The Increase in Alcohol Dehydrogenase Activity and Ethanol Content during Ripening of Banana Fruit

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Summary
Alcohol dehydrogenase (ADH, Alcohol: NAD⁺ oxidoreductase, Ec 1.1.1.1) activity markedly increased during ripening of banana fruit in association with the rise in ethylene production and the respiration climacteric. The marked rise in alcohol dehydrogenase activity was followed by a sharp rise in ethanol formation in the tissue.

Introduction
Marked changes in metabolism occur during ripening of fruit(9,10,11), i.e. rise in respiration, increase in ethylene production, starch breakdown to soluble sugars and acceleration of glycolysis. In banana fruits, rise in ethylene production is accompanied by rise in respiration, reaching a maximum (climacteric peak) and then declining(3). These increases in ethylene production and respiration are accompanied or followed by features of ripening such as sweetness and softening of flesh, yellowing of peel and production of aroma (8). In climacteric fruits such as banana, pear, avocado, apple, melon and tomato, the climacteric is considered as the stage which delineates between maturation and senescence(2). As senescence proceeds, the formation of eshanol is accelerated. Ethanol accumulates in postclimacteric banana fruits in a large amount(7).

In the present paper, we demonstrate the increasing patterns of alcohol dehydrogenase (ADH) activity and ethanol in the pulp tissue of banana fruit during ripening and senescence. We also report some enzymatic properties of ADH.

Materials and Methods
Green banana fruits (Musa AAA group, Cavendish subgroup cv. Giant Cavendish) gassed by application of about 300 µl/l ethylene for 1 day at 20°C were obtained from a local fruit company. They were allowed to ripen in the laboratory at 25°C in a flowing stream of humidified air at RH 90%. During the incubation period ethylene production and respiration (CO₂ evolution) increased and reached a maximum on day 6 (5). Ripening was assessed by scoring changes in peel color, the extent of appearance of brown spotting and flesh firmness, rating from 1 (all green) to 8 (overripe) as described elsewhere (5).

At appropriate intervals during the course of ripening, the pulp tissue was extracted for determination of ADH activity and ethanol accumulation. Cross-sectional slices (5 g) of flesh were ground in 30 ml of 0.02 M Tris-HCl buffer, pH 7.5, containing 1 mM dithiothreitol and 0.5 g Polyclar AT with a mortar and pestle in ice bath. The homogenate was centrifuged at 8,000 x g for 20 min at 2°C. The supernatant was passed through a Sephadex G-25 column and the protein fraction collected was used for ADH assay. The presence of dithiothreitol in the extraction and elution buffer and removal of low molecular weight compounds by Sephadex G-25 column were prerequisite for obtaining the stable ADH activity. ADH reaction was started with addition of 0.1 ml
of enzyme preparation to the reaction medium consisting of 0.02 M Na-pyrophosphate buffer, pH 7.5, 0.1 M acetaldehyde and 0.13 mM NADH in a total of 3 ml, and the decrease in absorbance at 340 nm at 22°C was recorded. As for measuring the oxidation of ethanol, 0.1 ml of enzyme preparation was added to the reaction medium consisting of 0.02 M Na-pyrophosphate buffer, pH 9.5, 0.1 M ethanol and 0.5 mM NADH in a total of 3 ml. The readings of the increase in absorbance at 340 nm at 22°C were taken. Enzyme activity was expressed as μmol of NADH formed or oxidized per min per g fresh weight of pulp tissue.

Another slice of flesh weighing 5 g was ground in 30 ml of H2O with a mortar and pestle in ice bath. The homogenate was centrifuged at 8,000 × g for 20 min at 2°C. The ethanol content in the supernatant was assayed by gas chromatography according to the method described by Davis and Chace(4). The glass column containing 25% polyethylene glycol 20 M Chromosorb W was used.

