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Angiotensin converting enzyme inhibitors from ripened and unripened bananas

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Ripened and unripened nendran, rasthali, poovan, robusta, bontha and safed velchi bananas were investigated for inhibition against angiotensin converting enzyme (ACE) using Hip–His–Leu as substrate. The inhibition of ACE by different ripened banana cultivars was much more than that of unripened banana cultivars. The ACE inhibitory activity of ripened and unripened poovan was heat stable and stable to extreme acidic pH and alkaline pH. The ACE inhibitory activity of ripened and unripened banana cultivars was reduced to 25% and 33% respectively on dialysis.

THE angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC 3.4.15.1) and plays an important role in the regulation of blood pressure[1]. Several potent inhibitors of this enzyme have been reported to be orally active antihypertensive agents[2]. ACE inhibitors derived from casein[3,4], sardines[5], tuna[6], bonito[7] and maize protein[8] are shown to be effective in lowering blood pressure[9]. Since these food themselves lacked ACE inhibition and only their derivatives showed ACE inhibitory activity, their antihypertensive role is difficult to assess. Since ripened bananas are consumed raw and unripened bananas are cooked, studies on ACE inhibitors from bananas are helpful in establishing antihypertensive role to food stuffs. Potato, a well known source of serine and cysteine protease inhibitors, contains carboxypeptidase inhibitors[10,11]. Earlier study from this laboratory indicated the presence of serine and cysteine protease inhibitors in ripened and unripened bananas[12]. In addition, bananas have been reported to be useful in the treatment of hypertension and other cardiac diseases in the indigenous system of medicine in India[13,14]. A recent report indicated that feeding of ripened banana to rats prevented an increase in blood pressure induced by deoxycorticosterone[15].

In view of the above-mentioned observations, an attempt was made to identify ACE inhibitors from ripened and unripened bananas consumed all over the world. This communication reports the presence of inhibitors to ACE in six ripened and unripened banana cultivars and some of their properties.

Ripened and unripened bananas nendran-*Musa* (AAB), rasthali-*Musa* (ABA), poovan-*Musa* (BAA), robusta-*Musa* (AAA), bontha-*Musa* (ABB) and safed velchi-*Musa* (AB) were procured from local sources. Angiotensin converting enzyme was prepared by using a modification of the method described by Cushman and Cheung[16]. Albino rats of both sexes were used in these experiments. After the animals were decapitated, the lungs were isolated and washed with ice-cold 100 mM borate buffer, pH 8.3, containing 50 mM KCl and frozen until further use. One g of lung tissue was diced and homogenized in 10 ml of the same ice-cold buffer using a motor driven teflon/glass homogenizer at 4°C. The homogenate was centrifuged at 20000 *g* for 20 min at 4°C. The supernatant was collected and dialysed for 12 h against 20 volumes of the same buffer at 4°C to remove endogenous low molecular weight inhibitors. The dialysed supernatant was used as the enzyme source for angiotensin converting enzyme. Protein content of the supernatant was measured by the method of Lowry *et al.*[17] using bovine serum albumin as standard. Banana extract was prepared by homogenizing 5 g of ripened or unripened banana without the outer skin in 5 ml of 50 mM borate buffer, pH 8.3. The homogenate was centrifuged at 10000 *g* for 20 min at 4°C. The supernatant was collected and tested for inhibitory activity.

ACE activity was measured by modification of the method described by Schnaith *et al.*[18] using hippuryl-L-histidyl-L-leucine (HHL) as substrate. The reaction mixture contained 0.2 ml of 5 mM HHL prepared

in 200 mM borate buffer, pH 8.3, containing 1000 mM KCl, lung extract and distilled water in a volume of 1.0 ml. Lung extract in a volume of 50 µl or less was added to initiate the reaction and reaction mixtures were incubated for 30 min at 37°C. After the reaction was stopped by adding 2 ml of 100 mM N-(2-hydroxy ethyl)-piperazine-N′-2-ethane sulfonic acid (HEPES), pH 9.0, containing 2.5 mM EDTA, 1 ml of 136 mM cyanuric chloride prepared in 1,4-dioxane was added to the reaction mixture and mixed vigorously by vortex mixing for 15 s. The absorbance of the yellow colour that developed was measured at 405 nm. The specificity of the reaction for ACE was tested by adding 10 µl of 10 µM captopril to the incubation mixture. Under the assay conditions captopril blocked 99 % of ACE activity. One unit of ACE was defined as the amount of enzyme catalysing the release of 1 n mole of hippuric acid from HHL per min at 37°C. Under the assay conditions 25 µl of lung extract (100–120 µg protein) released 150 n moles of hippuric acid equivalent to an absorbance of 0.6 from HHL.

