Pergamon

PII: S0031-9422(97)00211-2

Phytochemistry, Vol. 46, No. 1, pp. 57–60, 1997

0031-9422/97 $17.00+0.00

PUTRESCINE ACCUMULATION IN BANANA FRUIT WITH RIPENING DURING STORAGE

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(*Received in revised form* 5 *February* 1997)

Key Word Index—*Musa* AAA group; Musaceae; banana; polyamine; putrescine; ethylene.

Abstract—Considerable accumulation of the free polyamine, putrescine (Put), was found in both pulp and peel tissue of unchilled and rewarmed bananas (*Musa* AAA group) during normal fruit ripening with climacteric ethylene production, while free Put remained at the original level in continuously chilled fruit. The bound Put content in both tissues of the unchilled and rewarmed fruits increased *ca* 3–10 fold. No competitive relationship was observed between Put and ethylene production in the banana fruit. © 1997 Elsevier Ltd. All rights reserved

INTRODUCTION

The polyamines, putrescine (Put), spermidine (Spd) and spermine (Spm), act as important regulators of the functions of plant senescence and response to stress [1–3]. Ethylene is known to be required for fruit ripening [4] and expression of antisense RNA to the 1-aminocyclopropane-1-carboxylate synthase in the biosynthetic pathways of ethylene inhibits fruit ripening [5]. Ethylene is also reported to control the activity of arginine decarboxylase (ADC, EC 4.1.1.19), a key enzyme in polyamine biosynthesis [6]. Polyamine levels decline before the onset of climacteric ethylene production in avocado [7], pea [8] and tomato [9, 10]. In contrast, increases in polyamines and their related enzymes were observed in nonclimacteric fruits, such as orange [11, 12] and non-ripening mutant tomato fruits [10, 13] with not ethylene production. Polyamines and ethylene may have opposite effects in plant ripening and senescence [2, 6 and 7].

Little is known about the polyamines of climacteric fruit at the post-harvest stage. To clarify the relationship of polyamines and ethylene in fruit during storage, we chose the banana fruit, a typical climacteric fruit in which ripening is ethylene dependent and associated with a burst in ethylene production. We report here that polyamines are accumulated in the banana fruit at ambient temperature.

RESULTS AND DISCUSSION

Ethylene production and fruit ripening

Figure 1 shows changes in ethylene production in whole banana fruit during storage. Both unchilled and rewarmed fruit began to produce ethylene on the 4th day, with a peak observed on the 6th day of storage, when the fruit turned yellow and soft. In contrast, no ethylene was detected in the continuously chilled fruits. Thus, ethylene production in the fruit was inhibited under cold conditions during 10 days of storage. The colour of the peel of continuously chilled banana fruit changed from green to deep brown, indicating the typical symptom of chilling injury.

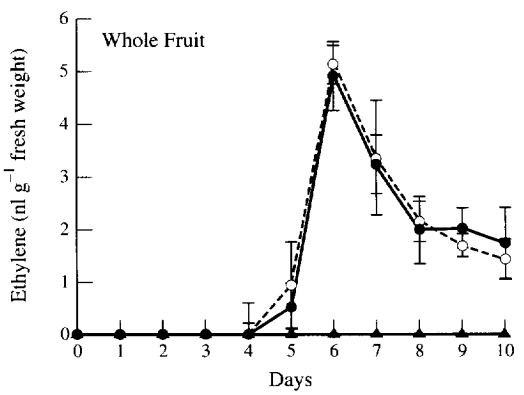

Fig. 1. Ethylene evolution in unchilled (●), chilled (▲) and rewarmed (○) whole banana fruit. Data points are means ±s.d. ($n = 4$).