Results and Discussion

Fig. 1 shows increasing patterns of ADH, ethanol and color score. During ripening of banana fruit as represented by development of color index and symptom of senescence, there was a marked increase in ADH activity followed by a sharp rise in ethanol content. After 2 days of storage at 25°C, ADH activity began to increase at a rapid rate, reached a peak on the 6th day and then declined. The increase in ADH activity preceded the rise in ethanol formation. Ethanol was accumulated in a large amount at the overripe stage. The amount of acetaldehyde in the fruit also increased during ripening (data not shown). Skakoun and Daussant(14) reported that ADH increased in activity much higher in postclimacteric banana fruit than in preclimacteric fruit. They also found using an immunochemical method that there was an increase in ADH protein in parallel with that in ADH activity, which may indicate that the rise in ADH

![Graph of Results and Discussion](image-url)
activity results from increased synthesis of ADH protein.

Optimum pHs of ADH were 7.5 and 9.5 for reduction of acetaldehyde with NADH and for oxidation of ethanol with NAD\(^+\), respectively (Fig. 2). At pH 7.0, the rate of reduction of acetaldehyde was about 15 times that of oxidation of ethanol, suggesting that the reaction is highly favorable for ethanol formation in the tissue. Km values for ethanol and NAD\(^+\) at pH 9.5 were 3.6 mM and 44 \(\mu\)M, respectively. ADH from banana fruit was highly specific for ethanol and \(n\)-propanol, followed by \(n\)-butanol and \(n\)-pentanol. It showed no activity for methanol and isopentanol. The enzyme activity was strongly inhibited by Hg\(^{2+}\), \(p\)-chloromercuribenzoate and moniodoacetate, which suggests that sulfhydryl groups are responsible for the active site. ADH was partially purified by DEAE-cellulose column chromatography. A single peak having ADH activity was obtained. Recently Igaue and Yagi(6) isolated and highly purified ADH from cultured rice cells. Its molecular weight was 76,000, consisting of two subunits. The rice enzyme contained zinc, and sulfhydryl groups were essential for the enzyme activity.

Degradation of sugars by glycolysis is accelerated in banana fruit during ripening. Barker and Solomos(1) showed that there was a 20-fold increase in the content of fructose 1,6-diphosphate in association with the 4- to 5-fold increase in rate of respiration. Salminen and Young (12, 13) examined changes in levels of glycolytic intermediates between preclimacteric and climacteric peak stage. They showed that there was the greatest change in the level of fructose 1,6-diphosphate. A crossover plot based on the changes in the levels of some glycolytic intermediates indicated that the crossover point was between fructose 6-phosphate and fructose 1,6-diphosphate. They also found that the activity of phosphofructokinase increased 2.5-fold(13).

We examined the change in activity of pyruvate decarboxylase and for the preliminary results found that there was little change in the activity during the course of ripening, although acetaldehyde content increased in the tissue. It appears that pyruvate decarboxylase is not rate-limiting and that the induction of ADH is largely responsible for the formation of ethanol in banana fruit. The rate of ethylene production increases until fruits reach the climacteric. It may be probable that the accumulation of high concentrations of ethylene in the tissue has something to do with the induction and formation of ADH.

**Literature Cited**


バナナ果実追熟中のアルコール脱水素酵素の活性増大及びエタノールの増加

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摘 要

バナナ果肉中のアルコール脱水素酵素の活性は、追熟に伴って、エチレンの生成や呼吸の増大と共に、著しく增大した。それに伴って果肉中のエタノール含量は著著に増加した。バナナ果肉のアルコール脱水素酵素の最適pH はアセトアルデヒドの NADH による還元では 7.5、一方エタノールの NAD⁺ による酸化は 9.5 であった。また pH 7.0 では、アセトアルデヒドの還元の速度がエタノールの酸化の速度より 15 倍も速かった。これらのことばはバナナ果肉中では、アルコール脱水素酵素のエタノール生成の方向に有利に働いていると考えられる。バナナ果肉は、追熟に伴い、解糖系による糖の分解が促進される。特にフルクトース 1,6-二リン酸の増加が著しい。ビルピン酸デカルボキシラーゼの活性はほとんど変化はみられなかった。したがって追熟に伴う果肉中のエタノールの増加は、アルコール脱水素酵素の活性増大が大きな起因をなしていると考えられる。