Changes in Pectic Components, Ascorbic Acid, Pectic Enzymes and Cellulase Activity in Ripening and Stored Guava (*Psidium guajava* L.)

Golam MOWLAH* and Saburo ITOO*

* *Laboratory of Postharvest Physiology and Preservation of Fruits and Vegetables, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890*

This study was designed to observe chemical changes along with pectin-degrading and cellulolytic enzyme activities in two cultivars of guava during fruit maturation, ripening and storage. Total pectin content was 345.9-396.1 mg/100 g during maturation and 704.8-804.0 mg/100 g in ripening in both cultivars. Protopectin and water soluble pectin were predominant in matured and ripened fruits, respectively. Alcohol insoluble solids and acidity decreased in both cultivars; and pH was 4.2-5.0 in white and 4.0-5.2 in pink varieties during maturation, ripening and storage. Guava polygalacturonase and cellulase showed maximum activity at pH 5.5 and 6.0, respectively. Ripe guava showed polygalacturonase activity recognized only remarkably at full ripe and the activity increased further in stored samples. Guava indicated a little pectinesterase and cellulase activity and these were found to increase sharply at the stage from maturation to ripening. During the stages from immature to full ripe on the tree, ascorbic acid content increased gradually in both cultivars. During stages from ripe to full ripe, and storage for 10 days at 20°C; in white cultivar total ascorbic acid, ascorbic acid and dehydroascorbic acid contents were 149.9-273.5, 126.7-224.0 and 23.3-49.5 mg/100 g, respectively; and in pink cultivar these were 172.6-270.3, 146.2-203.8 and 26.4-66.5 mg/100 g, respectively. Ascorbic acid content was found to increase remarkably in stored fruits until decay or senescence of the fruits started.

Pectic substances are drawing attention because of their role in maintaining the texture of fruits¹⁾ and in influencing the quality of products. Numerous studies were made regarding the effect of pectins on firmness of fresh fruits and processed fruit products²⁻⁴⁾ and in assessing the pectic constituents of fruits⁵⁾⁶⁾. Peaches were found to have different proportions of water soluble and water insoluble pectin depending on their varieties at different stages of maturation and ripening³⁾⁷⁾. Thickening and gelling properties of pectin present in fruits affect the rheological properties of jam and nectar products. Until now no precise information is available on the pectic constituents of guava during maturation and ripening as characterized by varietal changes but this type of information is earnestly demanded by the guava processors. Further-

more, fruits ripening and softness are attributed to the activities of pectic enzymes⁸⁾⁻¹²⁾. In case of tomato fruit cellulase was found to be associated with softening¹³⁾¹⁴⁾ and evidences of cellulase activity was found also in various fruits¹⁵⁾⁻¹⁷⁾.

During the previous studies investigations were made on polyphenols and polyphenol-oxidase; and sugar components and related enzymes invertase and amylase at stages of fruit maturation, ripening and storage in two cultivars of guava¹⁸⁾¹⁹⁾. The present work was performed with the same varieties at their different stages of maturation, ripening and storage to determine quantitatively the changes in pectic and cellulolytic enzymes along with some chemical analysis.

* Present address where correspondence is to be made: Laboratory of Food Science & Technology, Faculty of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156

Materials and Methods

Samples and sample preparation

Guava (*Psidium guajava* L.) samples of white C-1-16 and pink C-1-40 seedling varieties were collected and graded as immature (picked on September 24), mature (picked on October 8), ripe (picked on October 8), full ripe (picked on October 22) and stored according to the procedure described previously^{18,19}. The fruits picked on October 8 were graded mature and ripe depending on size and degree of ripeness as mentioned earlier¹⁸. The term stored was used for full ripe guava samples stored for 8 days at 20°C in small polyethylene bags with opened mouth. For storage experiment (1-11 days) the same procedure was followed with guava harvested on November 4. The strawberry (cv. *Harunoka*) samples were picked at their picking time of consumption (April 7) and stored (1-7 days) at the same storage conditions used for guava.

Preparation of alcohol insoluble solids (AIS)

AIS were prepared and collected according to the procedure of SHEWFELT²⁰ with boiling ethanol to inactivate the enzymes from each of the representative samples and the prepared AIS were stored in desiccator at 10°C.

