エチレン吸収剤がカキ果実のポリガラクチュロナーゼ活性に及ぼす影響

誌名	園藝學會雑誌
ISSN	00137626
著者	松井, 年行 北川, 博敏
巻/号	57巻4号
掲載ページ	p. 697-701
発行年月	1989年3月

J. Japan. Soc. Hort. Sci. 57(4) : 697—701. 1989.

Effects of Ethylene Absorbent on Polygalacuturonase Activity of Persimmon Fruit

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Summary

The inclusion of an ethylene absorbent in the polyethylene bag is known to be effective in extending the storage period of persimmon. The polygalacturonase (PG) activity in the persimmon fruit placed in polyethylene bags with or without abosrbent was compared during storage at room temperature (about 20°C) and 5°C. PG is considered to decrease the quality and hardness of persimmon fruit during storage by its action on the pectic substances. Therefore, the activity of PG has been used as an index of the quality and hardness of the fruit. The PG activity in the insoluble fraction of persimmon fruit was higher in the presence than in the absence of an ethylene absorbent at room temperature. In contrast, no significant differences were observed in its activity in the soluble fraction. PG activity in the soluble fraction at 5°C tended to be higher than at room temperature in the presence of an ethylene absorbent. Although ethylene absorbent prevents the decomposition of total pectin at room temperature, PG activity was hardly affected by temperature or the presence of ethylene absorbent. It may be concluded that the effect of ethylene absorbent on PG is less significant than that of invertase (10) and that PG is not closely related to the softening of the fruits.

Introduction

Softening is the most prominent deteriorative change in persimmon fruit. It lowers the market value of the fruit and is thus a serious problem during marketing and distribution. Maotani *et al.* (7) were able to prolong the storage time of persimmon fruit by placing them in polyethylene bags containing an ethylene absorbent. In an earlier study (10), it was shown that ethylene absorbent can be used irrespective of the temperature of storage. PG is considered to decompose polygalacturonic acid and polygalacturonan into galacturonic acid and its derivatives after harvest and decrease the quality and hardness of persimmon fruit during storage. In order to confirm the change of the activity in the soluble and insoluble fractions of the flesh during storage, these fractions were separated and the PG was analyzed. The PG activity was used as an index of the quality and softening of the fruit. As there are differences in the enzyme activity and components of individual sample fruit after harvest, the differences among samples were eliminated by random sampling and the test differences of two variances were estimated on the basis of the F-value (3, 5).

Received for publication January 6, 1988.

Materials and Methods

1. Materials

Fruit of the persimmon cultivar 'Fuyu' was harvested in the field of Kagawa University located in Nagao, Kagawa Prefecture on the 25th of October, 1985. After harvestabnormal and deformed fruits were removed, and the fruits were sampled at random for the following experiment.

Each 10 kg sample of persimmon fruit was placed in a polyethylene bag 0.05 mm in thickness (size 130×85 cm) and the bag was degassed. "Crisper 101", an ethylene absorbent coated with bromide on activated carbon was obtained from Ohoe Chemical Co. The conditions for storage were as follows: ER, 10 kg of fruit was placed in a polyethylene bag containing ethylene absorbent (about 38 g) and stored at room temperature; NER, the same as ER except that ethylene absorbent was not used; E5, the

same as ER except that the fruit were stored at 5°C; NE5, the same as E5 except that the ethylene absorbent was not used.

2. Enzyme extraction

The fruit samples were mixed with 15% of Polyclar AT in proportion to the total sample weight and the mixture was ground with a cooled mortar and pestle with twice the volume of chilled water and 5 g of sea sand (8). The resulting homogenate was filtered through 4 layers of cotton cloth and the filtrate was centrifuged at 6,000×*g* for 10 min. The residue was rinsed with the same volume of water and then centrifuged in the same way. The total supernatant was dialyzed with 5 mM of phosphate-citrate buffer (pH 7.4) for 12 h and the fraction was designated as soluble fraction (S. f.). On the other hand, the residue was re-extracted with twice the volume of 0.2 M phosphate-citrate buffer (pH 8.0) containing 5% NaCl for 12 h with occasional stirring. The resulting homogenate was centrifuged at 20,000 ×*g* for 20 min. The supernatant was dialyzed in the same way and the fraction was designated as insoluble fraction (Is.f.). The extraction was carried out at 4°C.

