Seasonal Changes in α - and β -Amylase Activities in Relation to Starch Content in Kiwifruit

Toshiyuki MATSUI*, Hirotoshi KITAGAWA* and Shinichi DOI**

* *Department of Agroindustrial Science, Faculty of Agriculture,
Kagawa University, Miki-cho, Kagawa 761-07*

** *Agricultural Experiment Station of Kagawa Prefecture,
Fuchu Branch, Fuchu-cho, Sakaide, Kagawa 762*

This paper describes the seasonal changes in starch content, and α - and β -amylase activities of two cultivars of kiwifruit, 'Koryoku' and 'Hayward'. The main purpose of the study was to investigate the seasonal changes in starch content in relation to α - and β -amylase activities and also to examine the properties of these two enzymes in both cultivars. The highest activity of α -amylase in the insoluble fraction was observed in September for 'Koryoku' and in October for 'Hayward', whereas that of β -amylase in the same fraction was observed in October for both cultivars. Both enzyme activities were higher in the insoluble than in the soluble fraction. The highest starch content in 'Koryoku' was observed in the middle of October, while in 'Hayward' it was observed in the later part of October or early November. In both cultivars, α -amylase activity peaked one month earlier than starch content.

Since it is difficult to judge maturity of kiwifruit by appearance, Brix degree is used as an indicator of the optimum time to harvest. But this method could give erroneous values in that these do not necessarily reflect the starch content. HARMAN¹⁾ showed that the optimum harvest index of kiwifruit for export in New Zealand was between 6.2% and 7.5% soluble solids, while OKUSE *et al.*²⁾ and several workers³⁾⁻⁶⁾ analyzed starch and sugar contents during the growth period of kiwifruit. However, no reports have been published as to the relationships between starch content and amylase activities in this fruit. In this study, the seasonal changes were investigated in α - and β -amylase activities in relation to starch content in kiwifruits.

Materials and Methods

Materials

Kiwifruit cultivars Koryoku and Hayward were harvested in the fields of the Agricultural Experiment Station of Kagawa Prefecture

located in Fuchu-cho, Sakaide, Kagawa Prefecture. Samples were collected on the 17th of each month from June to October, and on the 30th of October, 1986. Fifteen fruits were harvested at random.

Methods

Enzyme extraction: The fruit pulp was grated using a cooled plastic grater and mixed with 15% polyclar AT (w/w) and added with chilled water, twice the amount of sample (w/v). The resulting homogenate was filtered through 4 layers of cotton cloth and the filtrate was centrifuged at 10 000 G for 10 min after adjusting to pH 6.0 with 1 N ammonia solution. The residue was rinsed with the same amount of water and centrifuged under the same condition. The total supernatant was dialyzed with 5 mM phosphate-citrate buffer (pH 7.0) for 12 h in Visking tube (size 36/32, 0.025 mm in thickness) and the inner solution was designated as soluble fraction (S.f.). On the other hand, the residue was re-extracted with 0.2 M phosphate-citrate

buffer (pH 7.0), twice the amount of sample for 12 h with occasional stirring. The resulting homogenate was centrifuged at 10 000 G for 10 min. The supernatant was dialyzed in the same way and the inner solution was designated as insoluble fraction (Is.f.). The extractions were carried out at 0–4°C.

Enzyme assay : The standard assay medium for α -amylase consisted of 0.2 ml of 0.004 M phosphate-citrate buffer (pH 7.0) containing 0.4 mg soluble starch, 0.1 ml of crude enzyme and 0.1 ml of water. The reaction mixture was incubated at 30°C for 10 min, followed by mixing with 0.2 ml of 0.1 M HCl for stopping the enzyme reaction, and added with 1 ml of iodine solution (the stock solution contains 500 mg of iodine and 5 g of potassium iodide filled up to 100 ml with water. For daily use, it was diluted 10 times with water.⁷⁾). After 10 min, the colored solution was centrifuged at 3 000 rpm for 10 min to remove unreacting starch and measured at 660 nm against the mixture of iodine solution and 0.7 ml of water. In the case of β -amylase, the assay medium consisted of 0.2 ml of 0.02 M phosphate-citrate buffer (pH 7.0) containing 2 mg of soluble starch, 0.2 ml of 0.2 M acetate buffer (pH 4.5) and 0.1 ml of crude enzyme. The reaction mixture was incubated at 30°C for 15 min⁷⁾. After neutralization, the reducing sugar was estimated by the method of SOMOGYI⁸⁾. Soluble protein content was determined according to the method of LOWRY *et al.*⁹⁾. Both enzyme activities were presented as the amount of maltose liberated per min per mg of protein.

