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The Relationship Between Pulp-Soaking Symptom in Pummelos *(Citrus grandis* L. Osbeck) and Their Structure, Calcium Content, Pectin Methylesterase Activity and Internal Gas Composition

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ABSTRACT

Structurally, juice-sacs of Khao-thongdee cultivar contained epidermal cells which have thicker outer wall than Khao-pan, and also contained fewer layers of subepidermal cells. No change was found in the outer wall of epidermal cells but the side walls of both epidermal and subepidermal cells were weaken after storage. Calcium content in Khao-thongdee was about double the amount in Khao-pan. Pectin-methylesterase activity was also significantly higher in Khao-thongdee. The relationship between calcium content, pectin-methylesterase activity and pulp-soaking symptom, however, could not be clearly explained. Difference in internal concentrations of carbon dioxide and ethylene between the two cultivars were small and were not likely to be the factors involving the development of the symptom.

INTRODUCTION

Shelf life of pummelos is quite long. In fact, according to the Thai consumer, pummelos taste better if stored for about one or two weeks after harvest. This is true since acid content continues to decline once pummelos are picked from the tree (Sinclair, 1984, and Siriphanich *et al.*, 1987). However, during their shelf-life, the texture of juice-sacs also changes. Juice-sac membrane becomes easily broken while peeling, causing a watery appearance. This symptom is termed 'pulp-soaking'. Complete and undamaged segments of pummelos could not be easily obtained. These pummelos are considered poor in quality even though their appearance and taste might still be acceptable. Symptom similar to that described above is also found in other citrus fruits particularly canned citrus segments (Blundstone *et al.*, 1971).

The seriousness of this textural problem in pummelos varies with cultivars. Khao-thongdee is the most susceptible one while Khao-pan and Khao-poung are less affected (Siriphanich *et al.*, 1987). It may be due to the structural differences among different cultivars. The juice-sacs of the Thongdee cultivar appeared to be juicier than other cultivar even at the time of harvest. By looking with naked-eye the juice-sac membrane seem to be thinner and easier to be broken. Thus, an anatomical study was conducted to determine this possibility.

To avoid pulp-soaking problem, it is recommended to apply calcium in the form of calcium nitrate or phosphate to the plant (Chaiwongkiat, 1984). However, the result of this cultural practice is not consistently obtained. In the case of canned citrus segment, calcium chloride was used (Blundstone *et al.*, 1971). In this phenomena, changes in the cell wall are likely to occur in the

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similar way to that found in the softening of many other fruits. Cell wall materials particularly pectic substances in the middle lamella are degraded by various enzymes such as pectin methylesterase and particularly polygalacturonase (Huber, 1983). However, what happens in citrus is not clear. There were conflicting reports about the existence of polygalacturonase in citrus (Riov, 1975). Calcium is known to prevent or delay fruit softening (Bangerth *et al.,* 1972) probably by cross-linked between molecules of the cell wall components (Furguson, 1984). Thus, besides the anatomical study, calcium content, pectin methylesterase activity and internal gas composition in pummelos were investigated. If the mechanism on the development of this phenomena can be illustrated, it will be very useful for adapting the pre or post-harvest treatment to alleviate this problem. In addition, it may also be useful for plant breeder in their breeding program to select pummelos cultivar which have little or no pulp-soaking symptom.

MATERIALS AND METHODS

All pummelos used in this study were obtained directly from orchards in Sam Phran district, Nakhon Pathom province. The experiments were carried out at the Postharvest Research Unit, Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaeng Saen campus, Nakhon Pathom.

To understand factors that influence juice-sacs deterioration, a 2 × 2 × 2 factorial in completely randomized experimental design was carried out. Comparisons between pummelos cultivars, orchard, and storage period were made using Khao-thongdee and Khao-pan, the most and the least susceptible cultivars for the 'pulp-soaking' problem, Pummelos were harvested from two orchards (A and B) in the same district in August, then washed in 1000 ppm Benzimidazole solution for 5 minutes, dried, and kept under room condition (30°C and 60% relative humidity, in average). Structural and chemical investigtions were carried out at harvest and again after 1 month in storage at 10°C. The experiment was repeated again in October, however, pummelos obtained were from different orchards (A and C).

