

CHANGES IN POLYGALACTURONASE AND α -AMYLASE
ACTIVITIES IN MELON FRUIT

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This paper reported the changes in total (TP) and water soluble pectin (WSP), and starch contents in three melon cultivars (*Cucumis melo* L.) in relation to polygalacturonase (PG) and α -amylase activities. The properties of these enzymes in 'Ginsen' were also determined. The PG activity in the water soluble fraction increased whereas that in the insoluble fraction decreased. However, there were no significant differences in the TP and WSP contents between each harvest period. The PG and α -amylase activities in both fractions, TP, WSP and starch contents cannot be used as indices of optimum harvest period in place of firmness.

The PG and α -amylase of 'Ginsen' had a molecular weight of ca. 24,000, and 52,000, optimum pH of ca. 5.5 and 5.0, and K_m value of 0.14 and 3.3 mg/ml for polygalacturonic acid and starch, respectively. Both enzymes had an optimum temperature of 45°C.

Key Words : total pectin, water soluble pectin, polygalacturonase, α -amylase, melon.

Introduction

Melon fruit is known to soften with ethylene treatment after harvest due to an enhancement of some degradative enzymes^(1,2). PG is found to decompose polygalacturonic acid and polygalacturonan into galacturonic acid and its derivative⁽³⁾ whereas α -amylase degrades starch into maltose and its derivative. The soluble solids content and firmness were used as maturity indices of melon fruit⁽⁴⁾. This study was therefore conducted to determine the changes in PG and α -amylase activities in relation to TP, WSP and starch contents of melons at each indicated suitable harvest date after anthesis.

Materials and Methods

Materials. The growing conditions were the same as in the previous paper⁽⁴⁾. The fruits were harvested at 5–10 days interval.

Enzyme extraction (PG and α -amylase). PG and α -amylase were extracted from about 10 g of fruit pulp, according to the methods of MATSUI and KITAGAWA^(1,2).

Enzyme assay. The assay media for PG and α -amylase were prepared as in the previous paper^(1,2).

Partial purification of PG and α -amylase. 'Ginsen' harvested at 20 days after anthesis was used for partial purification of PG and α -amylase as described in the previous paper^(1,2).

Estimation of the molecular weight of the enzyme by HPLC. The estimation procedure was carried out as described in the previous paper⁽⁵⁾. The elution volumes of the following molecular markers; cytochrome c (indicator protein, mol. wt. 1.25×10^4), albumin (bovine, mol. wt. 6.7×10^4) and aldolase (rabbit muscle, mol. wt. 1.6×10^5) were plotted against the

molecular weight of the marker.

Determination of total pectin (TP) and water soluble pectin (WSP). TP and WSP were analyzed according to the methods described in the previous paper^(1,2).

Determination of starch. Starch content was analyzed by the same method as in the previous paper⁽²⁾.

Measurement of Firmness. The fruit was cut longitudinally into two near the center. The firmness of pericarp at six points was measured using a penetrometer with a 5 mm conical plunger (Kiya Seisaku Co.Ltd).

Results and Discussion

Properties of polygalacturonase and α -amylase in melon fruits

1) Optimum pH and temperature. Fig. 1 shows the optimum pH (A) and temperature (B) for the activities of partially purified PG and α -amylase. The optimum pH for PG and α -amylase of 'Ginsen' was ca. 5.5 and ca. 5.0, respectively, and their optimum temperature was ca. 45°C. The optimum pH of PG in 'Ginsen' was the same as that of kiwifruit while the optimum temperature was slightly lower. On the other hand, the optimum pH and temperature of α -amylase in 'Ginsen' were slightly lower than that of kiwifruit.

2) Km value for galacturonic acid and soluble starch. The LINEWEAVER-BURK reciprocal

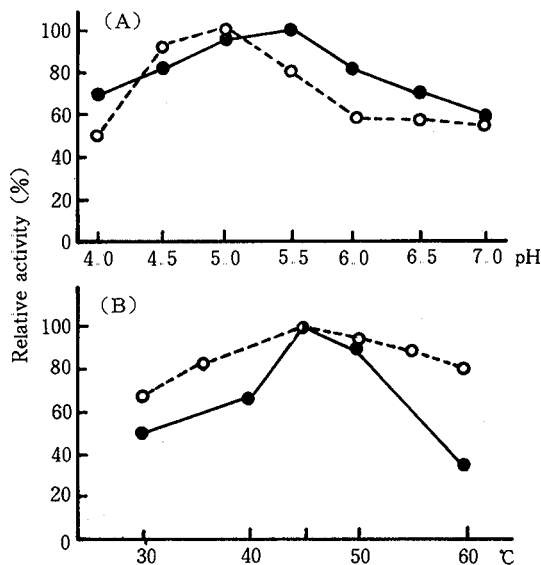


Fig.1 Optimum pH (A) and temperature (B) for the activities of polygalacturonase and α -amylase in melon cv Ginsen ●, polygalacturonase; ○, α -amylase.