Determination of pectic substances

The colorimetric carbazole method of McCOMB and McCREADY²¹ was followed according to the modification made by BITTER and MUIR²² for the determination of pectic substances and the results were expressed as mg of α -D-galacturonic acid monohydrate (AGA) per 100 g of fruit (mg/100 g). Versene-pectinase carbazole method of McCREADY and McCOMB⁶ was used to determine the total pectin with the following modification. AIS were added with 5% versene solution (ethylenediamine tetraacetic acid tetrasodium salt), and the mixture was adjusted to pH 11.5 with 1N NaOH and de-esterified at 30°C for 2 h and then acidified to pH 3.0 with 1N HCl. To the acidified mixture pectinase (10% of AIS) was added. After adding a few drops of toluene, the mixture was allowed to stand overnight at 25-27°C and then filtered and the

filtrate was made up to the required volume and then was used for determination of total pectin.

Protopectin determination

Protopectin was considered to be water insoluble fraction but soluble in versene-pectinase. Water soluble pectin from the AIS was extracted according to the procedure of POSTLMAYR *et al.*²³ and the residue was used as the material for protopectin determination and the results were found to be similar to that determined by the method of difference.

Determination of acidity and pH

Titratable acidity was determined with phenolphthalein as the indicator at pH 8.1. The pH of the extracted juice was determined with a single electrode pH meter.

Analysis of ascorbic acid

As guava contained dehydroascorbic acid (DHAA), the indophenol method of BESSEY²³ alone was not sufficient to investigate the total ascorbic acid (TAA) content of guava. TAA was determined according to the DNP colorimetric method of ROE and OESTERLING²⁴ with some modifications. The weighed amount of minced guava samples was extracted with 5% metaphosphoric acid (HPO_3) containing 1% thiourea. The aliquot portions were first added with 0.02% of 2,6-dichloroindophenol sodium salt solution and then with 2% thiourea solution (2% thiourea in 5% metaphosphoric acid). Then 2% DNP solution (2% solution of 2,4-dinitrophenylhydrazine in 9N H_2SO_4) was added. The reaction mixtures were warmed up at 50°C for 70 min, cooled sufficiently at ice temperature and then added with 85% H_2SO_4 taking care that the temperature did not rise. After color development absorbance of the reaction mixtures was measured at 520 nm with a Shimadzu Spectrophotometer. This procedure gave TAA (ascorbic acid plus DHAA). Ascorbic acid (AA) was represented by the difference between the amount for TAA and that determined without addition of 2,6-dichloroindophenol solution a procedure for determining the DHAA. The concentration of ascorbic acid was determined from a standard curve of L-ascorbic acid and expressed as mg per 100 g fruit.

Preparation of acetone powders and samples

Acetone powder, prepared as mentioned in the earlier paper¹⁸⁾, was extracted with 4% sodium chloride in presence of insoluble polyvinylpyrrolidone (10% of acetone powder) by centrifuging at 14 000 rpm for 15 min at -5°C . The same extraction was repeated two times more. The pooled extract was made up to the desired volume (protein content 45.1–127.7 $\mu\text{g}/\text{ml}$).

Enzyme assay

Polygalacturonase (PG) (*poly- α -1,4-galacturonide glycanohydrolase*, EC 3.2.1.15): Routinely the enzyme reaction mixture (3 ml) consisted of 1 ml of enzyme extract, 1 ml of 0.25% pectic acid solution and 1 ml of 0.1 M acetate buffer (pH 5.5). The increase in reducing groups was measured by the copper reduction method²⁶⁾. The activity was given as increased reducing power and expressed as the liberation of microgram equivalent of reducing groups taking α -D-galacturonic acid as a standard in 24 h under the above specific reaction conditions.

Pectinesterase (PE) (*pectin pectyl-hydrolase*, EC 3.1.1.11): The acetone powder was dispersed in 0.15 M sodium chloride in presence of insoluble polyvinylpyrrolidone at pH 7.5 and the dispersion was stirred continuously for 15 min and then centrifuged for 15 min at 14 000 rpm at -5°C . The clear supernatant was used to determine the pectinesterase activity according to the principle of KERTESZ²⁶⁾ as follows. Citrus pectin solution (1%) was prepared with 0.15 M sodium chloride, adjusted to pH 7.5. The stock solution of 1% citrus pectin was kept in a refrigerator at 4°C with the addition of a few drops of toluene and warmed up to room temperature of 26 – 30°C prior to use. Thirty ml of 1% citrus pectin solution of pH 7.5 was warmed at 30°C in a constant temperature water bath. Then 5 ml of the enzyme extract was added and the pH was immediately readjusted to 7.5 and the time was noted. The alkali was added at the rate required to keep the mixture at pH 7.5 for 15 min. PE unit was expressed as ml of alkali multiplied by normality of alkali consumed by

1 ml of the added enzyme solution in 15 min which represents the mEq of ester hydrolyzed per 15 min per ml of enzyme solution.