ACE inhibitory activity was measured by including suitable aliquots of banana extracts in the assay system. Reduction in ACE activity was the measure of inhibition. One unit of inhibitory activity was the amount that suppressed enzyme activity by one unit.

To study the effect of temperature on the inhibitory activity of poovan, 3 ml of ripened or unripened poovan banana extract was exposed to temperatures of 70°C and 100°C for 10 min. After cooling, aliquots were assayed for inhibitory activity.

Effect of pH on the inhibitory activity of poovan was carried out by incubating ripened and unripened poovan banana extract (volume: 0.1 ml) with 0.1 ml of different buffers for 48 h at 4°C: 0.1 M HCl (pH 1.0); 0.1 M HCl/KCl (pH 2.0); 0.1 M acetate (pH 4.5); 0.1 M borate (pH 7.0 and 8.0); 0.1 M bicarbonate (pH 10.0) and 0.2 M NaOH (pH 12.5). After incubation, 0.4 ml of 200 mM borate buffer, pH 8.3, was added and the extract was assayed for inhibitory activity.

Four ml of ripened and unripened banana extracts were dialysed (MWCO 12–14,000) for 12 h against 200 ml of 50 mM borate buffer, pH 8.3, at 4°C and dialysate and outer solution were assayed for inhibitory activity. Controls without banana extract were run simultaneously in all cases.

Crude extracts of six ripened and unripened banana cultivars were found to inhibit ACE. The data on inhibition of ACE activity by different ripened and unripened banana extracts are summarized in Table 1. Ripened nendran, rasthali, poovan, robusta, bontha and safed velchi strongly inhibited ACE. But all six unripened banana cultivars showed weak inhibition against ACE.

Among the ripened bananas, nendran showed highest inhibition of ACE. Rasthali, poovan and robusta affected ACE activity to some extent but bontha and safed velchi affected moderately. Regarding the inhibition of ACE by different unripened banana cultivars, nendran showed strong inhibition of ACE. Rasthali, poovan, bontha and safed velchi affected ACE activity equally and robusta affected moderately. Further, inhibition of ACE by ripened nendran was two times higher than the rest of the ripened bananas. Unripened nendran was three times more potent in blocking ACE activity compared to the remaining unripened banana cultivars. The greater inhibition of ACE by both ripened and unripened nendran indicates that it is the best source of ACE inhibitors.

Poovan was chosen for further studies because of its availability round the year. The inhibitory effect of poovan banana extract on ACE is shown in Figure 1. Inhibition of ACE by ripened poovan was linear upto 80% and complete inhibition was obtained. In contrast, inhibition of ACE by unripened poovan was linear upto 60%, beyond this the inhibition was sluggish and maximum inhibition obtained at high concentration of banana extract was only 90%. The amount of banana extracts required to cause 50% inhibition of ACE activity are listed in Table 2. These values substantiate conclusions

Table 1. Inhibition of angiotensin converting enzyme activity by banana extracts

Banana cultivar	Ripened banana extract (Inhibitory units/ml)	Unripened banana extract (Inhibitory units/ml)
Nendran	41.00 ± 2.30	30.00 ± 1.50
Rasthali	23.00 ± 1.50	10.00 ± 0.80
Poovan	22.00 ± 2.00	10.00 ± 0.70
Robusta	22.00 ± 1.50	7.50 ± 0.40
Bontha	20.50 ± 1.00	10.00 ± 0.80
Safed velchi	19.00 ± 0.90	9.00 ± 0.00

Data are means ± SE of five experiments.