(A) The activity at pH 5.0 (α -amylase) and pH 5.5 (PG) was taken as 100%.

(B) The activity at 45°C was taken as 100%

Values are means of duplicate assays.

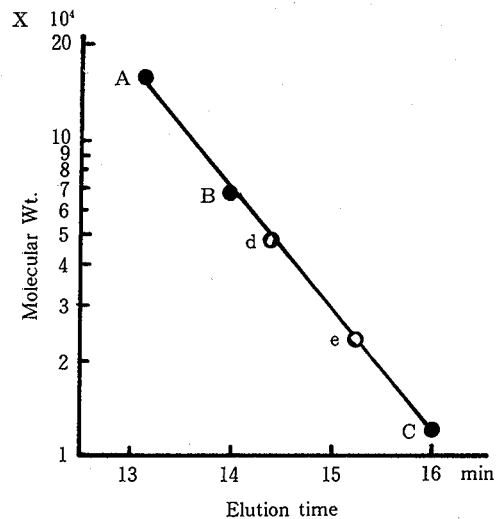


Fig.2 Molecular weight of polygalacturonase and α -amylase as determined by HPLC (Shim-pack Diol-150) Molecular weight makers; A, aldolase (1.6×10^5); B, albumin (6.7×10^4); C, cytochrome c (1.25×10^4) e and d indicate the molecular weight of polygalacturonase and α -amylase in melon cv. Ginsen, respectively.

plot for PG and α -amylase as a function of polygalacturonic acid and soluble starch concentrations were linear and the km value was 0.14 mg/ml at pH 5.0 and 45°C, and 3.3 mg/ml at pH 7.0 and 30°C, respectively.

3) **Molecular weight of PG and α -amylase.** The molecular weight of PG and α -amylase in 'Ginsen' was estimated as ca. 24,000 and 52,000, respectively by Shim-pack Diol (Fig. 2).

Changes in PG and α -amylase activities during growth of fruit. The suitable time to harvest 'Honey Dew', 'IW-57' and 'Ginsen' was reported⁽⁴⁾ to be about 50, 45 and 35 days after anthesis, respectively. The average weight of these cultivars at harvest was 1,400, 750 and 490 g, respectively. The PG activity in the water soluble fraction increased during maturity but this cannot be used as a maturity index. There were no significant differences in the PG activity in both the soluble and insoluble fractions between each harvest time⁽⁶⁾ (Table 1). On the other hand, α -amylase activity in both fractions was almost zero.

Changes in TP, WSP and starch contents, and firmness during growth of fruit. The TP content in melon fruit was higher than WSP in all harvest period. In 'IW-57', the TP was significantly higher than the WSP content at 40 days after anthesis. No significant differences in other cultivars were observed between 35 and 45 days after anthesis (Table 2). The highest TP content did not coincide with the period of highest PG activity. These indicate that PG activity is not closely related to softening of the fruits and TP and WSP contents are not suitable chemical indices of the harvest maturity. The starch content in melon appeared not to influence greatly the quality of the fruit since the increase in sucrose was less than 27 mg/100 g if the starch is completely converted to sucrose⁽⁷⁾ (Fig. 3). On the last harvest date, 'IW-57' was the most firm followed by 'Honey Dew' and 'Ginsen' (Fig. 3). The suitable firmness of fruit for consumption however, was changed when melon was treated with ethylene after harvest⁽⁴⁾.

Table 1. Changes in PG activity in the water soluble and insoluble fractions of three melon cultivars harvested at different days after anthesis*

Cultivars	Galacturonate (μ mol/min/mg protein)/Days after anthesis					
	10	20	30	35	40	45
	<u>Soluble fraction</u>					
'Honey Dew'	0.03 \pm 0.06 ^c	3.30 \pm 0.50 ^{ab}	4.00 \pm 0.06 ^a		3.87 \pm 0.18 ^{ab}	2.47 \pm 0.32 ^{ab}
'IW-57'	0.03 \pm 0.05 ^b	1.51 \pm 0.56 ^b	1.19 \pm 0.04 ^b	2.60 \pm 0.01 ^{ab}	4.22 \pm 0.64 ^a	1.99 \pm 0.33 ^{ab}
'Ginsen'	0.60 \pm 0.33 ^b	3.49 \pm 0.60 ^a	2.78 \pm 0.27 ^{ab}	2.94 \pm 0.08 ^{ab}	3.10 \pm 0.51 ^{ab}	
	<u>Insoluble fraction</u>					
'Honey Dew'	1.20 \pm 0.46 ^a	1.41 \pm 0.23 ^a	0.30 \pm 0.03 ^a		0.29 \pm 0.03 ^a	0.20 \pm 0.01 ^a
'IW-57'	0.31 \pm 0.10 ^a	0.10 \pm 0.01 ^a	1.51 \pm 0.56 ^a	0.30 \pm 0.03 ^a	0.30 \pm 0.02 ^a	0.20 \pm 0.01 ^a
'Ginsen'	1.49 \pm 0.02 ^a	0.35 \pm 0.05 ^b	0.05 \pm 0.02 ^b	0.05 \pm 0.01 ^b	0.50 \pm 0.06 ^b	

