 5min. Then each flask was sealed with a rubber serum cap for 3 hours and ethylene was determined as above. EFE activity was expressed as ethylene production. On the other hand, after infusion in vacuum, some pulp discs were coated with 1.5% Pro-long coating solution immediately and then sealed as above for ethylene determination.

2.5 Analysis of chlorophyll

A 4g banana peel sample was homogenized and extracted with 80% acetone. Ammonium hydroxide (three to four drops) was added to stabilize the chlorophyll. The extract was filtered with a Buchner funnel at reduced pressure. The filtrate was diluted into 50ml. The solution was read at 645nm and 663nm and the amount of chlorophyll in the sample calculated with the following equation: $C_{(a+b)} = (20.2 \times A_{645}) + (8.02 \times A_{663})$. Micrograms of chlorophyll per gram fresh weight was calculated by multiplying by the dilution factor.

2.6 Analysis of firmness

The firmness of banana pulp and peel was analyzed by TA-XT2 Texture Analyser with XTRA Dimension software package (SMS Stable Micro Systems, Unit 105, Blackdown Rural Industries, Haste Hill, Haslemere, Surrey GU27 3AY, England).

2.7 Statistical analyses

All data are presented as means of measurements from triplicate experiments. Analysis of variance was used for least significant difference at the 5% level.

3 Results

3.1 Effect of Pro-long treatment on chlorophyll contents and firmness

In the beginning stage of storage, the chlorophyll contents of the peels in both the control and Pro-long treatment were high, decreasing slowly with the development of ripening, and that of Pro-long treatment decreased more slowly than that of the control (Figure 1). At day 10 of storage, the chlorophyll contents of the control and treatment were 33.52% and 71.62% of that at the beginning respectively. Then, they fell to low levels.

During the beginning stage of storage, the firmness of peels and pulps in both the control and Pro-long treatment were high. The firmness of peels and pulps in the control started to decrease quickly after the 8th day, while that in the Pro-long treatment remained high, starting to decrease by the 15th day (Figure 2). The patterns of changes of the firmness in both the control and the treatment are similar.

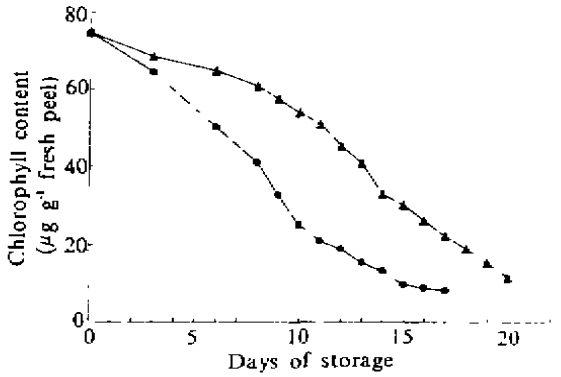

Fig. 1 Changes in chlorophyll content of banana fruit peel during ripening
—●— Control;
—▲— 1.5% Pro-long

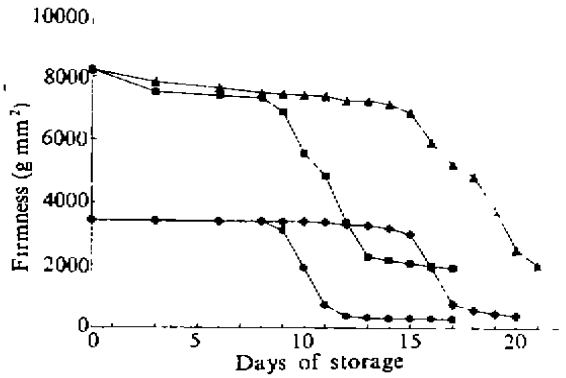

Fig. 2 Changes in firmness of banana fruit peel and pulp during ripening
—■— Control Peel; —●— Control Pulp,
—▲— 1.5% Pro-long Peel; —◆— 1.5% Pro-long Pulp

3.2 Effect of Pro-long treatment on ethylene production

In whole fruit, ethylene production in both the control and the Pro-long treatment was very low before day 4 and day 9 of storage respectively. Ethylene production in the control started to increase greatly after day 4 of storage and reached a peak at day 9 of storage, while that in Pro-long treatment started to increase after day 9 of storage and then reached a peak at day 14 of storage (Figure 3). Afterwards, ethylene production in both the control and Pro-long treatment decreased quickly. They have similar pattern of ethylene evolution.