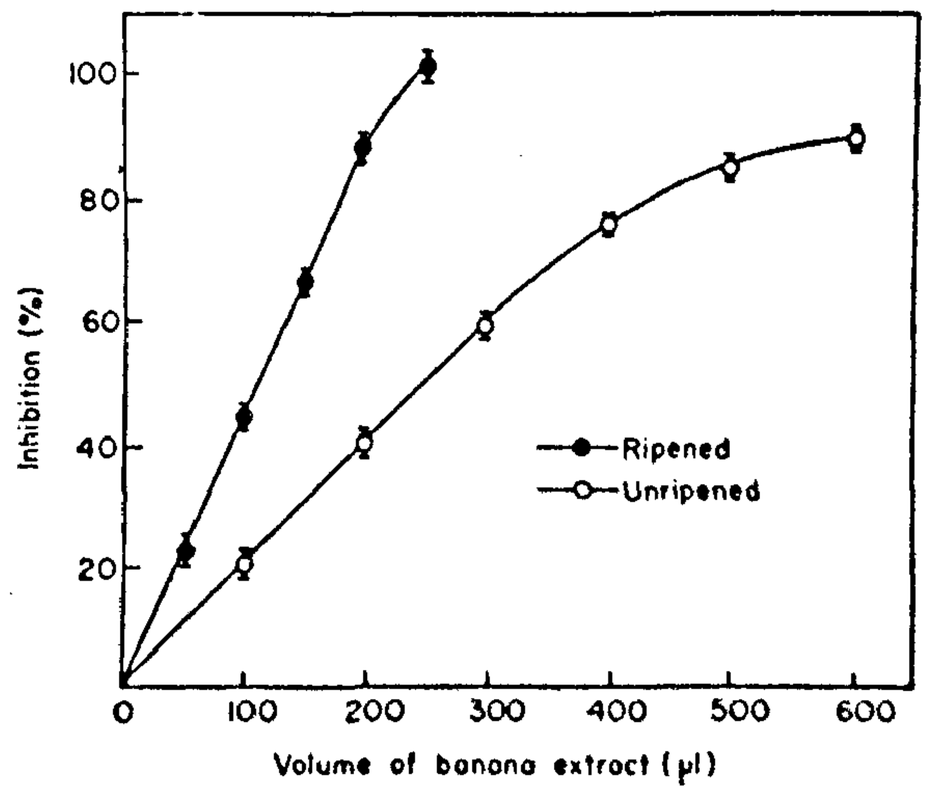

Figure 1. Inhibitory effect of poovan extract on angiotensin converting enzyme. Data are mean ± SE of three experiments.

Table 2. Banana extract required to cause 50 % inhibition of angiotensin converting enzyme activity

Banana cultivar	Banana extract (µl)	
	Ripened	Unripened
Nendran	55.00 ± 3.00	80.00 ± 4.00
Rasthali	110.00 ± 4.00	250.00 ± 15.00
Poovan	120.00 ± 9.00	230.00 ± 12.00
Robusta	115.00 ± 6.60	335.00 ± 25.00
Bontha	125.00 ± 8.75	250.00 ± 15.00
Safed velchi	130.00 ± 5.00	275.00 ± 15.00

Data are mean ± SE of five experiments.

Table 3. Effect of dialysis on inhibition of angiotensin converting enzyme activity by banana extracts

Banana cultivar	Ripened banana extract		Unripened banana extract	
	Native extract IU*/ml	Dialysate IU*/ml	Native extract IU*/ml	Dialysate IU*/ml
Nendran	40.00 ± 3.50	10.00 ± 0.60	27.00 ± 1.50	9.50 ± 0.50
Poovan	20.00 ± 1.50	5.00 ± 0.40	9.00 ± 0.50	3.00 ± 0.20
Safed velchi	18.00 ± 1.00	6.00 ± 0.20	9.00 ± 0.60	2.50 ± 0.15

*Inhibitory units.
Data are mean ± SE of three experiments.

reached based on linear range of inhibition for different ripened and unripened banana cultivars (Table 1).

ACE inhibitory activity of ripened and unripened poovan was highly heat stable. No loss of inhibitory activity was observed even after exposure to 100°C for 10 min. The ACE inhibitory activity of ripened and unripened poovan was stable to both extreme acidic pH and extreme alkaline pH. The data on the effect of dialysis on ACE inhibitory activity of ripened and unripened banana cultivars are presented in Table 3. The ACE inhibitory activity of dialysed ripened and unripened banana extracts was only 25 % and 33 % respectively of native extracts. The outer solutions were found to contain ACE inhibitory activity which was not quantitated.

This report establishes the presence of ACE inhibitors in six ripened and unripened banana cultivars. The crude extracts of ripened and unripened bananas inhibited ACE like crude extract from *Hibiscus sabdariffa*[19]. Our study suggests that reported antihypertensive effect of ripened banana may be due to ACE inhibitors[15]. It was reported that protease inhibitory activity of ripened poovan was unstable to alkaline pH[12]. Therefore, the stability of ACE inhibitory activity and unstability of protease inhibitory activity of ripened poovan to alkaline pH strongly suggests that inhibitory factors of ACE and proteases were different in banana. Captopril, an antihypertensive agent is a -SH containing ACE inhibitor[2]. The stability of banana inhibitors to alkali which would not be expected of a thiol-dependent compound suggest that banana inhibitors do not inhibit ACE by a mechanism involving the -SH group of the inhibitor[20]. Since unripened bananas are consumed as vegetables, the stability of banana ACE inhibitors to heat indicates that cooking may not destroy the antihypertensive action of bananas. Further, the stability of banana ACE inhibitors to extreme acidic pH indicates that they are stable to the acidic environment in the stomach and may be effective in controlling hypertension after absorption. In a study involving human volunteers, we observed 10 % fall in blood pressure after consumption of two bananas (pooven) per day for a week. Hence, consumption of single banana per day may not produce a fall in the blood pressure. Our findings suggest that consumption of bananas may prove beneficial to hypertensive individuals. However, further investigations using isolated compounds are required to confirm antihypertensive action of bananas.

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