Changes of Polyphenol Oxidase Activity of Banana (*Musa sapientum* L.) Fruits during Ripening Treatment by Ethylene

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Changes of polyphenol oxidase (PPO, *o*-diphenol : oxygen oxidoreductase, EC. 1. 10. 3. 1) activity of banana (*Musa sapientum* L.) fruits during ripening treatment with and without ethylene were determined. The total and soluble PPOs of pulp, which were extracted with and without detergent (Tween 80), were about 100 and 2 unit/g fresh weight, respectively. Both total and soluble pulp PPOs were remarkably increased and showed a maximum peak like a climacteric peak of respiration during the ethylene-ripening treatment. However, in the non-treated fruits, both total and soluble pulp PPOs changed a little during the treatment. In banana peel, total and soluble PPO were about 110 and 0.7 unit /g fresh weight, respectively. Both total and soluble PPO activities of peel were little affected by the ethylene-ripening treatment. These results suggested that the banana PPOs were mainly presented in pulp and peel as a "latent form", and only "soluble pulp PPO" was affected by ethylene-ripening treatment.

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Banana (*Musa sapientum* L.) is one of the most important world traded tropical fruits. In Japan, mature-green fruits are imported from such tropical countries as Philippine, Ecuador and Taiwan, and ripened by the treatment with ethylene, which is well known as a fruit-ripening hormone in plants and also as a growth-rate regulating hormone in vegetative organs of plants. During the treatment, the pulp sugar content was increased¹⁾ and peel color was degreened²⁾. In addition, internal ethylene was formed and affected the ripening of the fruit^{3),4)}. During the ethylene ripening treatment, many enzymes were activated and/or induced by ethylene in banana

fruit^{2),5)~7)}. Polyphenol oxidase (PPO, *o*-diphenol:oxygen oxidoreductase, EC. 1. 10. 3. 1) catalyzes the oxidation of phenolic compounds resulting in a formation of undesirable browning of banana fruit during postharvest storage or processing. Characterization of banana PPO have been conducted by many workers^{8)~12)}, however, little is known about the effect of ethylene treatment on PPO of banana fruit. In the present study, effects of the ethylene treatment on PPO activities of banana pulp and peel were investigated.

Materials and Methods

1. Materials

Mature-green banana fruits, which were imported from Philippines by Dole Co., were purchased from a distribution center in Saga city, Japan. The fruits of the same maturity without being damaged were used as materials. Standard ethylene gas was obtained from GL Sciences Co., Tokyo, Japan. Other reagents were purchased from Wako Pure Chemical Co., Osaka and Katayama Chemical Co., Osaka, Japan.

2. Ethylene treatment

Ethylene-ripening treatment of banana was performed according to the method of MA *et al*⁽²⁾. The green banana fruits were sealed up for 16 hr at 25°C in a 20 ℓ plastic bucket with and without 150 ppm ethylene. Then, the buckets were opened and completely aerated to remove ethylene and carbon dioxide, and further incubated at 25°C in darkness for 1~5 days in ethylene-free air for ripening the fruits.

3. Determination of peel color

The L, a and b values of banana peel during ethylene ripening treatment were determined by using a color reader (MINOLTA, CR-13, Minolta Co, Tokyo, Japan). The color change is expressed with $(L+b)/2 + a$ value, following after MA *et al*⁽²⁾.

4. Assay of PPO Activity

One hundred g of banana pulp or peel (20 g of the middle portion of each 5 samples were pooled) were homogenized in 60ml of 0.1 M sodium phosphate-0.1 M potassium phosphate buffer (0.1 M PB, pH 7.0) with or without 3 % Tween 80. After filtration of the homogenate through cotton cloth, the filtrate was centrifuged at 10,300 g at 2 °C for 20 min, and the supernatant was used as stock solution of PPO. For PPO assay, the stock solution was diluted 10 to 250-fold by 0.01 MPB. PPO activity was determined by the colorimetric method according to YANG *et al*⁽¹²⁾. One unit of the enzyme activity was defined as an increase in absorbance of the mixture at 420nm (ΔA_{420}) of 0.1 per min (1 cm light path).

In this experiment, PPO was extracted with (so called "total PPO"; "latent PPO" + "soluble PPO") or without detergent (so called "soluble PPO"), and the

total and the soluble PPO activities were determined.