3.3 Effect of Pro-long treatment on ACC and MACC contents

Changes of ACC and MACC contents in pulp are shown in Figure 4 and Figure 5 respectively. At day zero of storage, ACC levels in the pulp were very low, and MACC contents were at significant levels. After day 5 of storage, ACC content in the control started to increase and reached a peak (23.59nmol g^{-1} fresh pulp) at day 8 of storage, while that in the Pro-long treatment started to increase after the 2nd day and increased gradually and reached a peak (27.53nmol g^{-1} fresh pulp) at day 13 of storage. Afterwars, ACC contents in both the control and Pro-long treatment fell quickly to low levels. Meanwhile, MACC contents in both the control and Pro-long treatment increased slowly. After day 5

of storage. MACC content in the control increased gradually and reached up to 5 52nmol g^{-1} fresh pulp at day 11 of storage, while MACC content in the Pro-long treatment increased gradually after day 9 of storage and reached up to 5.43nmol g^{-1} fresh pulp at day 16 of storage.

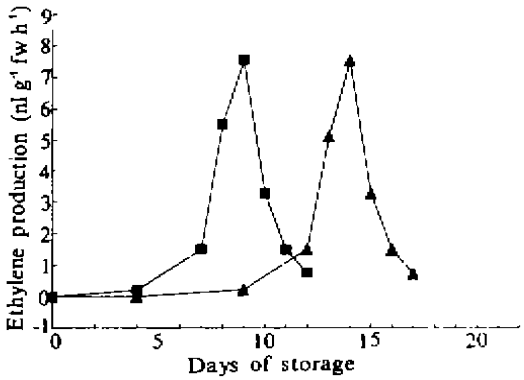

Fig. 3 Ethylene production of banana fruit during ripening

—■— Control; —▲— 1.5% Pro-long

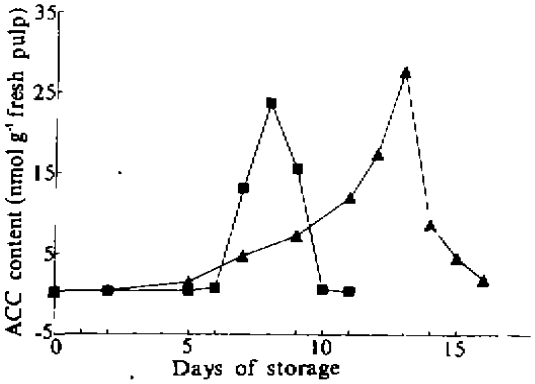

Fig. 4 Changes of ACC content in banana fruit pulp during ripening

—■— Control; —▲— 1.5% Pro-long

3.4 Effect of Pro-long treatment on EFE activities

Changes of EFE activities in pulps of both the control and Pro-long treatment of whole fruits druing ripening are shown in Figure 6. At day zero of storage, EFE activity was low (18.80nl g^{-1}fresh pulp h^{-1}). With the development of ripening, EFE activities in both the control and Pro-long treatment increased gradually, and that in the Pro-long treatment increased much less than that in the control before day 7. After day 7 of storage, EFE activity in the control increased greatly and reached a peak at day 9 of storage, which coincided with a peak in ethylene production, while EFE activity in the Pro-long treatment continued