For the structural study, juice-sacs from the middle part of pummelo segments were taken for freeze-sectioning according to the method described by Gahan *et al.* (1967) with 'Lipshaw' cryostat microtome Model 1500 at - 10°C.

Calcium content was determined from the whole juice-sacs and juice-sac membrane which was obtained after the juice-sacs were squeezed for juice. Each fraction was dried in an oven at 70°C until there was no reduction in weight. The dried materials were digested with 10 : 1 conc. HNO_3 and conc. H_2SO_4 solution, according to that described by Matoh *et al.* (1986). Total calcium was analysed by Jackson's method using Atomic Absorption Spectrophotometer (Jackson, 1986).

Internal gas of pummelo was collected by vacuo under water. Carbon dioxide and ethylene concentrations were measured with a TCD and FID Gas Chromatograph (Shimadzu, GR-RIA) respectively.

Pectinesterase activity of the juice-sac membrane was determined according to the method described by Hagerman and Austin (1986).

Pummelos quality was evaluated for soluble solids content using hand refractometer, and titratable acidity by titration with 0.2 NaOH with an 'Atago' Auto-titrator. Firmness of the pulp was measured at the center of pummelos segments, after making a cross section of the whole fruit, with a firmness tester constructed from a commercial scale (Siriphanich, 1986). Percentage of extractable juice was determined by squeezing only the pulp of pummelos for juice. While peeling and separating pummelos into individual segments, the degree of pulp-soaking symptom was evaluted by giving scores from 0 (nil) to 4 (very serious).

RESULTS

Textural Deterioration

When pummelos were evaluated for the degree of pulp soaking, no symptom was found right after harvest in both cultivars. After 4 weeks of storage at room temperature, however, the symptom was clearly developed, particularly in Khao-thongdee from both orchards as expected. When the evaluation was repeated in October, similar result was obtained (Figure 1). (Data shown were from orchard A only). In addition, there was significant difference between pummelos from the two orchards while such difference was not found in the earlier experiment (Table 1).

Firmness and extractable juice of pummelos were also determined in an attempt to use these two parameters as objective parameters of accessing the 'pulp-soaking' symptom. However it was not successful. The results are shown in Figures 2a and 2b. It could be seen that there was no clear difference between either cultivars or time in storage (Table 1). Although, there seemed to be a reduction in firmness and an increase in extractable juice with time in storage.

Soluble solid content of pummelos slightly increased during storage while titratable acidity decreased, resulting in an increasing sugar-acid ratio (Figure 2c). However, there were no differences in sugar-acid ratio between cultivars or time of harvesting, but there was a difference between orchards in the late season experiment (Table 1).

Juice-Sac Structure

Anatomical study of pummelos juice-sacs revealed that the juice-sac composed mainly of enlarged parenchyma cells and covered with two types of cells, the epidermal cells with very thick outer wall, and 2-4 layers of subepidermal cells (Figure 3a). In some section, colored materials was observed at the surface of epidermal cells. This material was probably cuticle on the surface of juice-sacs similar to that reported by Fahn *et al.* (1974). Difference between the two cultivars was apparent. In Khao-thongdee, the outer wall of the epidermal cells was about twice as thick as that of Khao-pan. However, Khao-thongdee contained only 2-3 layers of subepidermal cells while Khao-pan contained 4-5 layers (Figures 3a and 3b). No other structural difference was found between the samples from different times of storage, times of harvesting or from different orchards. After one month of storage no structural change was found in Khao-pan while in Khao-thongdee, more broken side walls of the epidermal and subepidermal cells could be clearly observed (Figure 3c).