Average of three replications \pm standard error.

* Any two means having a common letter in the same cultivar are not significantly different at 5% level, DMRT.⁽⁶⁾

Table 2. Changes in total and water soluble pectin contents of three melon cultivars harvested at different days after anthesis*

Cultivars	Days after anthesis					
	10	20	30	35	40	45
	Total pectin content (mg/100g fresh wt.)					
'Honey Dew'	421 ± 53 ^a	300 ± 28 ^b	249 ± 27 ^b		195 ± 9 ^b	233 ± 28 ^b
'IW-57'	887 ± 213 ^a	501 ± 11 ^c	554 ± 5 ^{bc}	402 ± 4 ^c	854 ± 26 ^{ab}	401 ± 6 ^c
'Ginsen'	303 ± 57 ^a	315 ± 13 ^a	292 ± 10 ^a	274 ± 9 ^a	251 ± 2 ^a	
	Water soluble pectin content (mg/100g fresh wt.)					
'Honey Dew'	250 ± 2 ^a	201 ± 2 ^b	190 ± 2 ^b		186 ± 6 ^b	190 ± 6 ^b
'IW-57'	252 ± 22 ^{abc}	261 ± 2 ^{abc}	299 ± 2 ^a	283 ± 4 ^{ab}	50 ± 3 ^d	200 ± 3 ^c
'Ginsen'	85 ± 13 ^e	170 ± 4 ^{bc}	249 ± 11 ^a	200 ± 11 ^{ab}	169 ± 8 ^{bc}	

Average of three replications ± standard error

*Any two means having a common letter in the same cultivar are not significantly different at 5% level, DMRT.

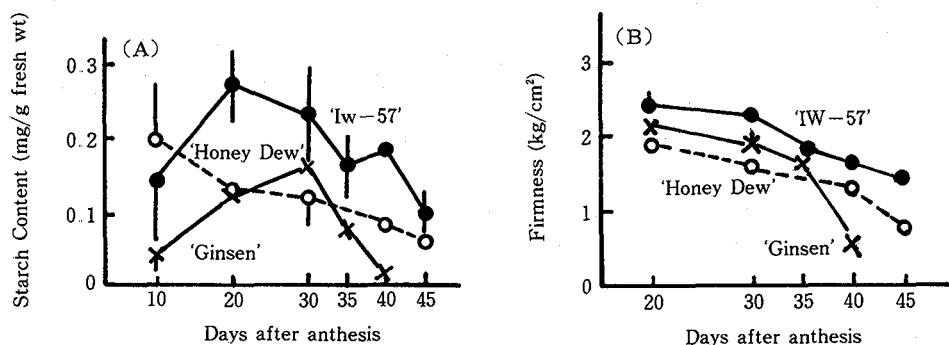


Fig.3 Changes in starch content (A) and firmness (B) during growth of melon fruit. (A) Values are means with S E (n=3). (B) Values are means with S E. (n=6).

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メロン果実のポリガラクトクロナーゼと α -アミラーゼの活性変化

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要 約

本報告は、メロン果実、3品種のポリガラクトクロナーゼ (PG) と α -アミラーゼ活性に関係する全ペクチン (TP)、水溶性ペクチン (WSP) とデンプン含量の変化について検討した。また、'銀泉'のこれら酵素の性質についても同様に検討した。

可溶性画分におけるPG活性は増大し、一方不溶性画分では減少した。しかしながら、各収穫適期とTP、WSP含量の間には有意差は認められなかった。両画分のPGと α -アミラーゼ活性、TP、WSP、デンプン含量は硬度に代わる最適収穫期の指標として利用できない。

'銀泉'のPGと α -アミラーゼ活性は各々分子量24,000と52,000、至適pH5.5と5.0、ポリガラクトクロン酸とデンプンに対する k_m は、各々0.14と3.3mg/mlであった。両酵素共に至適温度は45℃であった。