Cellulase [1,4-(1,3;1,4)- β -D-glucan 4-glycanohydrolase, EC 3.2.1.4]: Cellulase activity was measured according to HINTON and PRESSEY¹⁶⁾ using the same enzyme extract of PG. Enzyme reaction mixture was composed of 1 ml of enzyme solution, 5 ml of 0.5% carboxymethyl cellulose sodium salt (CMC salt) solution with 0.1 M citrate-phosphate buffer of pH 6.0 and incubated at 30°C in water bath in glass tubes. A control containing 1 ml of extract heated for 5 min in boiling water was run with each sample. After 24 h incubation the tubes were heated in boiling temperature for 10 min, cooled, centrifuged and then 5 ml of the supernatant was put into an OSTWALD Viscometer placed in a constant temperature water bath at 30°C and the viscosity was measured. The percent loss of viscosity was supposed as being proportional to the cellulase activity. The CMC salt stock solution was preserved at 4°C with the addition of a few drops of toluene.

A study indicated that guava cellulase had maximum activity at pH 6.0.

Results and Discussion

Table 1 showed that there was a variation in pectin content in two cultivars of guava. Pectin contents changed throughout the whole period of maturation, ripening and storage in both varieties. In ripening pectin content was higher in white than in pink but during storage pectin content was slightly higher in pink than in white. It shows that pectin in pink is resistant to storage exposure than in white, which may be due to the differences in chemical composition. Protopectin content was 83.5–96.9% of the total pectin during development and maturation, and the WSP was 3.0–16.4% during these times in both the varieties. This high retention of protopectin with less transformation to WSP during guava maturation conforms with the findings of NIGHTINGALE *et al.*²⁷⁾ and POSTLMAYR *et al.*²⁾ in peach varieties. On the other hand, WSP increased with the beginning of ripening and

Table 1 Pectic substances in quava varieties during maturation, ripening and storage

Degree of maturation	TP (mg/100 g F)		Water soluble pectin				Protopectin			
			mg/100 g F		%TP		mg/100 g F		%TP	
	W	P	W	P	W	P	W	P	W	P
Immature	360.7	345.9	12.2	10.4	3.4	3.0	348.4	335.4	96.5	96.9
Mature	396.1	363.6	65.1	53.1	16.4	14.6	330.9	310.4	83.5	85.3
Ripe	791.9	704.7	486.5	409.4	61.0	57.3	315.3	305.3	39.0	42.6
Full ripe	804.0	720.0	604.1	560.7	68.9	70.9	299.9	259.2	31.1	29.0
Stored	723.6	727.2	616.9	580.9	84.5	79.8	116.6	146.3	15.4	20.1

W=white, C-1-16 seedling variety; P=pink, C-1-40 variety; TP=total pectin contents expressed as milligram per 100 gram fruit weight.

Table 2 Chemical composition of guava varieties during maturation, ripening and storage

Degree of maturation	AIS (%)		Acidity (%)		pH	
	W	P	W	P	W	P
Immature	10.60	17.99	—	—	—	—
Mature	8.90	8.47	0.42	0.56	4.2	4.0
Ripe	5.50	5.41	0.37	0.45	4.2	4.1
Full ripe	5.10	4.60	0.35	0.42	4.6	5.0
Stored	4.80	4.20	0.32	0.33	5.0	5.2

W=white, C-1-16 variety; P=pink, C-1-40 variety; Acidity expressed as citric acid; '—'=determination could not be made.

at full ripe it became 68.9-70.9% of total pectin, during storage it reached 79.8-84.5% of total pectin while protopectin was 29.0-31.1% at full ripe and 15.4-20.1% at storage, leading to marked softness of the fruits. The same phenomenon was also observed by DOLEND *et al.*²⁸⁾ in case of ripening avocado, and by STERLING and KALB²⁹⁾ in case of Elberta peaches. From the correlation between the changes in solubility of pectin and calcium, DOESBURG³⁰⁾ suggested that solubilization of pectin during ripening of apples was possibly caused by movement of calcium in the cell wall also resulting to changes in pH and organic acids³¹⁾. Chemical analyses with respect to AIS, pH and titratable acidity of the guava varieties are given in Table 2.