Calcium Content

Calcium content of pummelos juice-sacs was determined from the juice as well as the juice-sac membrane. Most of calcium was found in the juice-sac membrane as shown in Figure 4. Khao-thongdee contained significantly higher amount of calcium than Khao-pan. However, no clear difference was found between calcium content in pummelos from different harvesting times, storage times, and orchards (Table 2).

Gas Composition

Internal carbon dioxide and ethylene concentrations of both pummelos cultivars were similar but there seemed to be an increase in concentration of carbon dioxide with time of storage and time of harvesting but the difference was less than one percent (Figure 5a). For ethylene concentration the opposite trend was found, but it should be noted that there was quite a large variation in ethylene concentration inside pummelos (Figure 5b., Table 2).

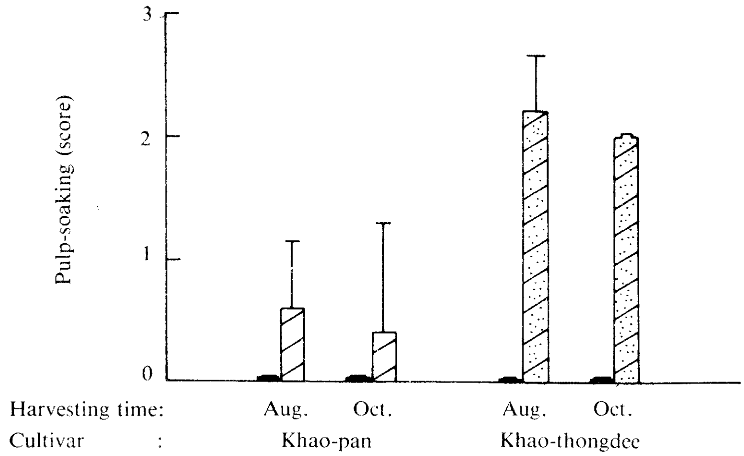


Figure 1 Degree of pulp-soaking developed in pummelos at harvest (▨), and after one month in storage at room temperature (▨). T on each bar indicates standard deviation.

Table 1 Summary of statistical significant levels for time in storage, orchard, and cultivar on pulp-soaking, firmness, extractable juice, and sugar-acid ratio of pummelos pulp.

Source of variation	Pulp-soaking	Firmness	Extractable juice	Sugar-acid ratio
August harvesting				
Time in storage (A)	**	**	**	*
Orchard (B)	NS	**	NS	**
Cultivar (C)	**	NS	**	NS
A × B	NS	NS	*	*
A × C	**	NS	NS	NS
B × C	**	NS	NS	**
A × B × C	**	NS	NS	NS
October harvesting				
Time in storage (A)	**	NS	NS	**
Orchard (B)	**	**	NS	NS
Cultivar (C)	**	**	NS	NS
A × B	**	NS	NS	NS
A × C	NS	NS	NS	NS
B × C	NS	*	NS	NS
A × B × C	NS	NS	NS	NS

*, **, NS; Significant at P = 0.5, 0.01, and nonsignificant, respectively.