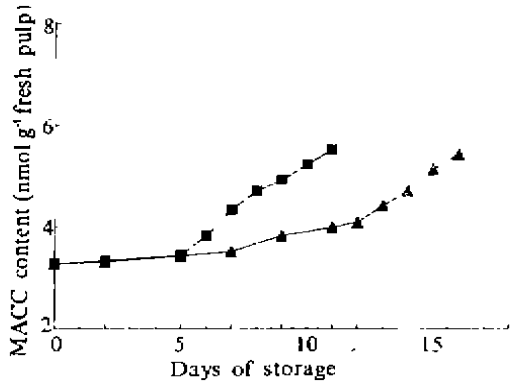

Fig. 5 Changes of MACC content in banana fruit pulp during ripening

—■— Control; —▲— 1.5% Pro-long

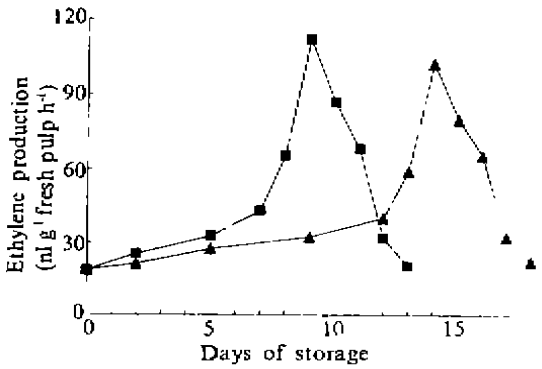

Fig. 6 Changes of EFE (ethylene forming enzyme) activities in banana pulp during ripening

—■— Control, —▲— 1.5% Pro-long

to increase slowly before day 12 of storage and then increased and reached a peak at day 14 of storage. Then, EFE activities in both the control and Pro-long treatment decreased to low levels.

Further to understand the effect of Pro-long coating on EFE in banana pulp discs, changes of EFE activities in banana pulp discs treated with 1.5% Pro-long were measured. The results are shown in Figure 7. EFE activities in banana pulp discs of both the control and treatment increased slowly and reached a peak at day 9 of storage, and then they fell quickly to low levels. Throughout these processes, EFE activity in banana pulp discs treated with 1.5% Pro-long remained lower than that in the control. EFE activities in the treated banana pulp discs were reduced by from 19.45% to 40.51% as compared with the control. At day 9, EFE activity in this treated discs was reduced by 38.85%.

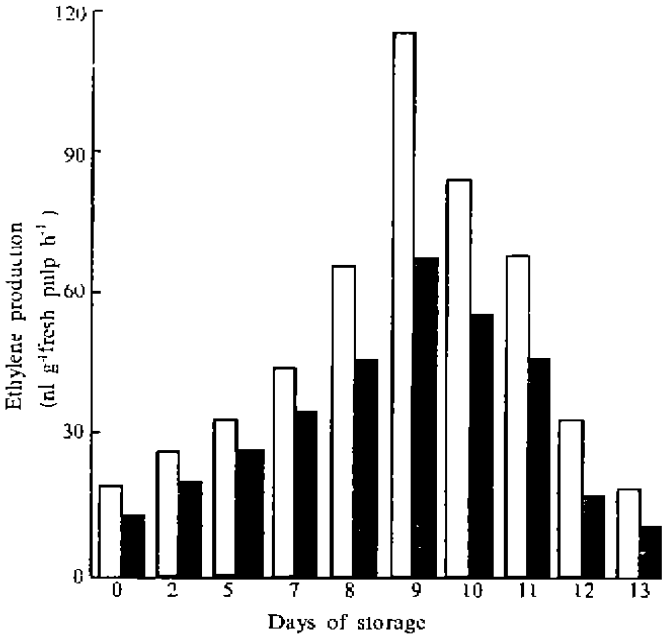

Fig. 7 Ethylene forming enzyme activities in banana pulp discs treated with 1.5% Pro-long coating

4 Discussion

During ripening of banana fruit chlorophyll contents in peels of both the control and Pro-long treatment decreased continuously. The rusults shown in Figure 1 indicate clearly that chlorophyll content in the Pro-long treatment decreased more slowly than that in the control. There is a significant difference between the control and Pro-long treatment ($P<0.05$). That is Pro-long treatment delayed chlorophyll breakdown in banana peel.

