Chapter 4

Effect of prestorage factors on quality of 'Nam Dok Mai' mango under low temperature storage

4.1 Introduction

Mango (*Mangifera indica* Linn.) is a highly perishable crop. In Thailand mango fruits are consumed locally and some are export. However the economic production of mango in Thailand for export has been limited because of a lack of high quality fresh fruit to satisfy consumers in both domestic and export markets. The relative importance of quality depends upon maturity at harvest before marketing, which is one of the most important factors to determine storage life and final fruit quality. Moreover, storage conditions such, as temperature are important factors because low temperature storage can generally improve storability, but unfortunately it sometimes induces the chilling injury of mango. Under storage below 10 °C the fruit of all cultivars of mango may be susceptible to develop chilling injury (Mendoza, 1981). However, different cultivars vary for the time required to develop the visible symptoms. Also, it has been shown that different harvesting time affected the duration of developing the chilling injury symptoms (Ketsa and Raksrithong, 1992).

VHT has been used against mango fruit fly, *Dacus occipitallis* Bezzi and melon fly, *Dacus cycyrbutae* (Mendoza and Wills, 1984).

VHT has been used for the export of mango cultivars such as 'Carabao' from The Philippines (Quimio, 1973), 'Nang Klangwan' from Thailand (Unahawatti et al.,1986), 'Harumanis' from Malaysia (Latifah et al., 1996) and 'Kensington' from Australia (Heather et al., 1991; Jacobi and Wong, 1992). For the long distances from Thailand to an export market, low temperature storage has been required during transit periods. Combinations of VHT and low temperature storage can affect mango fruit quality. This has been reported for 'Kensington' mango from different production regions and there were treated at 46.5 °C for 10 min (Jacobi and Wong, 1992).

Fruit responses to heat treatment have been reported in avocado (Woolf et al., 1995), citrus (Wild,1993; Rodov et al., 1995), lemon (Schirra and Mulus, 1995), mango (McCollum et al., 1993), persimmon (Burmeister et al., 1997), cucumber (McCollum et al., 1995; Chan and Linse, 1989), pepper (Mencarelli et al., 1993), zucchini (Wang, 1994), apple (Klein and Lurie, 1990) and Tomato (Lurie and Klein, 1991). However, the responses may depend on cultivars, location, preharvest environmental factors, maturity at harvest, and also the time and temperature to which commodities are exposed. In spite of the positive response of heat treatment to reduce the sensitivity of chilling injury, the treatment of mango may cause tissue damage symptoms such as poor color development, abnormal ripening, the lack of starch breakdown and internal breakdown (Mitcham and McDonald, 1993; Paull, 1995).

Moreover exposure to low temperature after heat treatment induces biochemical and physiological changes such as changes in

polyamines level. It was observed that pretreatments that increased polyamine levels induced more resistance to chilling injury. (McDonald and Kushad, 1986; Serrano et al., 1996). However, recent knowledge is not clear about the interrelationship between the development of chilling injury after heat treatment and physiological and biochemical changes during low temperature storage and after transferring to 20 °C. This study was conducted to clarify these points.

4.2 Physiological and biochemical changes in 'Nam Dok Mai' mango under low temperature storage

4.2.1 Materials and Methods

Fruits

Mango fruits were harvested from Thailand with 2 maturity stages (floating in 1% NaCl solution was considered as immature fruit and sinking was considered as mature). They were divided into two groups: Non-VHT and VHT. VHT procedure was conducted at the Center for Production Development and Pest Control located in Kasetsart University, Bangkok, Thailand. During VHT, the temperature reached 47°C in the inner mesocarp and chamber temperature was around 51.5 °C, when this temperature was held for 10 min. Then, the chamber was cooled down until reaching room temperature, and the VHT-treated mangoes were transported to Japan on the same day.

Storage condition

Fruits of each group, that is, mature and immature fruit either VHT and non-VHT, were kept at 5, 8, and 13 °C. At 7-day intervals, the fruits were taken and transferred to 25 °C for ripening until eating ripe for studies on fruit quality, storability and physiochemical changes as follows:

Peel color changes

Peel color was measured by a color difference meter model CR-100 (Minolta Camera Co., Ltd.).

Firmness

Flesh firmness was measured by a fruit rheometer (type NRM-202J) connected with a 7 mm diameter plunger which penetrated to a depth of 10 mm at the rate of 50 mm/min. The average value was calculated.

CO_2 evolution

CO_2 was determined by the same method as described in Chapter 2.

Chilling injury

CI was evaluated by means of an arbitrary scale of visual

symptoms, based on skin blackening, pulp browning and intensity of ripening: 1= normal, 2 = slight, 3 = medium and 4 = severe CI symptoms. A CI index was determined using the formula : Σ (CI symptom scale (1-4) x number of corresponding fruit within each class) / total number of fruits estimated.

Internal breakdown (IB)

Evaluation of IB was performed by slicing the fruit longitudinally along the seed coat and rating severity as a score, 1 = no IB; 2 = slight (10% of cut surface affected); 3 = moderate (10-25% of cut surface affected); and 4= severe (25-50% of cut surface affected).

Total soluble solid (TSS) and titratable acid (TA)

TSS was determined by a hand refractometer and TA was determined by titrating 10 ml of extracted juice with 0.1 NaOH and expressed as percentage of citric acid.

Determination of storage life

The number of days to ripeness (when fruit reached the softness determined by touch and peel color turned fully yellow) was determined.

Membrane permeability

Membrane permeability was determined by the same method as described in Chapter 3.

Polyamines analysis

The same method was employed as described in Chapter 3.

4.2.2 Results

Firmness

For VHT treated fruit, the initial firmness value was 10 kg/cm^2 in both ripening stages, while those of non-VHT treated fruit were 11.5 in both ripening stages, respectively. Generally, the higher the storage temperature the more rapid was the decrease in firmness (Fig. 4-1). This is particularly the case for the fruit stored at 25 °C, where the firmness decreased to about 2 kg/cm^2 within 4 days regardless of maturation and VHT treatment. VHT treated and non-VHT treated of the same maturity stage fruit showed a slight difference in firmness changes during storage; the decrease in firmness was more pronounced for the non-VHT fruits.

Peel color development

Fig. 4-2 shows that peel color development of fruit after VHT was advanced when stored at all temperatures especially at 13

°C. Thus, VHT treated fruits developed color better than non-VHT treated fruit during storage and after transferring for ripening. When compared with maturity, the mature fruits developed peel color faster than immature ones.

Total soluble solids (TSS)

TSS in the fruit showed the highest level in VHT-treated mature fruit, although the difference was rather small. Storage temperature, maturity and VHT had no effect on TSS when the fruits were transferred to 25 °C. The TSS value between the fruit stored at the low temperature and that of ripened fruit at 25 °C after storage was no different (Fig. 4-3).

Titratable acidity (TA)

VHT-treated fruit seemed to reduce TA levels more than non-treated ones when kept at 5 °C for 1 week and 8 °C for 1-2 weeks and transferred to 25 °C although the difference was small, while fruits stored at 13 °C did not change in TA at all treatments from 1 week until the end of storage (Fig. 4-4).

Electrolyte leakage

At the lower storage temperature, the higher leakage from the tissues was observed and immature fruit showed slightly more leakage. Non-VHT fruits showed an increase in leakage until the

end of the experiment (4 weeks) while VHT fruits showed an increase in leakage until 7 days and then did not change appreciably (Fig. 4-5).