Partial purification of α - and β -amylase :

To the insoluble fraction described above, powdered ammonium sulfate was added to give 80% saturation. The precipitate was collected by centrifugation at 10 000 G for 10 min and it was dissolved in a small amount of 50 mM sodium phosphate buffer (pH 7.4) and dialyzed against the same buffer for 12 h. The dialyzed enzyme was centrifuged at 10 000 G for 15 min and the resulting enzyme solution was applied to a Sephacry S-200 superfine column (2.3 i.d. $\times$ 53 cm) which had been equilibrated with

50 mM sodium phosphate buffer (pH 7.4). The column was eluted with the same buffer at a flow rate of 15 to 20 ml/h. Each 5 ml fraction was collected, monitored at 280 nm for protein, and α - and β -amylases were assayed (c.f. enzyme assay described above).

Determination of starch : One gram of peeled sample was homogenized in a POTTER's homogenizer with 10 ml of water. The homogenate was centrifuged at 3 000 rpm for 10 min and the supernatant was discarded. The residue was rinsed and centrifuged with 5 ml of water to remove soluble sugar. The residue was added with 100 ml of 0.75 N HCl and the mixture was boiled in a water bath for 2.5 h under a reflux condenser. The solution was neutralized and made up to 250 ml with water and filtered. The reducing sugar was determined by the SOMOGYI method and the amount of starch was given as the glucose value multiplied by 0.9¹⁰⁾.

Result and Discussion

Properties of α - and β -amylase in kiwi-

Fig. 1 Optimum pH (A) and temperature (B) for the activities of α -amylase in kiwifruit cv. Hayward

(A) The reaction mixtures containing 0.2 ml of 0.004 M phosphate-citrate buffer (pH 7.0), 0.4 mg soluble starch, 0.1 ml of crude enzyme and 0.1 ml of water were incubated at 30°C for 10 min. The activity at pH 6.0 was taken as 100%. (B) The assay conditions were the same as (A) except for the reaction temperature. The activity at 50°C was taken as 100%.

fruit : Figs. 1 and 2 show the optimum pH (A) and temperature (B) for the activity of partially purified α - and β -amylase of kiwifruit cv. Hayward, respectively. α - and β -amylase activities had the same pH optima at 6.0 and these had optimum temperatures of 50 and 35 °C, respectively. The optimum pH value for α -amylase of rye is reported to be 3.3–4.0, whereas that for β -amylase of sweet potato and wheat malt are reported to be 4.0–5.0 and 5.2–5.3, respectively¹¹⁾. The optimum pH for β -amylase in kiwifruit seems to be the same as that in soybean¹²⁾ but the optimum pH for α -amylase in kiwifruit was higher than that in rye and wheat. PRONIN and DOKER suggested that an increase in temperature shifts the pH optimum of β -amylase toward higher pH values¹²⁾. When the pH is measured in temperatures higher than 30°C, the optimum pH may shift near neutrality. The optimum temperature for α -amylase of kiwifruit was the same as that of fungus, whereas that for β -amylase

Fig. 2 Optimum pH (A) and temperature (B) for the activities of β -amylase in kiwifruit cv. Hayward

(A) The reaction mixtures containing 0.2 ml of 0.02 M phosphate-citrate buffer (pH 7.0), 2 mg of soluble starch, 0.2 ml of 0.2 M acetate buffer (pH 4.5) and 0.1 ml of crude enzyme were incubated at 30°C for 15 min. The activity at pH 6.0 was taken as 100%. (B) The assay conditions were the same as (A) except for the reaction temperature. The activity at 35°C was taken as 100%.

of kiwifruit was lower than that of tapioka and wheat. Fig. 3 shows the k_m values of α - and β -amylase for soluble starch in kiwifruit cv. Hayward. The k_m values of α - and β -amylase for soluble starch were 44.0 and 18.4 mg/ml, respectively. These values are comparable with the reported ones ; 0.6¹³⁾ and 1.0¹⁴⁾ mg/ml of α -amylase for soluble starch and 7.3×10^{-5} M¹⁵⁾ of β -amylase for amylose.