3. Enzyme assay

The standard assay medium for PG consisted of 0.2 ml of 0.2 M sodium phosphate-citrate buffer (pH 5.0), 0.1 ml of 1% polygalacturonic acid, 0.1 ml crude enzyme and 0.1 ml water. A blank experiment was carried out using water instead of the substrate. The reaction mixture was incubated at 45°C for 15 min. After neutralization, the reaction mixture was placed on ice, color producing reagent (Cu-reagent) was added and the mixture was boiled for 10 min. The concentration of the reducing sugars was estimated by the method of Somogyi (13). Soluble protein content was determined according to the method of Lowry *et al.* (6) using bovine serum albumin as the standard. The enzyme activity was expressed as the amount of galacturonic acid liberated per min per mg of protein.

4. Determination of total pectin (TP) and water soluble pectin (WSP) content

Ten grams of peeled, sliced sample was boiled in 80 ml of 95% ethyl alcohol for 30 min under a reflux condenser, followed by filtration. The residue was extracted for about 4 times in a similar way until the washings were free from soluble sugar by phenol-H_2SO_4 reagent (1). Then the residue was washed with a small amount of ethyl ether and dried at around 60 °C.

TP: The dried sample (0.5 g or less) was ground with a mortar and pestle with 1 g of sea sand. To the resulting powder 25 ml of 0.05 N HCl was added and the mixture was heated in boiling water for 1 h under a reflux condenser. After rapid cooling with running water, the sample was made up to 50 ml with water and was filtered (11).

WSP: The powdered sample (0.5 g or less) was made up to 25 ml with water and was extracted at 30°C for 2–3 h with occasional stirring. The mixture was filtered and the filtrate analyzed. Pectin was determined as galacturonic acid by the Carbazole-H_2SO_4 method (1).

Results and Discussion

1. Change in weight of persimmon fruit during storage

Analysis of variance of weight of fruit was done (Table 1). Results indicate that fruits subjected to the different treatments did not show significant differences. It is well known that storage in polyethylene bags inhibits the transpiration of fruit and polyethylene regulates the concentrations of oxygen and carbon dioxide in connection with respiration (15). According to Tarutani (14), the amount of carbon dioxide and water vapor evolved were comparably stable at temperatures lower than 5°C but they fluctuated at temperatures higher than 10°C.

2. Change in PG activity of persimmon fruit during storage

PG has been reported to be presented as a cell wall-bound protein in tomato fruit (16). It was suggested that some soluble enzymes are absorbed on the cell surface or cytoplasmic particles during the fractionation procedure (2). However, here the water soluble fraction was defined as the soluble fraction, whereas the sodium phosphate-citrate buffer soluble fraction was defined as insoluble fraction (4) in order to facilitate the fractination procedure. In persimmon fruit, the PG in the soluble fraction exhibits a higher activity than that in the insoluble fraction along with the development

Table 1. Test difference of the two variances of PG activity as affected by ethylene absorbent.

Conditions	Comparison		Variances (V)		D. F.	F_0	Decision
R. T.	Total weight	O —X	414.10	539.25	20, 23	1.30	-
〃	PG activity in Is. f.	O —X	8.87	3.51	23, 20	2.53	*
〃	PG activity in Is. f.	X_b—X_a	5.45	1.28	11, 8	4.26	*
5°C	Total weight	O —X	369.61	484.58	11, 11	1.31	-
〃	PG activity in S. f.	O_b—O_a	23.14	2.53	5, 5	9.15	*
〃	PG activity in S. f.	X —X	113.58	9.81	5, 5	11.57	*

** $P<0.01$, $F(20, 23; 0.01) = 3.12$,
* $P<0.05$, $F(20, 23; 0.05) = 2.36$.
** $P<0.01$, $F(23, 20; 0.01) = 3.50$,
* $P<0.05$, $F(23, 20; 0.05) = 2.57$.
** $P<0.01$, $F(5, 5; 0.01) = 14.94$,
* $P<0.05$, $F(5, 5; 0.05) = 7.15$.

** $P<0.01$, $F(11, 11; 0.01) = 5.54$,
* $P<0.05$, $F(11, 11; 0.05) = 3.59$.
** $P<0.01$, $F(11, 8; 0.01) = 7.21$,
* $P<0.05$, $F(11, 8; 0.05) = 4.30$.

S. f.: Soluble fraction.
Is. f.: Insoluble fraction.
O: Presence of ethylene absorbent.
X: Absence of ethylene absorbent.
$_b$: Storage of 27 to 43 days.
$_a$: Storage of 5 to 22 days.
R. T.: Room temperature.