Respiration rate

The CO_2 production of the non-VHT treated fruits was generally higher than that of the treated ones. When stored at 8°C, non-VHT immature and mature fruit increased CO_2 production to the highest level on the 7th and 14th days, respectively, and then decreased until the end of storage (Fig. 4-6). When kept at 13°C the increase of CO_2 was shown only in non-VHT mature fruit, while fruits stored at 5 °C did not show any appreciable increase in CO_2 evolution.

Disease incidence

The serious disease problem after storage in this experiment was anthacnose, the severity of which differed markedly by difference in maturities (Fig.4-7). When stored at 5 °C for 14-21 days, disease incidence increased remarkably after transfer to 20 °C. Non-VHT treated fruit, when stored at 13 °C, showed marked disease incidence after 7 days in storage and transfer to 20 °C, particularly for mature fruit. Fruit stored at 8 °C showed a very low incidence of disease until 21 days of storage (Fig. 4-8).

Chilling injury

Chilling injury appeared in the fruits stored at 5 and 8 °C. The incidence was more severe for non-VHT fruits while VHT treated fruits, particularly mature fruits, showed only slight chilling injury (Fig. 4-9).

Internal breakdown

The incidence of internal breakdown was observed in VHT treated, but not in non-VHT fruit. As for VHT treated fruit, there was significantly higher IB in immature fruit ($p \leq 0.05$) than mature fruit when stored for 3 weeks at 5 and 8 °C and then transferred to ripen at 20 °C. The incidence of internal breakdown was affected by the length of storage time; the longer storage time, the more incidences there were (Fig. 4-10,11).

Polyamines levels

Free polyamine levels were analyzed in mango fruits when stored at different temperatures. Three types of polyamines were found, i.e., putrescine, spermidine and spermine.

In VHT-treated fruits the Put content increased and reached the maximum at the first and second weeks when stored at 5 and 8 °C, respectively and then decreased thereafter. A two fold increase in Put level was found in VHT treated mature fruits when kept at 5 °C as compared to non-VHT and immature fruits. Between

maturities, the mature fruits had the highest levels throughout the storage time. As for storage temperature, at 13°C non-VHT treated fruit remained very low in Put levels until the 3rd week of storage but VHT-treated fruit slightly increased to the highest levels at the second week (Fig. 4-12).

The Spd levels tended to decrease during storage time. VHT-treated mature fruits showed the highest Spd levels followed by VHT-treated immature fruits regardless of storage temperature. When stored at 13 °C the Spd level of non-treated fruits remained at a low level after the first week until the end of the storage time, while Spd in VHT fruits increased two fold by the 14th day (Fig. 4-13).

Spm level was initially much higher in VHT-treated fruits, particularly at 5 and 8 °C. A rapid decrease in Spm after storage for 1 week was observed, however, in VHT-treated fruit when stored at 5 and 8°C, and then it remained constantly low until the end of storage time. In fruit stored at 13 °C the initial Spm level was low and there was no difference among the treatments (Fig. 4-14).

4.3 Ultrastructure and microanalysis of mango fruit tissue.

4.3.1 Materials and Methods

For scanning electron microscopy, fruit samples were immediately frozen in liquid nitrogen and sections from the stem

end, middle and apex (Fig.4-15) were freeze-dried and sliced by a microtome (around 2 mm section thickness). For structural observation, the freeze-dried samples were sputter-coated with carbon and then observed by a JEOL T330 electron microscope equipped with an x-ray detection system (SED 882) for quantitative evaluation of x-ray spectra.

4.3.2 Results

Under the scanning electron microscope, the healthy and unripe tissue showed well-reserved cell integrity of the cells containing starch grains debonding each other (Fig. 4-16a). After VHT, the cells somewhat lost their integrity and the starch grains became dispersed, spreading out of the cells (Fig. 4-16b). When comparing the tissues of mesocarp from different parts, the density of Ca element demonstrated by digital mapping was the highest in the apex (Fig. 4-17, 18). There was no significant difference in Ca density between immature (Fig. 4-17) and mature fruits (Fig. 4-18). However, the tissue from immature fruit showed an obvious larger degree of cell separation at the apex where starch grains were loosely attached to the cell wall (Fig. 4-17c).

4.4 Discussion

Effect of maturity

This study confirmed that maturity at harvest was one of the most important factors that determined storage life and fruit quality when mango fruits were heat treated followed by low temperature storage. The maturity of fruit has been reported to strongly effect disease incidence and internal breakdown after heat treatment. VHT-treated immature fruit showed a higher susceptibility to internal breakdown following vapor heat treatment than mature ones (Esguerra and Lizada, 1990). The present observation also confirmed this as well as the report of the decreased CI of pepper with increasing maturity (Autio and Bramlage, 1986).

Effect of temperature

The most pronounced effects of different temperatures in this study were the reduction of both disease and the occurrence of chilling injury caused by storage at 8 °C. Also, the change in firmness during storage was influenced by temperature as well as maturity; non-VHT and immature fruit underwent softening faster than the other treatments when stored at 5 and 8 °C. In contrast, VHT-treated fruit showed slower changes in firmness. However, all of these fruits, after transfer to 20°C, rapidly decreased firmness to very low levels regardless of treatments so that no differences in level among the treatments were found, although they were still

acceptable in terms of texture and flavor characteristics after storage for less than 3 weeks (data not shown). This result shows that heat treatment might lessen the effect of chilling on softening. This coincides with the previous reports by Klein and Lurie (1990) that 'Granny Smith' and 'Anna' apples exposed to 38 °C for 4 days were firmer than non-heated control fruit. They suggested that this maintenance of firmness and the reduction in softening of fruit might be due to the inhibition of pectic hydrolysis. Heat treatment has also been reported to maintain firmness in papaya (Chan et al., 1981).

A two-fold increase in Put was found in VHT treated mature fruits when kept at 5 °C as compared to non-VHT immature ones. Temperature affected the accumulation of Put by delaying the peak for 1 week when stored at 8 °C. The decrease in this level was obvious after storage for more than 1 week at 5 °C and 2 weeks at 8 °C when the occurrence of chilling injury was investigated.

VHT-treated fruit also rapidly decreased Spd level after storage at 5 and 8°C for less than 2 weeks and 13°C for less than 1 week, then it increased slightly. The level of Spd in the VHT treated fruit was higher than that of non-treated ones.

Spermine showed no difference among the treatments with different temperatures. All together, the results obtained in this study indicated that the induction of higher levels of polyamines by heat treatment seems to be related with the decrease of chilling

injury in VHT treated fruits. McDonald and Kushad (1986) found that in lemons the conditioning reduced chilling injury symptoms and increased Put levels. Rodov et al. (1995) also mentioned that a significantly higher level of Put was found in hot-dip treated grapefruit as compared with untreated fruit stored for 4 weeks at the same temperature (2 °C). The reduction of CI symptoms by polyamines might be due to their capacity to preserve membrane integrity.

