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Biochemical Changes in Sugars and Cell Wall Degrading Enzymes during Ripening of Banana

- Research Note -

Min-Kyung Lee, Mi-Jeong Kim and Inshik Park†

Division of Food Science, Dong-A University, Busan 604-714, Korea

Abstract

Changes in reducing sugar and cell wall degrading enzymes during ripening of banana for 10 days were investigated. The amount of reducing sugar in bananas increased during storage at room temperature during the first 7 days, and decreased thereafter. However, starch content in banana decreased during ripening, and invertase and cell wall degrading enzymes such as cellulase, polygalacturonase and xylanase were most active after bananas were stored for 7 days at room temperature. When the bananas were stored at 4°C, the magnitude of changes were much less than during room temperature storage.

Key words: banana, reducing sugar, cell wall degrading enzymes

INTRODUCTION

Ripening of fruit is associated with textural changes and extensive softening of the tissues (1). Banana ripening is characterized by a peel color change from green to yellow and the softening of the pulp and peel (2). Biochemical, physiological and compositional changes associated with ripening, resulting in softening of bananas, have been reviewed extensively (3). Microscopic examinations show cell wall modifications in cells of the pulp during banana ripening (4). Unripe banana fruit has almost 20% starch which disappears during the ripening process concomitantly with sucrose accumulation (5). Subsequent studies demonstrated that the fluctuations in the sucrose/reducing sugar content in the flavedo cells resulted from changes in the activity of a soluble acid invertase, which develops during the winter but disappears in the spring (6). In this study, we investigated changes in the content of reducing sugar, invertase activity, and cell wall degradation enzymes such as cellulase, polygalacturonase and xylanase during ripening of banana fruit.

MATERIALS AND METHODS

Preparation of samples

A batch of bananas were obtained from a local market at the mature green stage. Carboxymethyl cellulose, xylan, polygalacturonic acid, and sucrose were obtained from Sigma Chemical Co. Two batches of bananas were allowed to ripen at either room temperature or 4°C. Two bananas were sampled every 2 or 3 days during ripening and homogenized in 50 mM phosphate buffer at pH 6.8 for 3 min. The homogenate was centrifuged at 15,000 ×g for 20 min, and the supernatant was collected. All steps were carried out at 4°C.

Measurement of reducing sugars and starch, and invertase activity

Reducing sugars were measured according to the method of Miller (7) using dinitrosalicylic acid (DNS). The starch content during ripening was measured by an iodometric assay method (8). Invertase activity was measured as described by Moriguchi et al. (9) with slight modifications. The assay mixture contained acetate buffer (100 mM, pH 4.5), sucrose (100 mM) and the enzyme preparation in a total volume of 1.0 mL. The reaction mixture was incubated for 30 min at 37°C. The amount of reducing sugar released was calculated from a calibration curve drawn using glucose as the standard. One unit of invertase activity was defined as 1 μmol of reducing sugar released per min at 37°C.

Measurement of polygalacturonase activity

Polygalacturonase activity was assayed by measuring the formation of reducing groups using the methods of Nelson and Somogyi (10,11). The reaction mixture contained 0.5 mL acetate buffer (100 mM, pH 4.5), 0.1 mL NaCl (200 mM), 0.3 mL polygalacturonic acid (1%, pH 4.5), and 0.1 mL of enzyme. The reaction mixture was incubated for 1 hr at 37°C. The formation of reducing groups was calculated using D-galacturonic acid as a standard. One unit of polygalacturonase activity is defined as the amount of enzyme producing 1 μmol of reduc-

†Corresponding author. E-mail: ispark@donga.ac.kr
Phone: +82-51-200-7322, Fax: +82-51-200-7535

ing groups per a minute at 37°C.

Measurement of cellulase activity

Cellulase activity was measured as described by Ahmed and Labavitch (12) with slight modifications. The reaction mixture consisted of acetate buffer (100 mM, pH 5.0), carboxy methyl cellulose (1.5% w/v) and enzyme in a final volume of 1.0 mL. The reaction mixture was incubated at 37°C for 16 hr. The amount of reducing sugar released was calculated from the calibration graph. One unit of cellulase activity was defined as the amount of enzyme liberating 1 μmol of reducing sugar per 1 hr at 37°C.

Measurement of xylanase activity

Xylanase activity was assayed as described by Singh and Singh (13) with slight modifications. The assay mixture consisted of acetate buffer (100 mM, pH 5.0), xylan (0.1%) and enzyme preparation in a total volume of 1.0 mL. The mixture was incubated for 1 hr at 37°C. The released reducing sugars were measured using DNS as for invertase (described above). One unit of xylanase activity was defined as 1 μmol of reducing sugar released per a minute at 37°C.

RESULTS AND DISCUSSION

Fig. 1 shows the changes in reducing sugar and starch contents during ripening of banana fruit when stored at room temperature or 4°C for 10 days. Reducing sugar content increased during storage at room temperature for the first 7 days, and then decreased thereafter. The production of reducing sugar was much higher when stored at room temperature than at 4°C. The starch content of the green bananas, which was 12% (w/w) before storage, decreased during storage.