Seasonal changes in α - and β -amylase activities in kiwifruit : The activities of α - and β -amylase in two cultivars are shown in Figs. 4 and 5. The α -amylase activity in the soluble fraction for the two cultivars was almost constant throughout the experimental period. The activity in the insoluble fraction was highest in September for 'Koryoku' and in October for Hayward. The early-ripening cultivar Koryoku reached the highest activity one month earlier than late-ripening cultivar, Hayward. In September or October, α -amylase activity in the insoluble fraction for the two cultivars was about 6 to 7 times as high as that in the soluble fraction. On the other hand, no difference in the β -amylase activity was observed in the two cultivars. Its activity in the soluble fraction was almost constant before September in both cultivars and then increased rapidly. In the insoluble fraction, it

Fig. 3 Reciprocal plot showing the effects of soluble starch concentration on α -(A) and β -amylase (B) in kiwifruit cv. Hayward

The assay conditions were the same as in Fig. 1.

increased slightly in September and then rapidly in October. The β -amylase activity in the insoluble fraction was about 5 times as high as that in soluble fraction.

Seasonal changes in starch content of kiwifruit: The seasonal changes in weight and

Fig. 4 Seasonal changes in α -amylase activity in the soluble fraction (A) and insoluble fraction (B) of the two cultivars of kiwifruit

Samples were collected on the 17th of each month from June to October, and on the 30th of October. ●—●, 'Koryoku'; ○---○, 'Hayward'. Vertical bars represent the SE of means of three determinations using three fruits.

Fig. 5 Seasonal changes in β -amylase activity in the soluble fraction (A) and insoluble fraction (B) of the two cultivars of kiwifruit

Symbols as shown in Fig. 4.

Fig. 6 Seasonal changes in weight (A) and starch content (B) of the two cultivars of kiwifruit

(A) Values are means with SE ($n=15$).

(B) Values are means with SE ($n=3$).

Symbols as shown in Fig. 4.

starch content of the two cultivars of kiwifruit are shown in Fig. 6. The fruit weight of 'Hayward' was apt to be higher than that of 'Koryoku'. The starch content of 'Hayward' and 'Koryoku' was around 6% and 8%, respectively, based on fresh weight. Fruits from the same trees had starch content of around 9.9% and 10.5%, respectively, in 1987¹⁶⁾. These values are comparable to the reported ones; around 6.2%⁵⁾ and 9.0%⁴⁾ for 'Hayward' and around 5.8%⁵⁾, 8.2%⁴⁾ and 8.3%⁶⁾ for early-ripening 'Monty', a cultivar similar to 'Koryoku'. These varying values indicate that the starch content of kiwifruit depends on many factors including time of flowering, climatic condition during growth and orchard¹⁾.

The starch content of 'Koryoku' was highest at the middle of October and decreased by 1% at the end of that month. In contrast, the starch content of 'Hayward' continued to increase until the end of October. The highest starch content was reported to be at the 10th of November¹⁶⁾. The period of highest α -amylase activity did not coincide with the time of highest starch content. α -Amylase activity in October was almost the same as in June or July in spite of the high starch content. The highest starch content observed did not always

fall within the 6.2–7.5% soluble solids reading used as the suitable harvest time¹⁾. On the other hand, β -amylase activity in 'Hayward' followed the pattern of increasing starch content but that in 'Koryoku' did not.

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キウイフルーツ果実の α -並びに β -アミラーゼ活性の 季節変化とデンプン含量との関係

松井年行*・北川博敏*・土居新一**

* 香川大学農学部農業生産学科

(〒761-07 香川県木田郡三木町池戸)

** 香川県農業試験場府中分場 (〒762 坂出市府中町)

本研究は、キウイフルーツの生長と成熟の指標を明らかにするため、主要成分であるデンプンと関連酵素活性やその性質について検討したものである。すなわち、'香緑'と'ヘイワード'種の2品種の果実の α -並びに β -アミラーゼ活性とデンプン含量の季節変化並びにこれら酵素の性質との関連性を調べ、次のような結果が得られた。不溶性画分の α -アミラーゼの最大活性は、'香緑'種で9月、'ヘイワード'種で10月に認められた。一方、同一画分の β -アミラーゼの場合は、両品種とも10月に最大活性が認められた。また、両活性とも可溶性画分よりも不溶性画分において活性が高かった。'香緑'種のデンプンの最大含量を示す時期は10月中旬、'ヘイワード'種では10月下旬から11月下旬であった。両品種とも、 α -アミラーゼ活性はデンプン含量が最大値の時期より1カ月早くピークを示した。