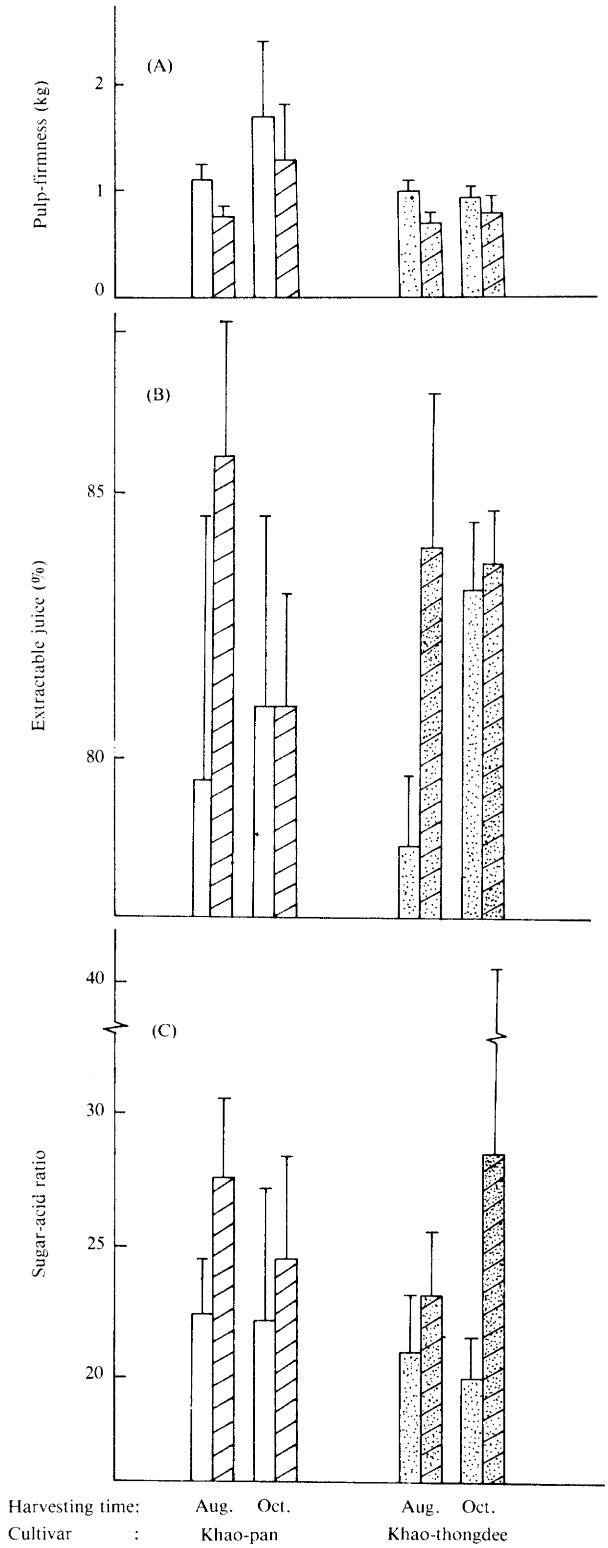
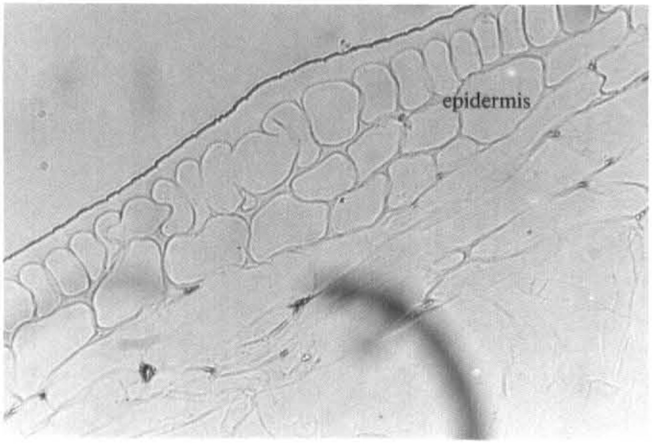
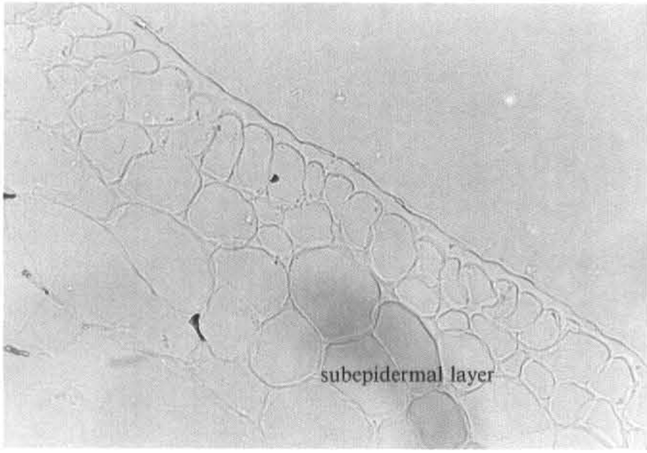