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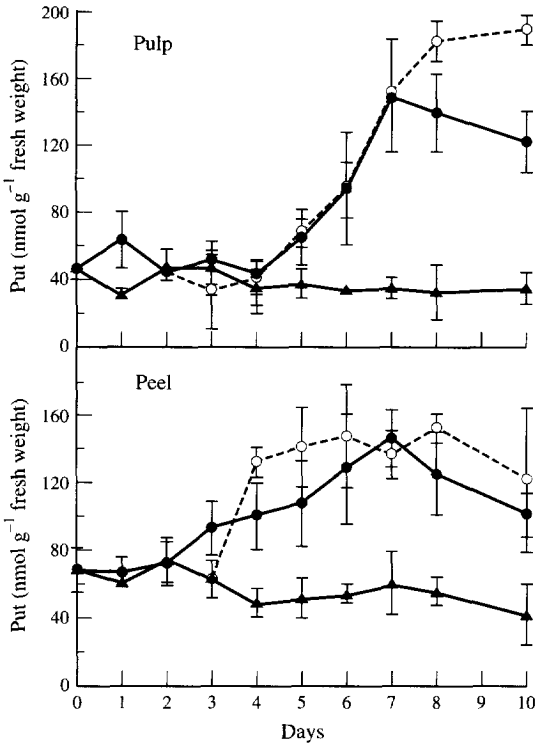

Fig. 2. Put contents in pulp and peel tissue of unchilled (●), chilled (▲) and rewarmed (○) banana fruit. Data points are means ±s.d. (n = 4).

Free polyamine levels in pulp and peel tissue

Polyamine content was determined by HPLC with fluorimetric post-column derivatization as described previously [14]. Put, Spd and agmatine (Agm) were mainly present in both tissues, however their levels were higher in peel than in pulp. The most striking changes in the level of Put in banana fruit occurred during ripening (Fig. 2). The Put content in pulp tissue of unchilled and rewarmed fruit started to increase on the 4th day of storage, reaching *ca* 2–4 fold the original level by 6–7th day. In contrast, the Put content in continuously chilled pulp tissue remained at *ca* 40 nmol g^{-1} fresh weight for the entire storage period. The increase in Put content in unchilled and rewarmed fruit occurred 1 or 2 days faster in the peel than in the pulp tissue.

The Agm content in the peel was *ca* 6–10 fold higher than that in the pulp. Except for the increase in the peel of the unchilled fruit, the Agm content decreased slightly over the storage period in both pulp and peel tissue. The Spd content in both tissues of the unchilled, chilled and rewarmed fruits remained at initial levels for the entire storage period.

Free and bound polyamine levels in pulp and peel tissue

Table 1 presents free and bound Put contents in both pre- and postclimacteric banana fruit. The Put content in the hydrolysates of supernatants from pulp and peel tissues are shown, since no Put was detected in the hydrolysed pellet. The free Put content in the pulp tissue in the climacteric stage (7 days, unchilled) was *ca* 5–12 fold higher than that of the pulp and peel tissue in the preclimacteric stage (0 day). The initial level of both types of Put in the tissues of the continuously chilled fruits were maintained for the entire storage period. In contrast, their contents in pulp and peel tissue of postclimacteric unchilled fruit increased *ca* 3–10 fold.

Winer and Apelbaum [7] demonstrated that the levels of Put, Spd and Spm decreased for a few days at the climacteric peak of ethylene production in avocado fruit during storage at 25°. Such an inverse relationship between polyamines and ethylene was also reported in tomato stored at 25° [10] and in broccoli [15]. Therefore, we expected an inverse relationship between the ethylene burst and a decline in the polyamine content in the banana, a climacteric fruit. However, the Put content during ripening under unchilled conditions increased markedly from the 4th day after storage, and a climacteric rise in the ethylene production was observed at the same time (Figs 1 and 2).

In cut carnation flowers, a significant increase in the content of Put has been reported at the stages of maturation and senescence [16]. Such a parallel increase in both Put and ethylene has not been reported yet for fruits.

MacDonald and Kushad [17] observed Put accumulation in lemons, grapefruits and peppers subjected to chilling injury. The Put accumulation occurred during hardening of wheat and alfalfa under a low temperature, whereas the Put content decreased during dehardening [18]. In this study, typical signs of chilling injury in the banana peel were evident, together with no increase in Put content in the continuously chilled fruit. In contrast, the Put content in the rewarmed fruits, which exhibited light chilling injury signs in the peel, was similar to that in the unchilled fruit. It appeared that the increase in Put content in both unchilled and rewarmed fruit reflects changes during normal ripening.