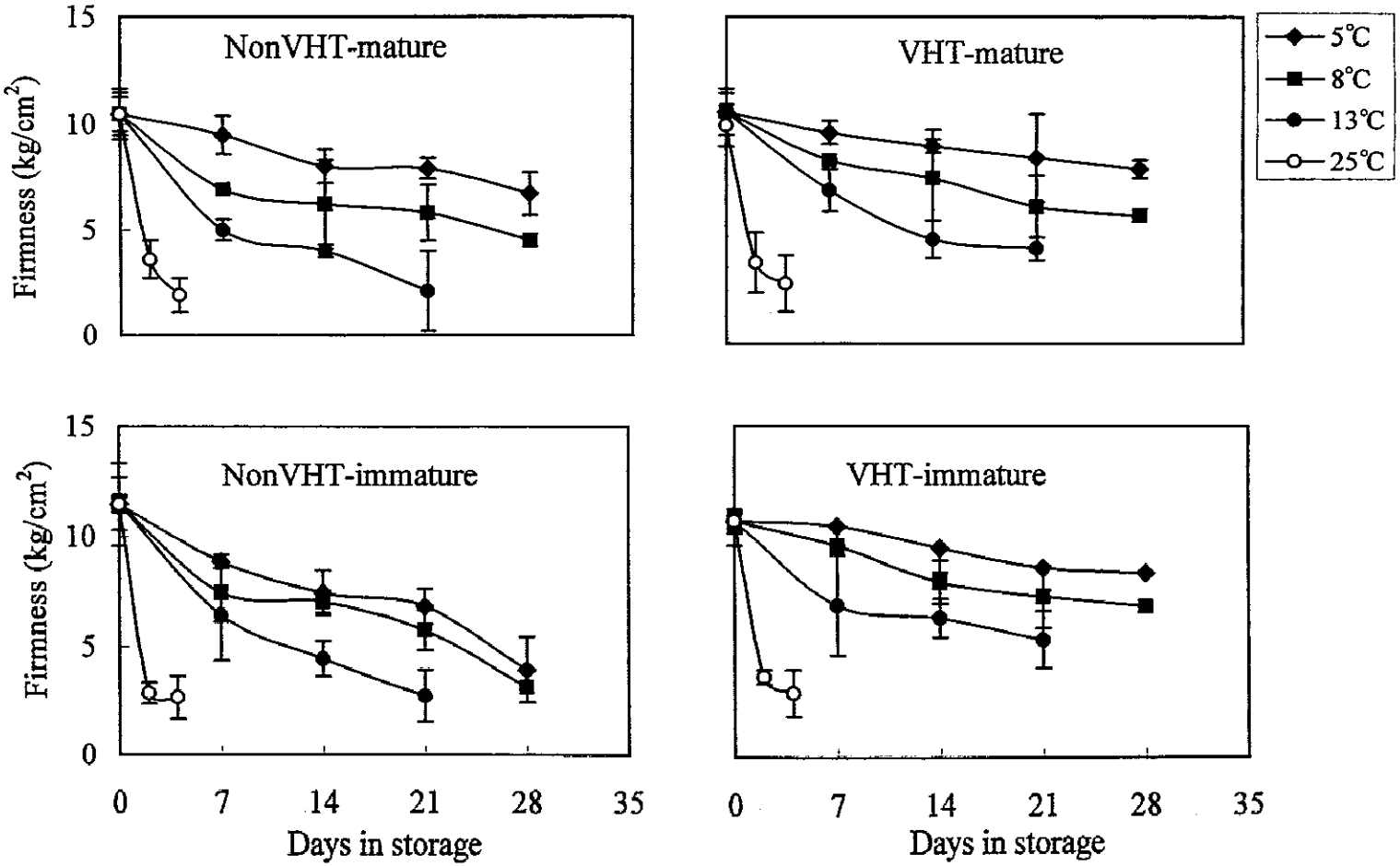

Fig. 4-1. Firmness of mango fruit with different maturities after VHT when stored at 5, 8 and 13°C.

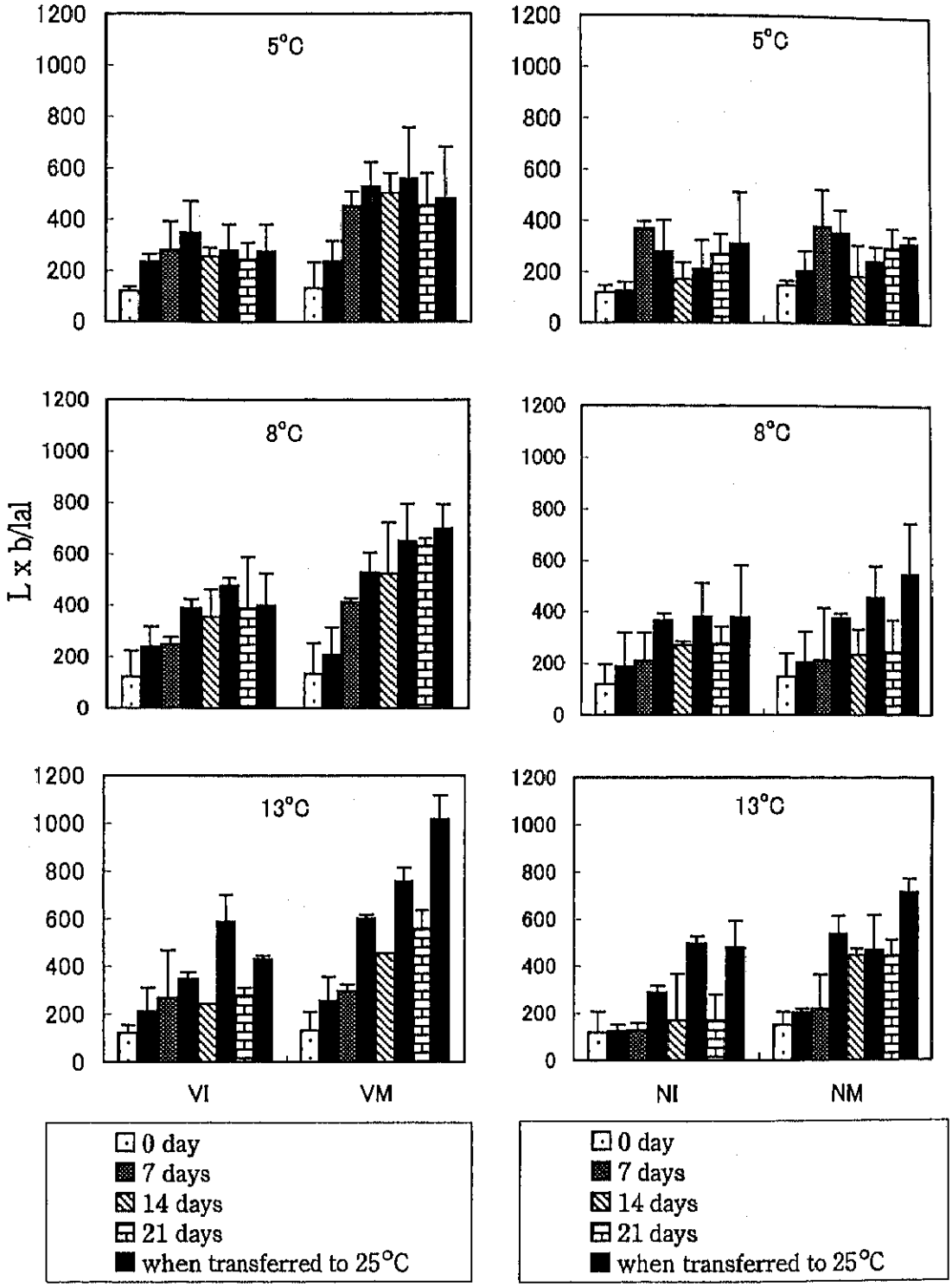

Fig. 4-2. Color changes of mango fruit with different maturities after VHT when stored at 5, 8 and 13 °C. (VI:VHT-immature, VM:VHT-mature, NI:Non VHT- immature and NM:Non VHT -mature)