Figure 2 **Pulp firmness (A), extractable juice (B), and sugar-acid ratio (C) at harvest (▨) and after one month in storage at room temperature (▨). T on each bar indicates standard deviation.**

(A)



(B)



(C)

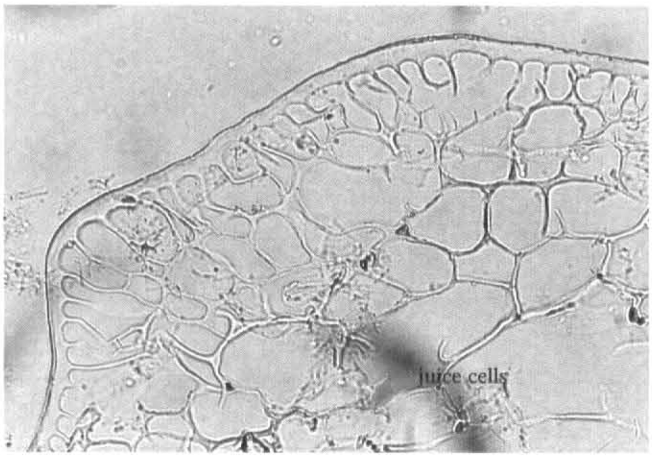


Figure 3 Cross sections of pummelo juice-sac (400 X) :

(A) Khao-thongdee at harvest (notice the thick outer wall of the epidermis).

(B) Khao-pan at harvest (notice number of subepidermal layer).

(C) Khao-thongdee one month after harvest (notice the broken side wall of both epidermal and subepidermal cells).

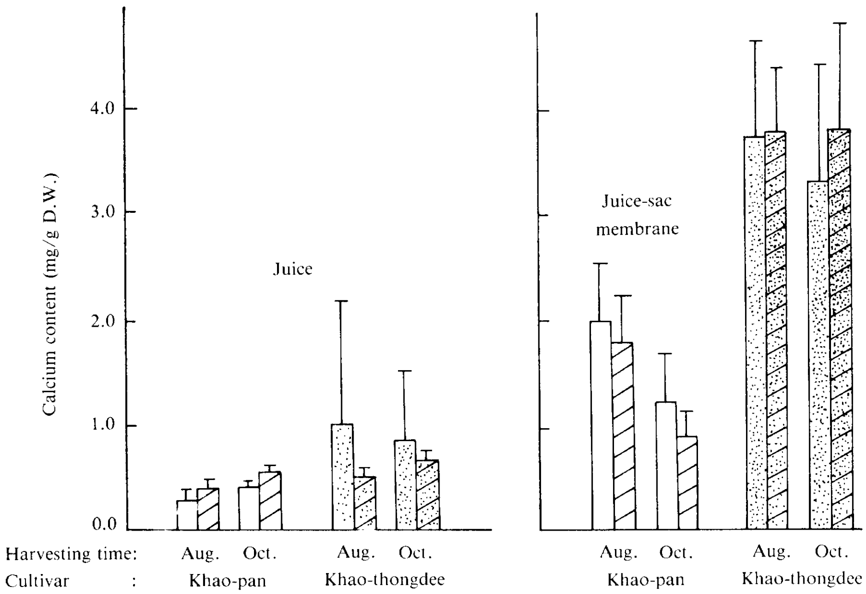


Figure 4 Calcium content determined from juice and juice-sac membrane of pummelos at harvest (▨), and one month after storage at room temperature (▨). T on each bar indicates standard deviation.