Changes in the activities of three cell wall degrading enzymes and invertase during ripening of banana fruit at room temperature and 4°C for 10 days are shown in Fig. 2 and Fig. 3, respectively. The 100% relative activities for each enzyme were 1.52, 0.96, 0.81, and 0.05 units/mL/min for invertase, polygalacturonase, xylanase and cellulase, respectively. The changes in cell wall degrading enzyme activities were also observed during storage at 4°C, but the magnitude of change was much lower

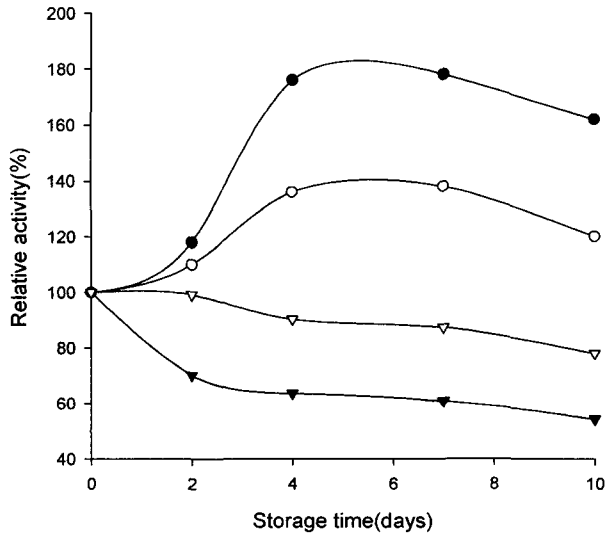

Fig. 1. Changes in reducing sugar and starch contents during ripening of banana fruit for 10 days.
Reducing sugar at room temperature (●); Reducing sugar at 4°C (○); Starch at room temperature (▼); Starch at 4°C (▽).

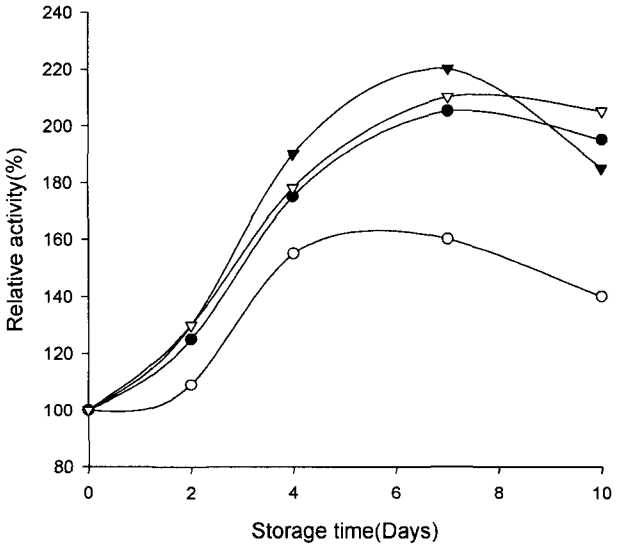

Fig. 2. Changes in cell wall degrading enzyme and invertase activities during ripening of banana fruit at room temperature for 10 days.
Invertase (●); Polygalacturonase (○); Cellulase (▼); Xylanase (▽).

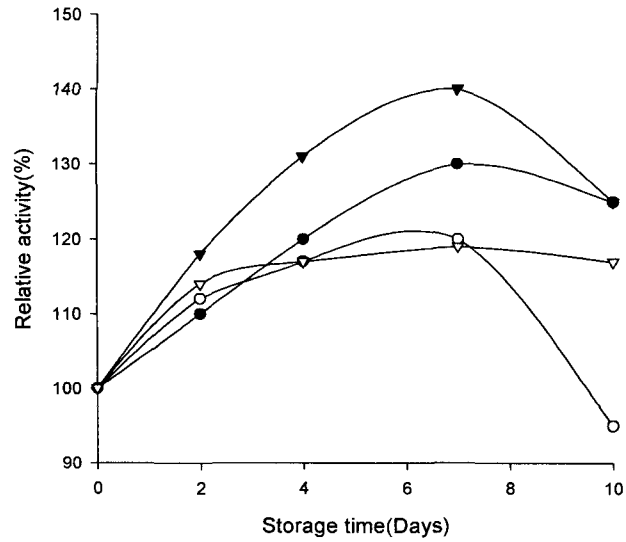

Fig. 3. Changes in cell wall degrading enzyme and invertase activities during ripening of banana fruit at 4°C for 10 days.
Invertase (●); Polygalacturonase (○); Cellulase (▼); Xylanase (▽).

than at room temperature storage. The data on cell wall degrading enzymes demonstrated that banana ripening was accompanied by an increase in all the three cell wall degrading enzymes, namely, cellulase, polygalacturonase, xylanase, during the first 7 days. Albert and John (14) reported that invertase activity increased in grapefruit flavedo tissue stored at 5°C and reducing sugar levels paralleled invertase activity while sucrose levels were inversely related to invertase levels. Manoj (15) suggested that banana ripening was accompanied by an increase in reducing sugar content and invertase activity concomitant with decreases in non-reducing sugar content. This accumulation of reducing sugars may be due to increased breakdown of starch during ripening as reported by Beaudry et al. (16). Priscila et al. (17) reported that mature green banana fruit can have a starch content as high as 20%, which is degraded during the ripening period in a complex process. Polygalacturonase is reported to be primarily responsible for ripening associated with pectin degradation and fruit softening (18). It has also been reported that carboxymethyl cellulase activity increases during ripening of tomato, strawberry, pear, peach and avocado (19).

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