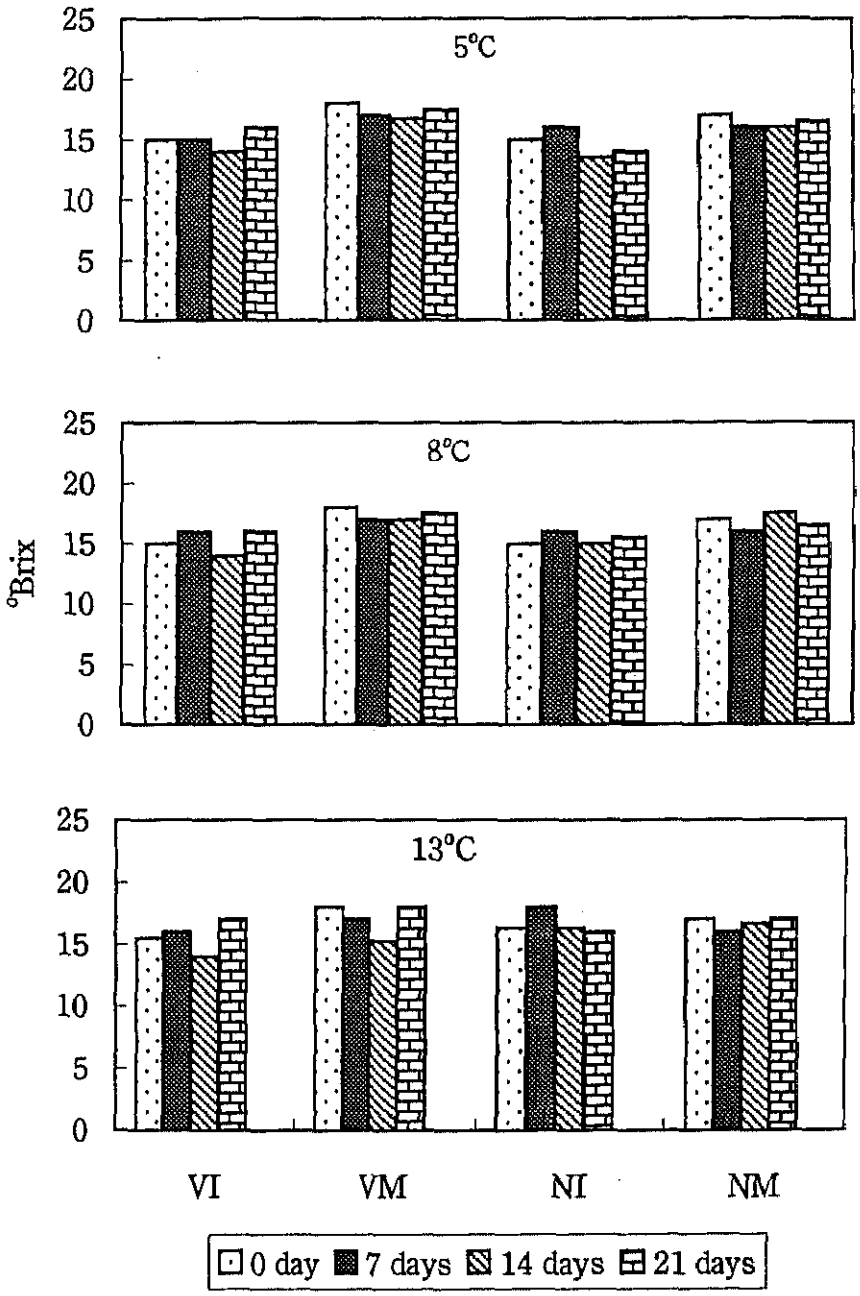

Fig. 4-3. Total soluble solids (TSS) of mango fruit with different maturities after VHT when stored at 5,8 and 13°C.
(VI:VHT-immature,VM:VHT-mature, NI:NonVHT-immature and NM:N on VHT- mature)