Table 2 **Summary of statistical significant levels for time in storage, orchard, and cultivar on calcium content, carbon dioxide and ethylene concentration, and pectin-methylesterase activity of pummelos pulp.**

Source of variation	Calcium content			Gas conc.		Pectin-methyl-esterase activity
	Juice	Juice-sacs membrane	Juice-sac	CO_2	C_2H_4	
August harvesting						
Time in storage (A)	NS	NS	NS	**	NS	—
Orchard (B)	NS	*	NS	NS	NS	—
Cultivar (C)	*	**	**	NS	NS	—
A x B	NS	NS	NS	NS	NS	—
A x C	NS	NS	NS	NS	NS	—
B x C	NS	NS	NS	NS	NS	—
A x B x C	NS	NS	NS	NS	NS	—
October harvesting						
Time in storage (A)	NS	NS	NS	NS	**	NS
Orchard (B)	NS	NS	**	NS	NS	NS
Cultivar (C)	*	**	**	*	*	**
A x B	NS	NS	NS	NS	*	**
A x C	NS	NS	NS	NS	**	NS
B x C	NS	NS	NS	NS	NS	NS
A x B x C	NS	NS	NS	NS	*	NS

*, **, NS Significant at P = 0.5 and 0.01 and nonsignificant respectively.