As shown in Fig. 1 guava PG and cellulase possessed maximum activity at pH 5.5 and

6.0, respectively.

Fig. 2 indicated that in fruit maturation PG activity was static but at the inception of ripening, activity of this enzyme started and rose sharply at full ripe and continued to increase during storage. Attempts were also made to determine PG activity of guava by viscosimetric method, but no detectable activity was observed. The determination of PG activity of guava by measuring the liberated reducing groups, therefore, was more sensitive, indicating that guava PG is an exo-PG which was also found to be true in case of citrus³²⁾. PG activity was recognizable with the initiation of ripening. The same phenomenon was also reported by BABBIT *et al.*³³⁾ in case of tomato ripening. However, the increase in PG activity at ripening imparts that the PG activity might lead the fruit to ripen, and also the PG activity may be responsible for some processing effects of guava products such as, cloud stability of guava juice. During storage PG activity increased inducing softness effect to the guava. PG activity was found to increase during softening of the tissues for dates³⁴⁾, avocados¹¹⁾ and tomatoes³⁵⁾. Among the two varieties the pink had higher activity than the white. The increased PG activity during ripening and storage of guava is likely due to the transformation of inactive insoluble protein to soluble protein³⁴⁾ caused by intrinsic auto-catalytic processes. Or the disintegration of the tissue during storage releases or opens the blockade of catalyst or active enzyme surface which as a whole increases the PG activity. Although

Fig. 1 The pH vs activity curves for polygalacturonase and cellulase of guava fruit

- : polygalacturonase; ○: cellulase
- pH: 4.0-5.5 with acetate buffer (0.1M)
- 6.0-6.5 with citrate-phosphate buffer (0.1M)
- 7.0-8.0 with phosphate buffer (0.1M)

Fig. 3 Pectinesterase activity in guava at stages of maturation and ripening

- : White variety, activity (units/mg protein)
- : Pink variety, activity (units/mg protein)
- ▲: White variety, activity (units/g fruit)
- △: Pink variety, activity (units/g fruit)

there is less possibility for the direct biosynthesis of enzyme for the increased PG activity, the disappearance, removal, or leaching

Fig. 2 Polygalacturonase activity in two varieties of guava at stages of maturation, ripening and storage

- : White variety, activity (units/g fruit)
- : Pink variety, activity (units/g fruit)
- ▲: White variety, activity (units/mg protein)
- △: Pink variety, activity (units/mg protein)

Fig. 4 Cellulase in guava varieties at maturation, ripening and storage

- : White and ○: Pink varieties

out of the PG inhibitory factor during softening of the tissue may have role in increasing PG activity during storage³⁴). Fig. 3 represents the PE activity in the two varieties of guava. Guava, as a whole, possessed low PE activity. It started at maturation and increased

faster at ripening than at full ripe which was different from PG.

As shown in Fig. 4, cellulase activity changed differently from PG and PE activity. It started to increase from immature stage with remarkable increase in maturation and ripening, and then slowly increased at full ripe and the increase was very slow during storage. The existence of cellulase activity in guava during fruit development and maturation coincided with the viewpoints of BABBIT *et al.*³³⁾ that cellulase activity was present in young fruit. It could be said that guava cellulase reached almost its maximum activity before full ripening of the fruit. As the enzymatic degradation of cellulose could be an important process during ripening, the increase in cellulase activity in ripening implied that cellulase in collaboration with pectic enzymes affects the ripening and thereafter softening of guava fruit. The same phenomenon was suggested also for peach¹⁶⁾ but with deviation from tomatoes as in case of tomato the cellulase activity increased only slightly at ripening³⁶⁾. These patterns of activity of PG and cellulase at the ripening paralleled the instances of invertase and amylase¹⁹⁾. PRATT and GOESCHL³⁷⁾ reported that the sharp increases of these hydrolytic enzymes at the early stage of ripening are a typical phenomenon occurring in climacteric fruits. Considering these changes coupled with the changes in chemical composition including carbohydrates¹⁸⁾¹⁹⁾ it can be suggested that these two cultivars of guava belong to climacteric group of fruits.