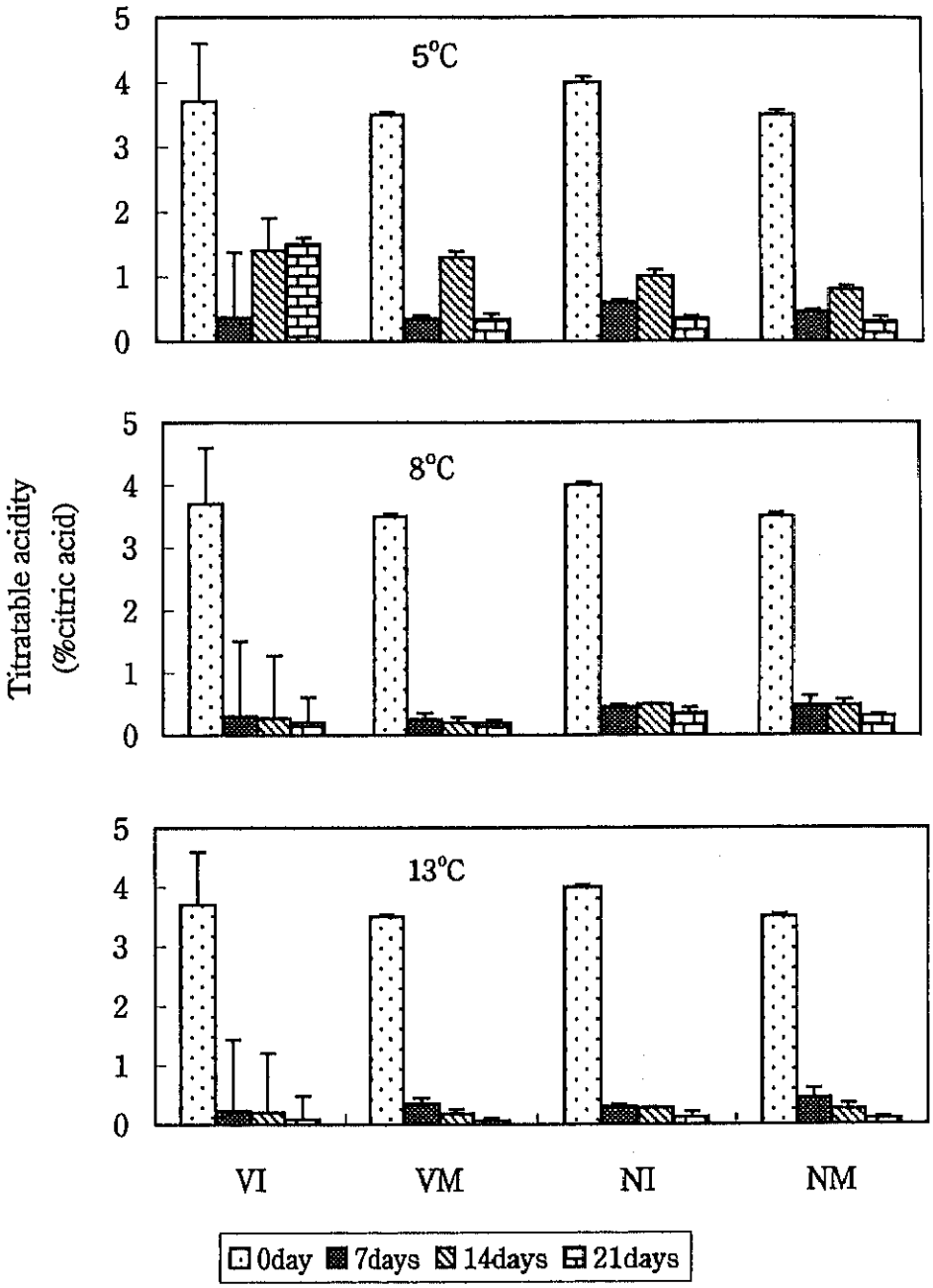

Fig. 4-4 Titratable acidity (TA) of mango fruit after VHT treated with different maturities when stored at 5, 8 and 13°C. (VI:VHT-immature,VM:VHT-mature,NI:NonVHT-immature and NM:NonVHT mature)

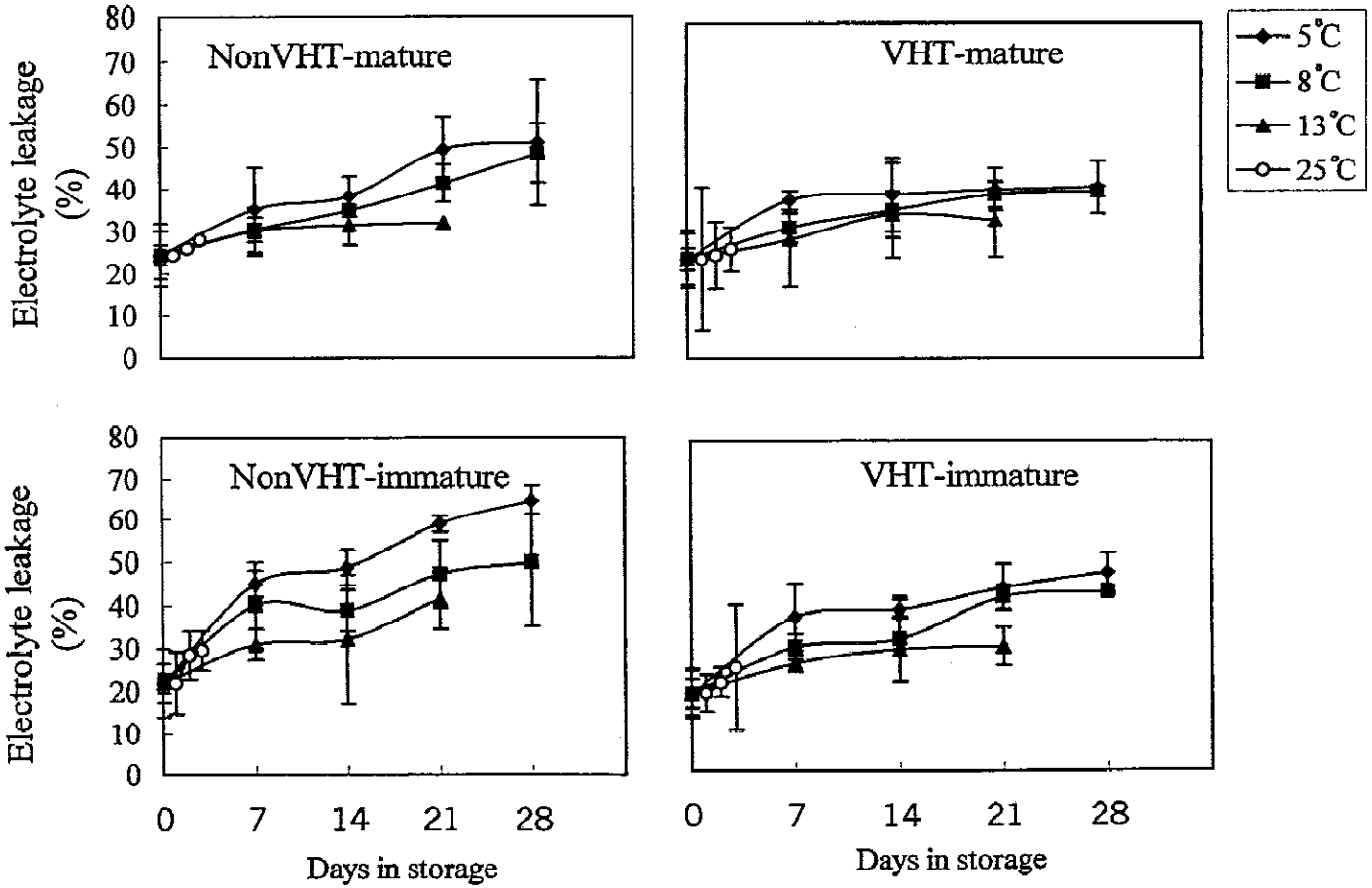

Fig. 4-5. Electrolyte leakage (%) of mango fruit with different maturities after VHT when stored at 5, 8 and 13 °C.