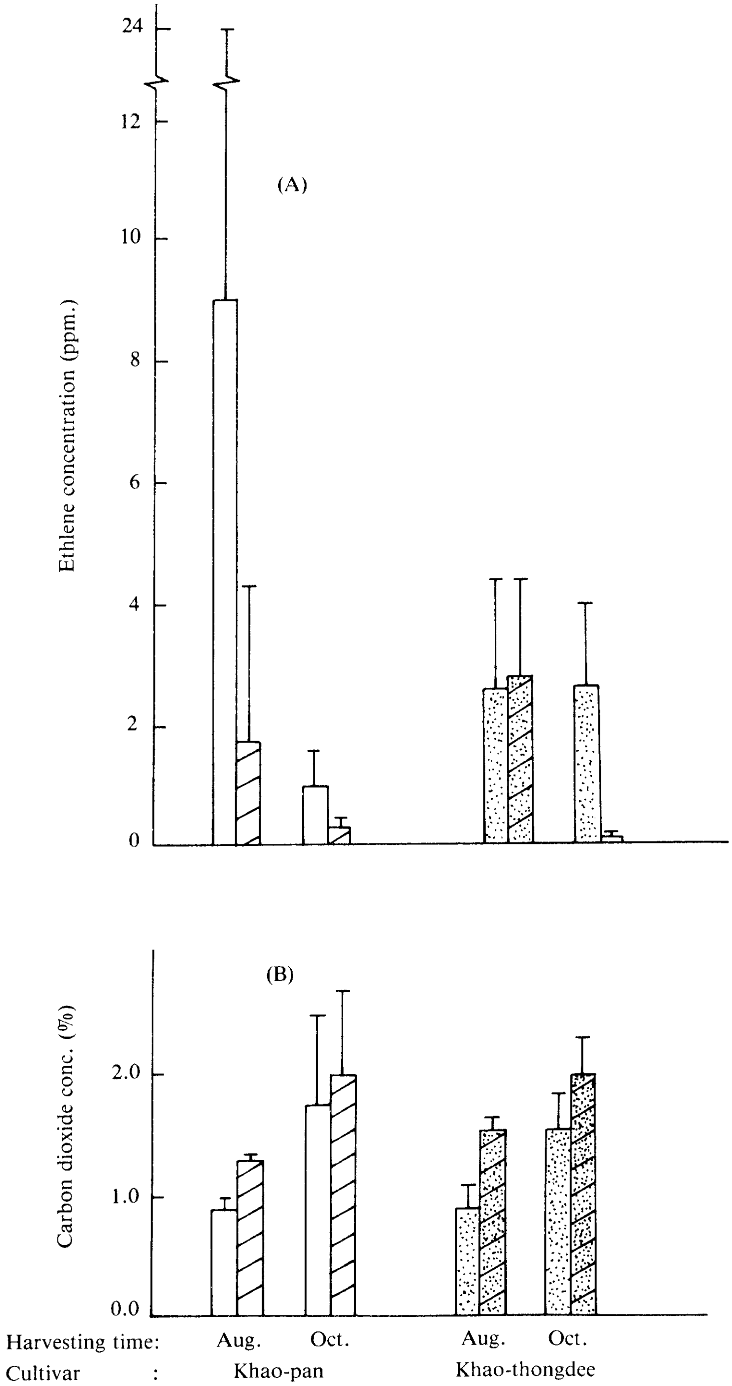


Figure 5 Internal ethylene (A) and cabon dioxide (B) concentration of pummelos immediately after harvest (▨), and one month at room temperature (▨). T on each bar indicates standard deviation.

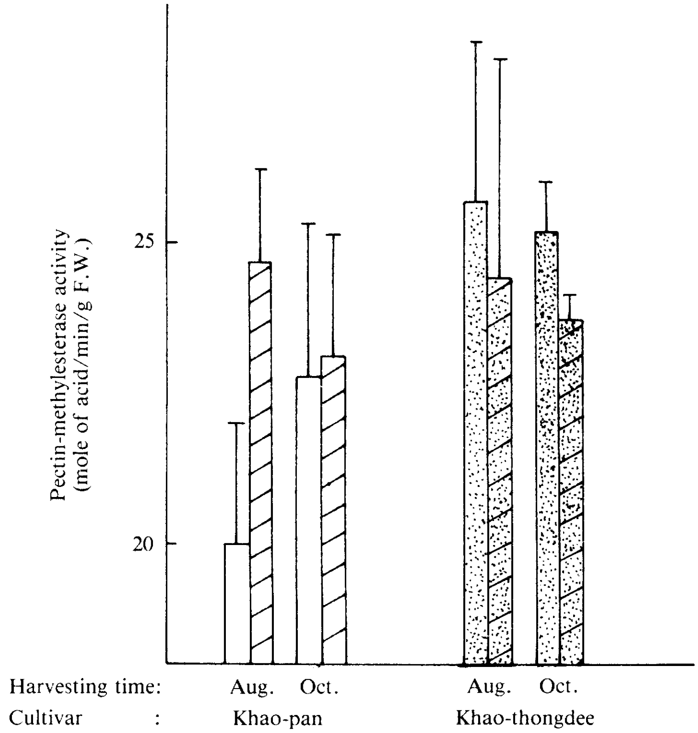


Figure 6 Activity of pectin-methylesterase of pummelos pulp at harvest (▩), and after one month at room temperature (▨). T on each bar indicates standard diviation.

Pectin-Methylesterase Activity

Very low pectin-methylesterase activity was found from the juice of pummelo. However, high activity was detected from the juice-sac membrane. Significantly higher acitivity of the enzyme was found in Khao-thongdee cultivar compared to that of Khao-pan. No difference was found between times of storage and between orchards (Figure 6, Table 2).

DISCUSSION

The development of pulp-soaking symptom in pummelos was more in the Khao-thongdee cultivar as expected (Figure 1). However, the symptom could not be clearly related to the firmness or extractable juice (Figures 2, 3 and Table 1). Thus, the measurement, of degree of pulp-soaking had to rely entirely on the judgement of the investigator instead of an objective procedure.

Structural study of pummelos juice-sac revealed a similar pattern of tissue organization to that reported by Shomer *et al.* (1988). When comparing the structure of the two cultivars and trying to explain the difference between the degree of pulp-soaking development, there were two structures that might have strong influence in the integrity of pummelos juice-sacs. One is the outer wall of the epidermal cells which was quite thick, the other was the number of subepidermal layers. The thicker the outer wall and the higher the number of subepidermal layers should give a stronger structure of the juice-sacs. Which of these two structures would give more effect on pulp-soaking development could be illustrated by considering the structure of Khao-thongdee cultivar. It had thicker wall and fewer numbers of subepidermal layers, but was very easily damaged while peeling after some time in storage. Thus it could be deduced that the number of subepidermal layer would be more influential than the thickness of the outer wall of the epidermis. In addition, after one month in storage, although the sectioning became very difficult, Khao-pan tissue was much easier to work with and no structural change could be observed from that immediately after harvest. On the other hand good slides from Khao-thongdee could not be easily obtained. Most sections of Khao-thongdee showed damaged inner wall of the epidermis and also the subepidermal cells (Figure 7). This evidence indicated for the first time in citrus that some changes occurred in the cell wall of juice-sacs and thus weakening the wall and the juice sac as a whole during storage. Although no change was oberved in the outer wall of the epidermis indicating its lesser role in juice-sacs deterioration, however, its involvement should not be completely ruled out. Ultrastructure study of pummelo juice-sacs should give more details information.