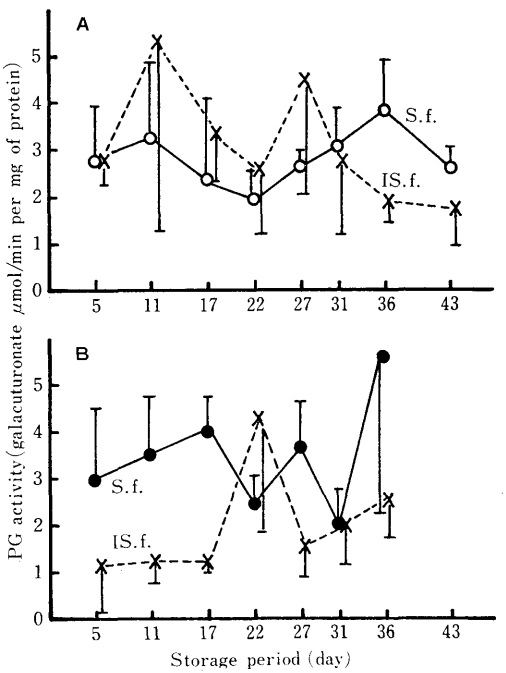

Fig. 1. Changes in polygalacturonase activity of persimmon fruit during storage with (A) or without (B) ethylene absorbent at room temperature. ○: soluble fraction with ethylene absorbent, ●: soluble fraction without ethylene absorbent, ×: Insoluble fraction. Values are means with S. E. ($n=3$).

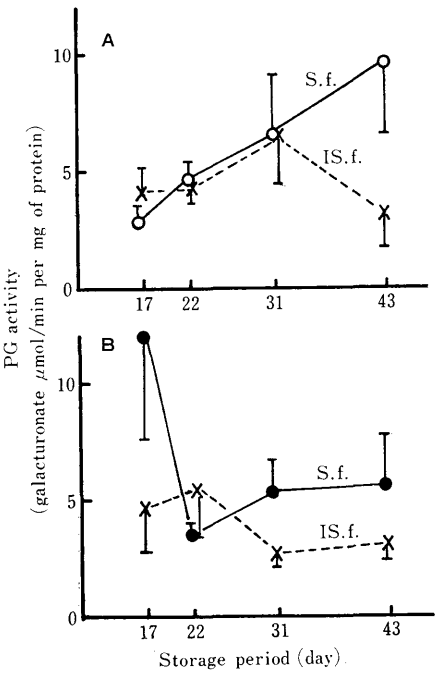

Fig. 2. Changes in polygalacturonase activity of persimmon fruit during storage with (A) or without (B) ethylene absorbent at 5°C. Symbols as shown in Fig. 1.

of the fruit (9). Figs. 1 and 2 show the changes in PG activity of persimmon fruit during storage at room temperature and 5°C, respectively. The activity of PG in the insoluble fraction increased rapidly at intial stage in the presence of ethylene absorbent at room temperature, whereas the activity in the soluble fraction increased moderately. PG in the soluble fraction showed a higher activity at the initial stage in the absence than in the presence of ethylene absorbent at 5°C. The activity of PG in the insoluble fraction was not significantly different between the initial and the later

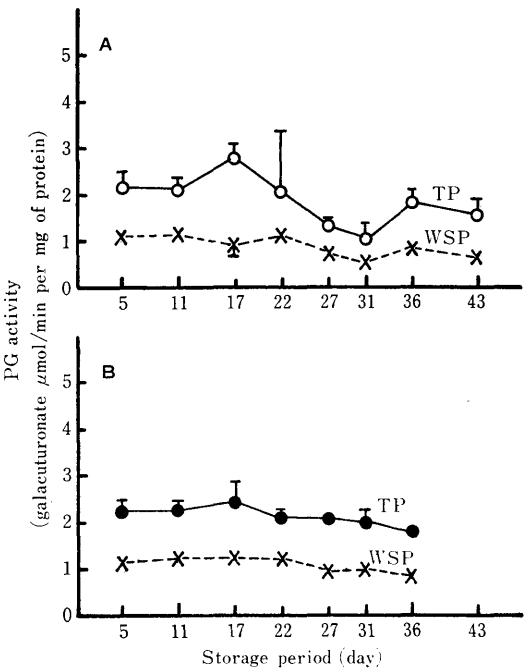

Fig. 3. Changes in the content of pectic substances in persimmon fruit during storage with (A) or without (B) ethylene absorbent at room temperature. ○: total pectin with ethylene absorbent, ●: total pectin without ethylene absorbent, ×: water soluble pectin.