In the preclimacteric stage, the firmness in peels and pulps of both the control and Pro-long treated fruits changed slightly, and then their firmness decreased quickly (Figure 2). Figure 2 indicates clearly that the Pro-long treatment delayed the decreases in the firmness of peel and pulp by 5 days. Also there are significant differences in the firmness of peel and pulp between the control and Pro-long treatment ($P<0.05$).

Our data indicate that the Pro-long treatment delayed the increase in ethylene production by 5 days (Figure 3), but the pattern of ethylene evolution in the Pro-long treatment appeared similar to that in the control.

The fact that the EFE activity increased gradually in the beginning stage indicates that the EFE system is active during the beginning stage (Figure 6). The great increase in EFE activity coincided with that of ethylene production. Figure 6 indicates that the Pro-long treatment delayed the great increase in EFE activity by 5 days. EFE is a key enzyme converting ACC to ethylene[2]. Banks[13]reported that the Km (O_2) for the conversion of ACC to ethylene in banana fruit pulp tissue as estimated by this system is 2.2%, ten times greater than that estimated by previous workers for discs of apple cortex. In our study, we applied Pro-long coating to banana pulp discs to reduce the supplement of oxygen thereby inhibiting EFE activity in banana pulp discs by from 19.45 to 40.51% during ripening (Figure 7) where there is a significant difference between the control and Pro-long treatment ($P<0.05$).

The ACC levels in pulps of both the control and Pro-long treatment in the beginning phase were low (Figure 4). Figure 4 shows that there was a slow accumulation of ACC in Pro-long treated fruit before the great increase of ACC content. The great increases of ACC content in both the control and Pro-long treated fruit took place a short time before the peaks of both EFE activities and ethylene production; the increase in ACC level in the Pro-long treated fruit was delayed by 5 days. Afterwards, because of the great increase in EFE activity, ACC was converted to ethylene and decreased quickly to low levels. Also there is a significant difference in the changes of ACC levels between the control and Pro-long treatment ($P<0.05$). We suggest that the following sequence of events occurs during ripening of banana fruit: ACC synthase is first synthesized producing ACC which is converted to ethylene by the already existing EFE system[15]. The ethylene produced stimulates additional ACC synthase activity and then, when ethylene production reaches a certain threshold level, it stimulates EFE activity, thereby further increasing ethylene production. Autocatalysis is a common feature of ripening in climacteric fruits[16]and some data indicates that ethylene enhances the induction of both ACC synthase[6] and EFE[5,17,18] in various fruits. In our study, Pro-long coating was used on banana fruit forming a gaseous barrier on the surface of the fruit to reduce the supplement of oxygen, thereby inhibiting EFE activity partly, which resulted in the accumulation of ACC content. However, because EFE activity was inhibited only in part, ethylene production continued, and when ethylene production reached a certain threshold level, it stimulated EFE activity so as to increase ethylene production further and then reached a peak.

It is well accepted that malonylation of ACC may play a role in the regulation of endogenous ACC level and enhance of ethylene production[3]. Preclimacteric banana fruits contain a significant level of MACC (Figure 5). This indicates that at least part of the ACC which is synthesized in the course of fruit growth is conjugated to MACC. Thus

malonylation of ACC may participate in regulating ethylene production during the preclimacteric stage. In contrast, it does not seem to play a role in regulating ethylene production at the climacteric stage. At this stage, MACC of both the control and Pro-long treated fruit increased only slightly as compared with the marked increase in ACC levels (Figure 4 and Figure 5). However, MACC content of the control increased earlier than that of 1.5% Pro-long treated fruit.

Banks[8,9] suggested Pro-long coating affects gaseous exchange of banana fruit surface thereby delaying ripening. Our results further clarify that effect of Pro-long coating on banana fruit ripening is a complex process, mainly affecting ethylene biosynthesis of banana fruit. This is through partly inhibiting EFE activity by reducing the supplement of oxygen which delays the great increase in ethylene production and so extends the ripening processes.

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