Results and Discussion

1. Confirmation of ethylene-ripening

Banana ripening was confirmed by determining color development in the peel. Irrespective of ethylene treatment for 16 hr under sealed condition, peel color did not changed. During the fruits storage in ethylene-free air for 1 ~ 5 days at 25°C, the peel

Fig. 1 Changes of color of banana peel stored after ethylene ripening treatment

color changed from green to yellow after ethylene treated. However, the color of peels not treated with ethylene hardly changed. As shown in Fig. 1, the " $(L+b)/2 + a$ " value expressed the color change of banana peel. The value increased more significantly in the ethylene treated fruits than in the non-treated ones. The same results were obtained in banana peel, in which chlorophyll degrading peroxidase was enhanced by ethylene^(2),7).

2. Effect of ethylene on PPO activity

GALEAZZI *et al*⁽¹¹⁾ reported that the banana pulp PPO presented as a "latent form" which was extracted by the buffer solution containing detergent. Therefore, the effect of ethylene treatment on the activity of "latent PPO" was also investigated.

Fig. 2 (A) shows the changes of total PPO activity of banana pulp during the storage after treated or not

treated with ethylene. Initially, total PPO activity of non-treated fruits was about 100 unit/g fresh weight and slightly increased in the experiment period. In contrast total PPO activity rapidly increased in the ethylene treated fruits. The activity increased after removing ethylene, reached maximum 2 days after, and decreased.

Fig. 2 (B) shows the effect of ethylene treatment on the activity of soluble PPO of banana pulp during storage. Only about 2 units/g fresh weight of the soluble PPO activity was detected. In the non-treated fruit, the soluble PPO activity slightly increased during the storage. However, the soluble PPO activity

was markedly accelerated by the ethylene treatment: the enzyme activity rapidly increased immediately after the ethylene treatment, reached a maximum 2 days after, and then decreased during storage. Banana is one of the climacteric fruits, and has a respiration peak at 3 days after 24 hr's ethylene ripening treatment¹⁾. These results indicate that the "soluble PPO" of banana pulp is induced by the ethylene ripening treatment, has both activity and breath peaks, and the activity decreases in the further maturation period.

Fig. 3 (A) shows the effect of ethylene treatment on the activity of total peel PPO. The activity was

(A); total PPO extracted with detergent, (B); soluble PPO extracted without detergent.

○ control (non treatment), ● ethylene ripening treatment,
△ control (non treatment), ▲ ethylene ripening treatment.

Fig. 2 Changes of polyphenol oxidase activity of banana pulp stored after ethylene ripening treatment

(A); total PPO extracted with detergent, (B); soluble PPO extracted without detergent.

○ control (non treatment), ● ethylene ripening treatment,
△ control (non treatment), ▲ ethylene ripening treatment.

Fig. 3 Changes of polyphenol oxidase activity of banana peel stored after ethylene ripening treatment

about 110 unit/g fresh weight and slightly increased during the experiment period, irrespective of the ethylene treatment. As shown in Fig. 3 (B), about 0.7 unit/g fresh weight of soluble PPO was detected and its activity slightly increased during the storage after the ethylene treatment. These results indicated that both peel PPOs were hardly affected by the ethylene treatment.

In conclusion, the results obtained in the present study suggested that the banana PPOs mainly presented in pulp and peel as a "latent form", and were dissolved by such detergent as Tween 80, and only "soluble pulp PPO" was affected by the ethylene-ripening treatment.

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エチレン処理後の追熟中の バナナポリフェノール酸化酵素活性の変化

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エチレン処理後の追熟中におけるポリフェノール酸化酵素 (PPO) の変化について追究した。抽出液中の界面活性剤の有無により、調製したバナナ果肉中の全 PPO および可溶性 PPO は新鮮果重 g 当たりそれぞれ 100 unit および 2 unit であった。両 PPO 活性ともエチレン処理後の追熟中に顕著に増加し、2 日後にクライマクテリック様のピークを示した。一方、エチレン無処理区では両活性とも変化量はきわめて低かった。バナナ果皮においては、新鮮果重 g 当たり 110 unit および 0.7 unit の全および可溶性 PPO 活性が認められたが、両活性ともエチレン処理による追熟中の変動は認められなかった。これらの結果はバナナ果肉および果皮の PPO の大部分が潜在型で存在することおよび果肉の可溶性 PPO のみがエチレンによる追熟処理によって影響を受けることを示唆する。

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