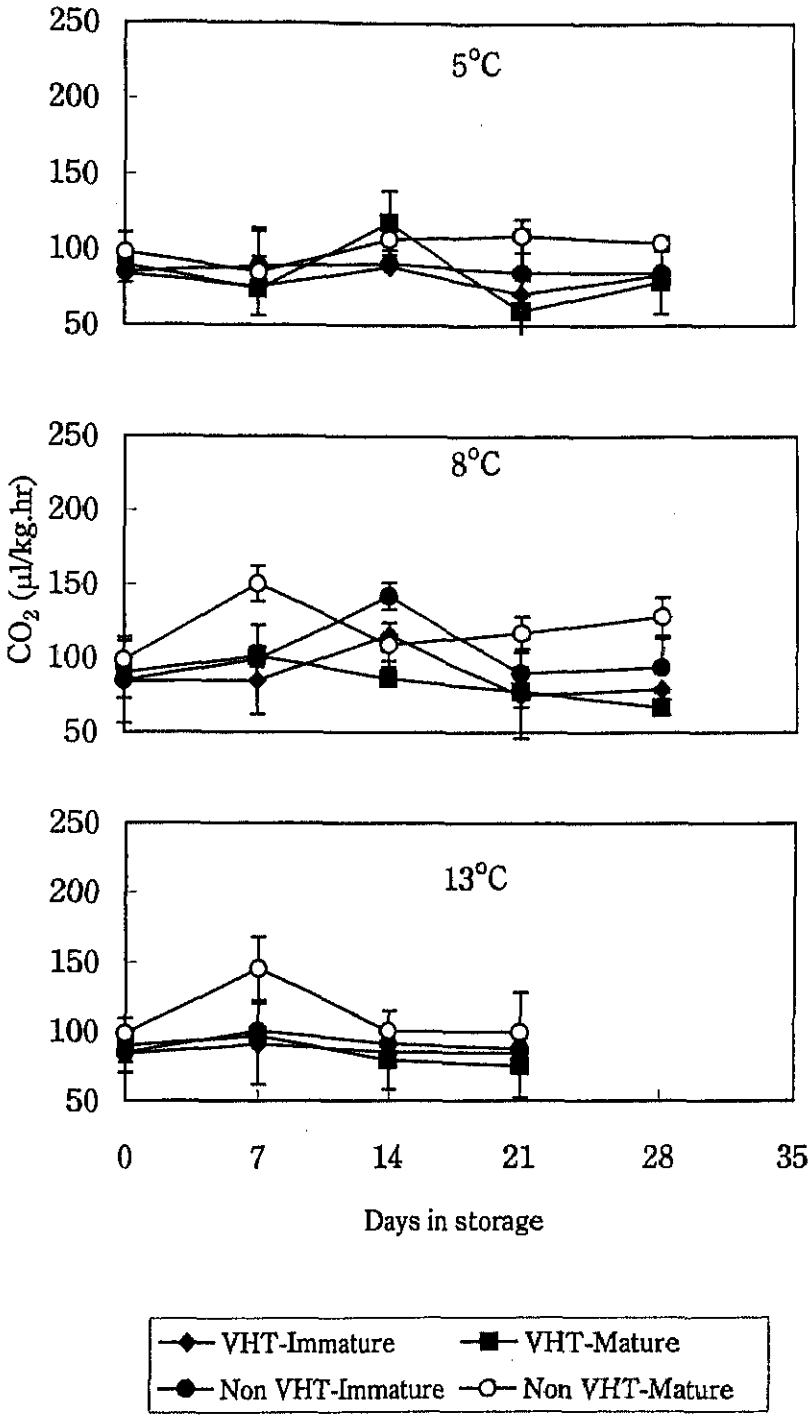

Fig. 4-6. Respiration rate of mango fruit with different after VHT maturities when stored at 5, 8 and 13°C.

Fig. 4-7. The ' Nam Dok Mai' mango fruits after VHT- treated (upper) and control (lower) with immature (left) and mature (right) fruits when stored at 5, 8 and 13°C for 2 weeks and transferred to ripen at 25 °C. (arrow marked the symptom of anthracnose in non-VHT fruits)

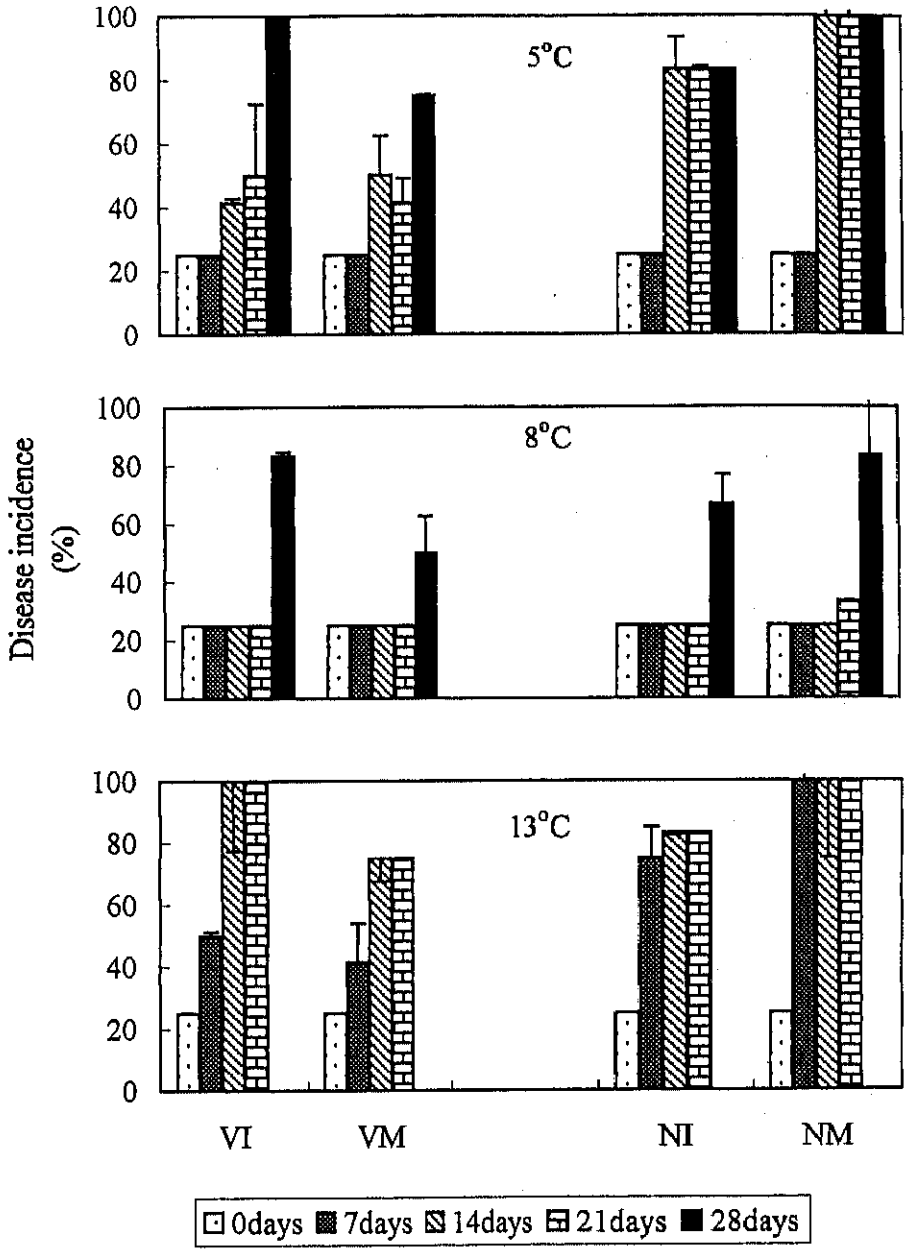

Fig. 4-8 Percent incidence of disease of mango fruit (with rating score, where 1=no occurence to 4=severe) with different maturities when stored at 5, 8 and 13°C (VI:VHT-immature, VM:VHT-mature, NI:NonVHT-immature and NM:NonVHT-mature).