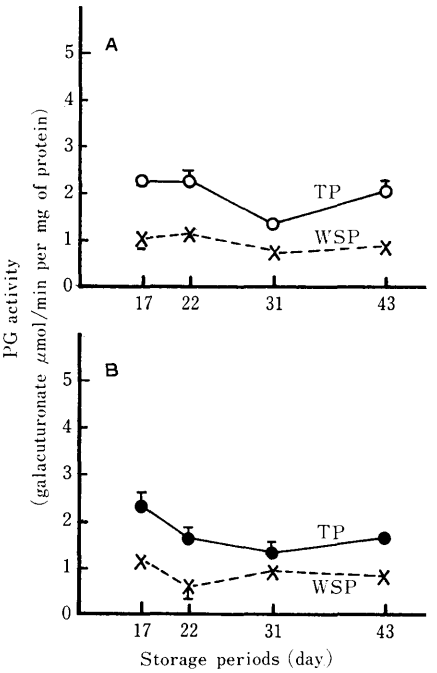

Fig. 4. Changes in the content of pectic substances in persimmon fruits during storage with (A) or without (B) ethylene absorbent at 5°C. Symbols as shown in Fig. 3.

stages regardless of the presence or absence of the ethylene absorbent at 5°C.

3. Changes in TP and WSP contents in persimmon fruit during storage

Figs. 3 and 4 show the changes in the TP and WSP contents in persimmon fruit during storage at room temperature and at 5°C, respectively. Table 2 shows the test differences of two variances of TP contents based on the F-value. At room temperature, only the TP content was significantly affected by the presence of ethylene absorbent. The TP contents were higher at the initial stage when the ethylene absorbent was present than at the later stage. TP content tended to decrease with time. There were no significant differences between the WSP contents at room temperature and 5 °C. In persimmon, TP which acts as the substrate of PG may be decomposed by PG at room temperature. The increase in the activity of PG increased the amount of WSP (12) in peach fruit unlike in persimmon fruit.

In the previous paper (10), invertase activity was higher in the absence of ethylene absorbent. Ethylene absorbent was thought to prevent sucrose decomposition. Due to the low activity of PG in persimmon fruit the WSP content appeared to decrease.

It may be concluded that the PG activity is not a suitable index of the quality and softening of persimmon although the presence of an ethylene absorbent works effectively in extending the storage life of the fruit.

Acnowledgment

We wish to thank Prof. S. KAWAMURA of

Table 2. Test difference of the two variances of the total pectin contents.

Condition	Comparison		Variances (V)		D. F.	F	Decision
R. T.	Total pectin content	O —X	0.85	0.169	23, 20	5.03	*
〃	Totol pectin content	O_b—O_a	1.16	0.24	11, 11	4.80	*

Symbols as shown in Table 1.

Kagawa-ken Meizen Junior College for the critical reading of this manuscript.

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エチレン吸収剤がカキ果実のポリガラクチュロナーゼ活性に及ぼす影響

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摘 要

エチレン吸収剤の入ったポリエチレン袋にカキ果実を包装すると，硬度の保持が良好で貯蔵期間を著しく延長することができることが知られている．そこで，室温と5℃ でのポリエチレンバッグ包装果実にエチレン吸収剤を使った場合と使わなかった場合におけるカキ果実のポリガラクチュロナーゼ（ＰＧ）に及ぼす効果について検討した．また，ＰＧ活性はカキ果実の品質や硬度を貯蔵中に低下すると考えられているので，ＰＧ活性を品質，軟化の指標として使用し得るかどうかを検討した．カキ果実の不溶性画分のＰＧは室温ではエチレン吸収剤を使用しなかった場合よりも使用した場合の方がより高い活性を示した．一方，可溶性画分では上記の区間に有意差は認められなかった．可溶性画分のＰＧ活性は室温でエチレン吸収剤を使ったものより5℃ で使ったものの方が高かった．エチレン吸収剤は室温で全ペクチンの分解を抑制するけれども，温度やエチレン吸収剤がＰＧ活性に対する影響はほとんど認められなかった．エチレン吸収剤に及ぼす作用はインベルターゼに対するよりも小さく，かつ，カキ果実の軟化とＰＧ活性との関係は少ないものと考えられる．