Polyamines are present in both free and conjugated forms in plants. In particular, bound polyamines are conjugated to various phenolic acids [19] and proteins [20]. No Put was detected in the hydrolysed $HClO_4$ precipitate pellet which contained proteins and condensed polyphenols in this study. The amount of the bound Put increased proportionally with the free Put in both pulp and peel tissue of unchilled fruit (Table 1).

The amount of free Put in avocado [7] and tomato [10] decreased in the climacteric stage, thereby limiting the amount of free Spd. In contrast, free Put accumulated at the climacteric peak without any change in free Spd content throughout ripening (Fig. 2). Flores and Galston [21] reported that accumulation of Put under stress conditions was due to a decreased rate of *de novo* biosynthesis of additional ADC activity and to a decreased rate of conversion of Put to Spd. The

Table 1. Free and bound putrescine contents of banana fruit stored at 23° and 2°

Days after storage Day	Putrescine (nmol g^{-1} fr. wt)*			
	Pulp		Peel	
	Free	Bound	Free	Bound
0	20.9±2.1	3.8±1.4	25.9±1.8	2.2±0.1
7 (2°)	27.1±1.7	2.9±1.3	27.5±1.8	3.7±2.4
(23°)	132±11.3	32.4±8.0	79.9±6.1	14.4±1.3

* Values are means ± s.d. ($n = 4$)

present findings indicate no inverse relationship between ethylene production and polyamine level in banana during ripening.

EXPERIMENTAL

Plant material and storage. Ungassed preclimacteric bananas (*Musa* AAA group, Cavendish subgroup cv. Giant Cavendish) were harvested and kept overnight at room temp. (20°) in the Agricultural product processing factory, Agricultural Cooperative Association of Okinawa, Japan. On arrival at the laboratory, each banana hand was sepd into individual fingers, which were then divided into 3 groups for storage. Each group was packed in a 65 × 80 cm low-density polyethylene pouch (30 μm in thickness). Half the samples were maintained at 2° (chilled) and the remainder were stored at 23° (unchilled) to serve as controls. Some of the fruits kept at 2° were rewarmed at 23° after a 2-day interval (rewarmed).

Extraction of free and bound polyamines. Pulp and peel from the equatorial part of the fruit were homogenized in ice-cold 5% $HClO_4$ (0.1 g fr. wt ml^{-1}). The macerated extracts were kept in iced polypropylene centrifuge tubes for 1 hr, then centrifuged at 23 500 *g* for 20 min. The supernatant which contained free polyamines was used immediately for HPLC analysis or stored at −20° in a plastic vial until analysis. The remaining pellet was washed twice in 5% $HClO_4$, then resuspended in 6 M HCl (0.25 g fr. wt ml^{-1}). In addition, 6 M HCl (0.1 g fr. wt ml^{-1}) was added to the −20° stored supernatant. Both suspensions from the supernatant and pellet were hydrolysed at 110° for 24 hr in sealed ampoules to release the bound polyamines. The hydrolysate was centrifuged and filtered through a 0.45 μm Millipore filter (Millipore Japan, Tokyo, Japan) before HPLC analysis.

HPLC determination of polyamines. Free and bound polyamines were determined as described in ref. [14].

C_2H_4 *analysis.* Whole fruits were enclosed in a gas-tight plastic jar equipped with a silicone rubber stopper for sampling, and 2 ml of gas sampled from the head space was analysed by GC. The concn of C_2H_4 in the samples was determined using a Shimadzu 6AM gas chromatograph equipped with an FID (with a 2 m × 3 mm i.d. column of Porapack Q, 70° column temp, and N_2 at a flow rate of 35 ml min^{-1}). To estimate the C_2H_4 produced by the whole fruit, each fruit was put in a 5.4 l gas-tight plastic jar equipped with a silicone rubber stopper for sampling, and the jars were incubated at each storage temp. for 3 hr. Then 2 ml of C_2H_4 sampled from the head space of the plastic jar was analysed by GC.

Acknowledgements—This work was supported in part by a Grant-in-Aid (Post Harvest Physiology Research Program) from the Ministry of Agriculture, Forestry and Fisheries of Japan. Y.T. is indebted to the Science and Technology Agency of Japan for the Award of a National Institute Post-Doctoral Fellowship. A.G.J.V. was a recipient of Research Awards for specialists from the Japanese Government.

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