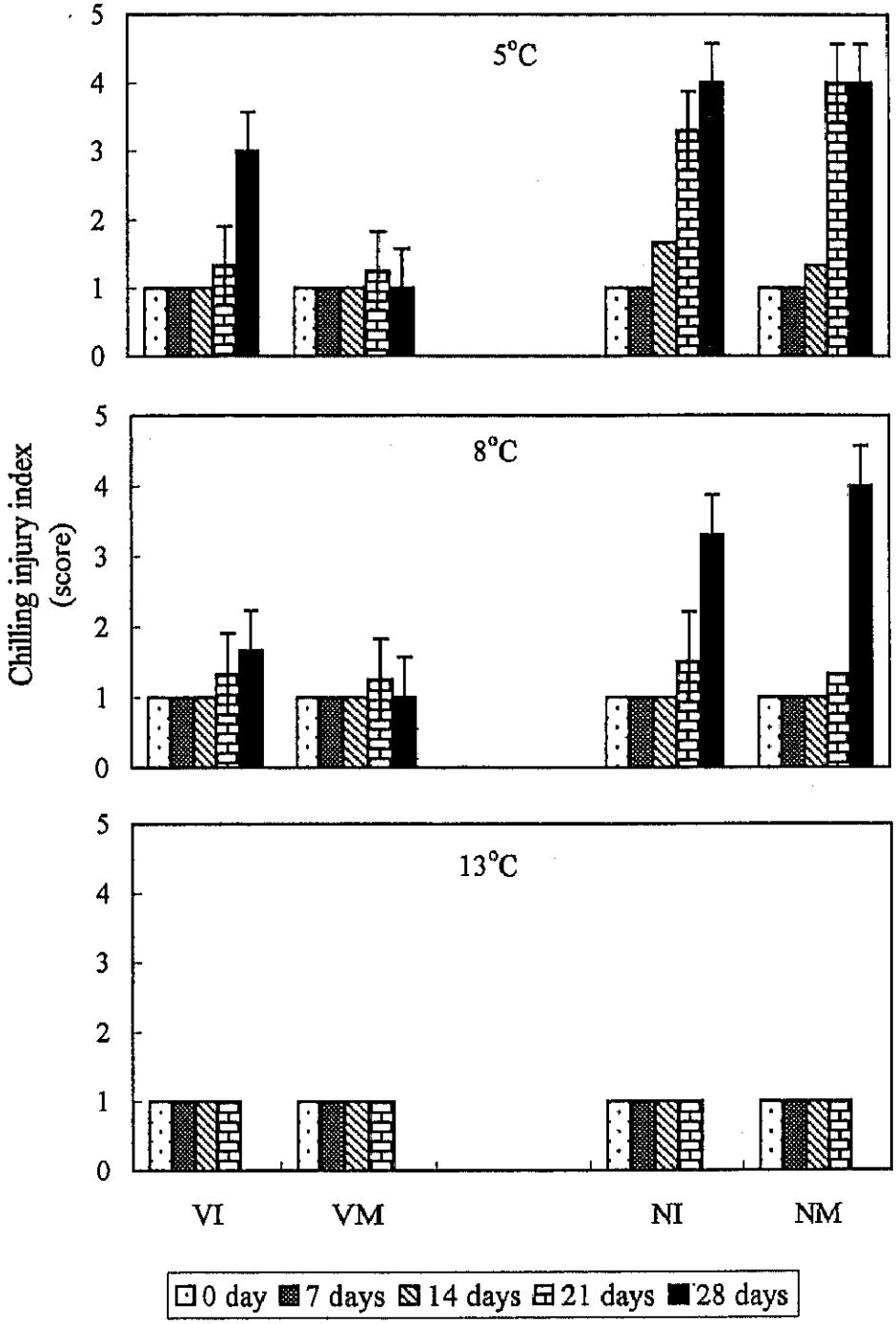

Fig. 4-9. Incidence of chilling injury symptom of mango fruit with different maturities with rating score, where 1=no symptom to 4=severe when stored at 5, 8 and 13°C. (VI:VHT-immature,VM: VHT-mature,NI:NonVHT-immature and NM:NonVHT-mature)

Fig. 4-10. 'Nam Dok Mai' mango showing symptom of internal breakdown after VHT with immature (left) and mature (right) when transferred to ripen at 25 °C.

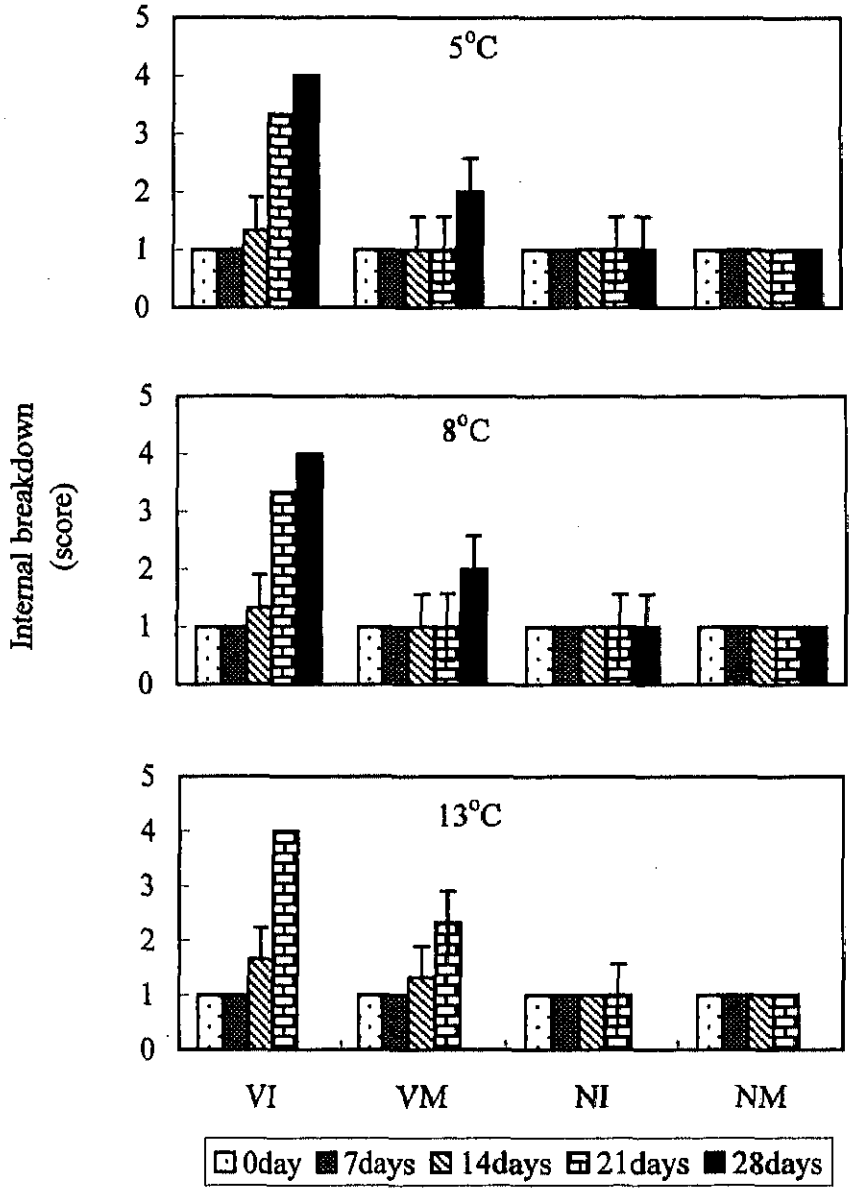

Fig. 4-11. Incidence of internal breakdown (IB) of mango fruit with different maturities (with rating score,where 1=no symptom to 4 =severe) when stored at 5, 8 and 13°C (VI:VHT-immature,VM:VHT-mature,NI:NonVHT-immature and NM:NonVHT-mature)