In general ascorbic acid contents of both cultivars varied widely at stages from maturation to ripe. TAA content was 96.4–149.9 mg/100 g, and AA content was 54.2–126.7 mg/100 g in white variety; and these were 106.5–172.6 and 72.5–146.2 mg/100 g, respectively, in pink variety (Fig. 5). However, stored samples showed increased ascorbic acid contents (e.g. white variety: TAA 273.5, AA 224.0, and DHAA 49.5 mg/100 g; and pink variety: TAA 270.3, AA 203.8, and DHAA 66.5 mg/100 g). It is evident that these two varieties of guava contained a quite high quantity of TAA as was found in other

Fig. 5 Ascorbic acids content of guava at maturation, ripening and storage at 20°C

- : White, total ascorbic acid (TAA)
- : Pink, TAA
- - ●: White, ascorbic acid (AA)
- - ○: Pink, AA
- ▲—▲: White, dehydroascorbic acid (DHAA)
- △- - △: Pink DHAA

varieties of guava^{38)–41)}. And they contained a small amount of DHAA. According to above stated results TAA increased considerably in stored samples. To investigate the increasing trend with storage, experiments were performed with two varieties of tree ripened guava fruit stored under the same experimental conditions and the results are shown in Fig. 6. The results indicated a sharp increase in ascorbic acid with storage time. For comparison with guava, the same postharvest experiment was done with strawberry (cv. *Harunoka*) during storage. As shown in Fig. 7, there was no increase of ascorbic acid in strawberry, although increase was observed in guava determined under the same conditions. It shows that ascorbic acid accumulation was continued in stored tree ripened guava. Increased PG activity in stored samples along with other biochemical changes may lead ascorbic acid synthetase or transformation

Fig. 6 Changes of ascorbic acids in ripe guava (harvested on Nov. 4) during post-harvest storage at 20°C

- : Pink, total ascorbic acid (TAA)
 ●—●: White, TAA
 ○---○: Pink, dehydroascorbic acid (DHAA)
 ●---●: White, DHAA

reaction to continue until decay or senescence of the fruit was started. It can be said that biosynthetic regulation along with translocatory system might be a pathway for ascorbic acid accumulation in guava. TANUSI and YAMAMOTO⁴²⁾ reported that citrus Sudachi can utilize D-galacturonic acid as a precursor of ascorbic acid when D-galacturonic acid was added with the homogenates of fruit stored under nitrogen atmosphere. This indicates that increased hydrolytic activity of PG resulting to liberation of galacturonic acid could have some effect on increasing of ascorbic acid in stored guava samples.

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Fig. 7 Postharvest (harvested on April 7) changes of ascorbic acid contents in strawberry (cv. *Harunoka*) during storage

- : Total ascorbic acid
 ---: Dehydroascorbic acid

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グアバ果実の成熟・追熟並びに貯蔵中におけるペクチン、アスコルビン酸、ペクチン関連酵素及びセルラーゼ活性の変化について

ゴースラム モーラ・伊藤三郎
(鹿児島大学農学部)

グアバの白色及び桃色2品種について成熟・追熟並びに貯蔵中における化学的变化、ペクチン関連酵素及びセルラーゼの変化を調べた。

1. グアバ果実中の全ペクチン量は両品種共に成熟時では345.9-396.1 mg%, 追熟時では704.7-804.0 mg%であった。なお、成熟及び追熟果におけるペクチンの主成分はプロトペクチン及び可溶性ペクチンであった。

2. アルコール不溶物量及び酸度は成熟・追熟並びに貯蔵中に減少した。一方、pHは白色種では4.20から5.00、桃色種では4.00から5.20に上昇した。

3. グアバ果実のポリガラクトナーゼ及びセルラーゼの最適pHは各々5.5及び6.0であった。ポリガラクトナーゼ活性は追熟及び貯蔵中に著しく増加した。ペクチンエステラーゼ及びセルラーゼは未熟果では、その活性はわずかであったが、成熟及び追熟中に増加した。

4. 未熟果から完熟果へと樹上で生育中にアスコルビン酸含量は次第に増加を示した。その後20℃で10日間貯蔵したところ、全アスコルビン酸、還元型および酸化型アスコルビン酸量は白肉種では各々149.9-273.5, 126.7-224.0及び23.3-49.5 mg%, 桃色種では各々166.5-270.3, 146.2-203.8及び26.4-66.5 mg%となり、著しく増加することを見出した。このビタミンC含量の増加は、貯蔵中に果実が老化、腐敗を生ずるまで続くことが認められた。