The result from calcium content determination was opposite to what was expected. Calcium was known to delay senescence and softening of many fruits (Buescher and Hobson, 1982). The main function of calcium in preventing softening was by cross-linking with pectic polymers in the cell wall and leaving reduced number of free carboxyl groups for the attack by polygalacturonase (Furguson, 1984). In tomato, it was shown that there was a removal of bound calcium from the middle lamella, thus allowing the degradation process to occur (Poovaiah, 1979). However, more recent information indicated that no such removal of calcium occurred during ripening. On the opposite, calcium content in the outer pericarp of tomatoes increased with ripening in relation with pectin-methylesterase activity which de-esterified the pectic polymers and exposed free carboxyl groups for calcium to bind (Burns and Pressey, 1987). The increase in calcium content thus made the outer pericarp of tomatoes firmer than the inner part of the fruit as well as that of peaches which was shown to contain low level of calcium and pectin-methylesterase. If calcium also plays a significant role in the deterioration of pummelos texture, one would expect to see lower calcium content in the susceptible cultivar 'Khao-thongdee'. Although our data showed the opposite result, it must be noted that our analysis showed only the total calcium content of the juice-sacs, and not of the cell wall or the middle lamella. The interpretation of our data in relation to the symptom, therefore, could not be done at this time. The calcium content of the cell wall or the middle lamella could be done by conventional method but it was thought to cause contamination of cell wall by calcium from the external media (Brady *et al.* 1985). A better technique reported by Burns and Pressey (1987) would give a better solution.

Another major factor influencing fruit softening that had already been shown in many fruits was polygalacturonase which cleaved pectic polymer into smaller molecules. Pectin-methyesterase was thought to support the function of polygalacturonase by de-esterified the methylated pectin. In citrus, no study involving tissue softening was conducted. However, very low activity of polygalacturonase was reported in grapefruit (Riov, 1975). Although pectin-methylesterase was thought to play a minor role in fruit softening, its role might be significant in tissue that contained very low level of polygalacturonase activity. Thus, the higher activity in Khao-thongdee could be interpreted in two ways; a) higher activity enhanced the de-esterification of pectic polymers and allowed polygalacturonase to degrade the wall more than that in Khao-pan, b) pectin-methylesterase also allow the binding of calcium to the de-esterified pectin. The interpretation of our data in relation to the development of pulp soaking symptom, therefore was quite difficult. More information is needed in term of changes in pectic materials, polygalacturonase activity, as well as the calcium content in the wall itself.

Atmoshperic condition was known to change the internal gas composition inside fruits and thus influencing the ripening process (Kader, 1985). Our data indicated a significant difference in both carbon dioxide and ethylene concentration that might resulted in a different rate of texture deterioration. However, the difference between the two cultivars was only about 1 percent for carbon dioxide and 1 ppm. for ethylene which was quite low to give significant physiological responses. In addition, after one month in storage carbon dioxide concentration seemed to increase while ethylene concentration decreased. The changes in concentration of both gases were in opposite direction to what it should be if they were involved in the development of pulp-soaking symptom. Since we learned from other fruits that higher concentration of carbon dioxide and low concentration of ethylene would delay the softening process (Smock, 1979), the involvement of carbon dioxide and ethylene content inside pummelos was not likely.

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