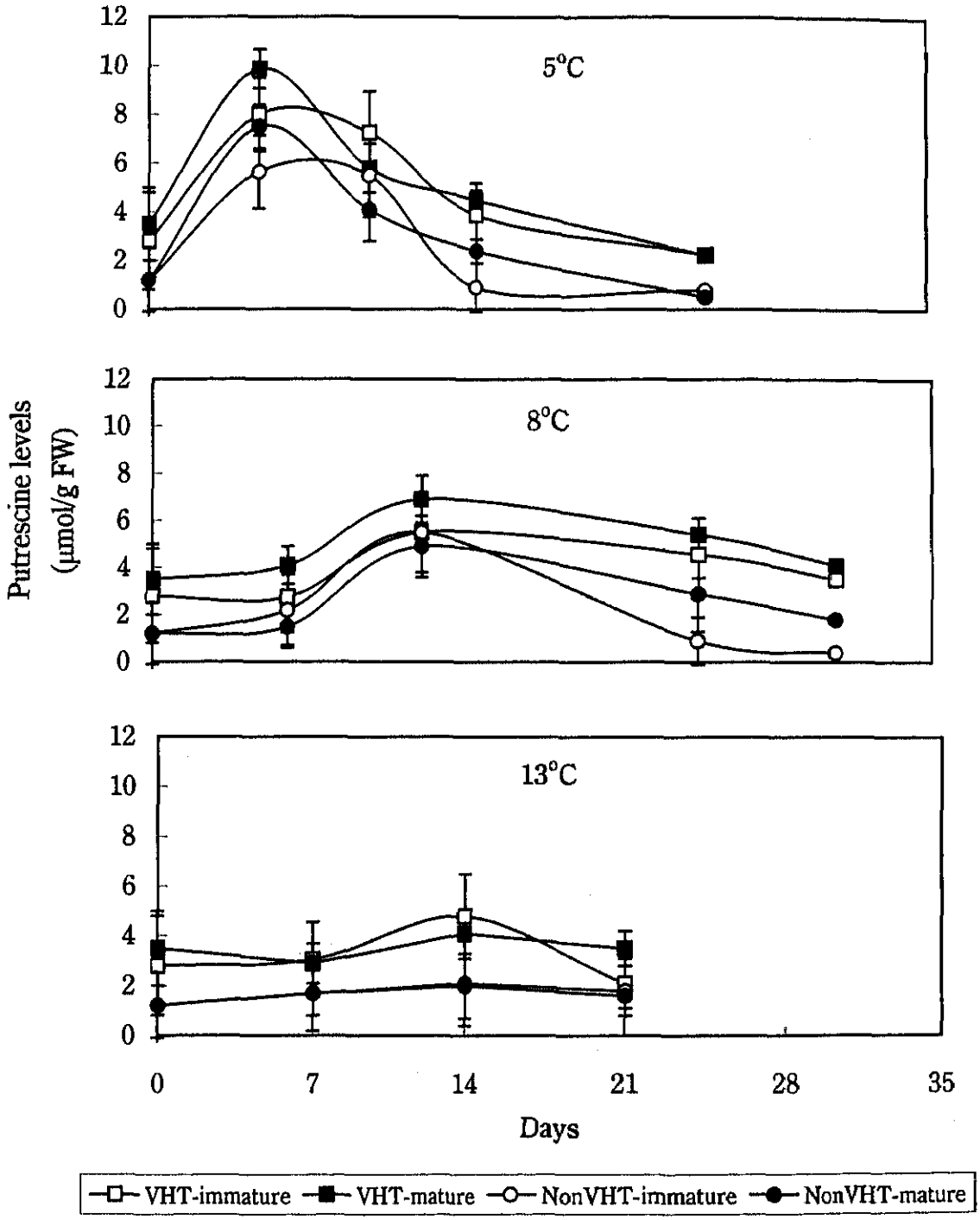

Fig. 4-12. Putrescine levels of mango fruits with different maturities after VHT when stored at 5, 8 and 13°C.

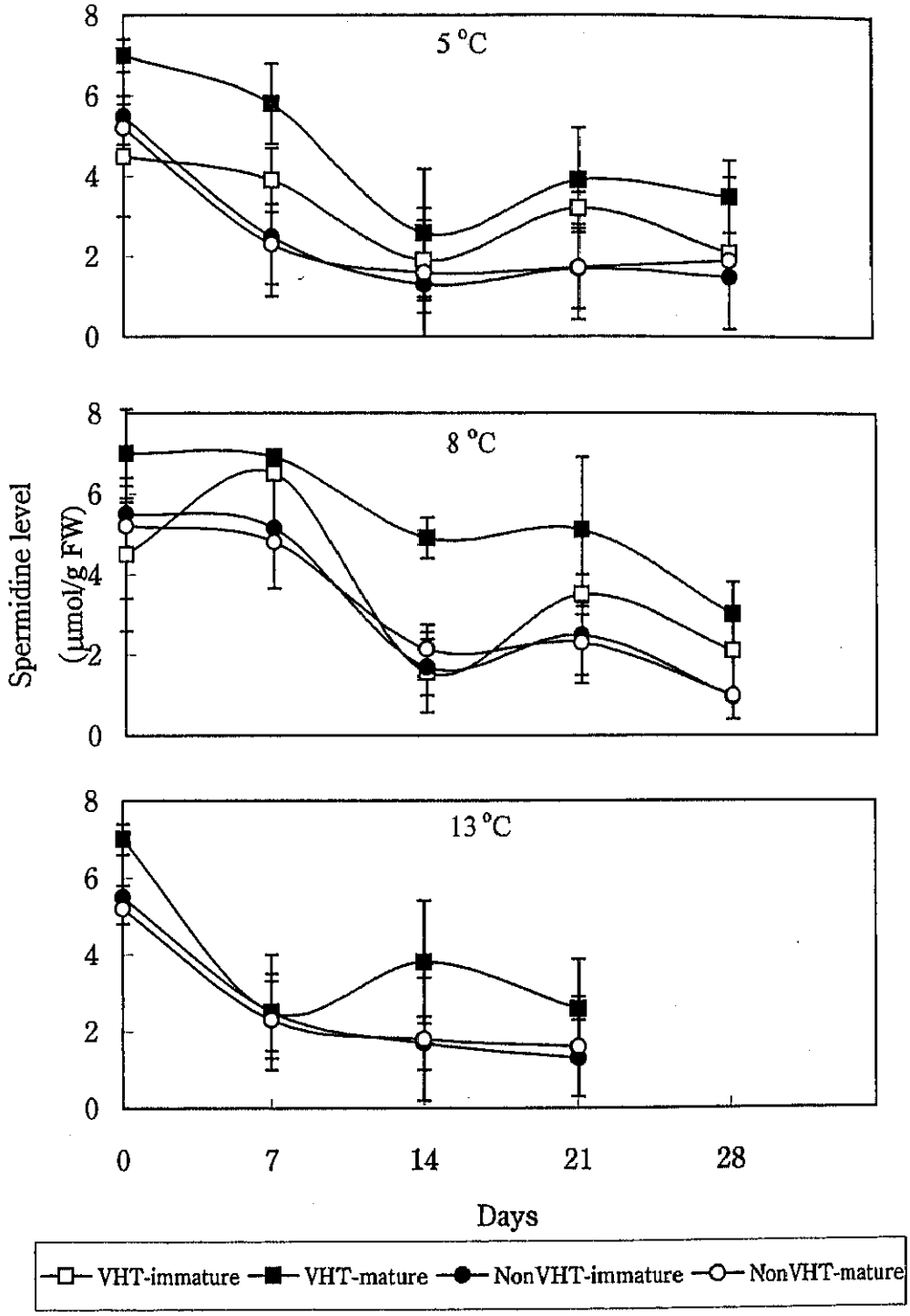

Fig. 4-13. Spermidine levels (μmol/gFW) of mango fruits with different maturities after VHT when stored at 5, 8 and 13°C

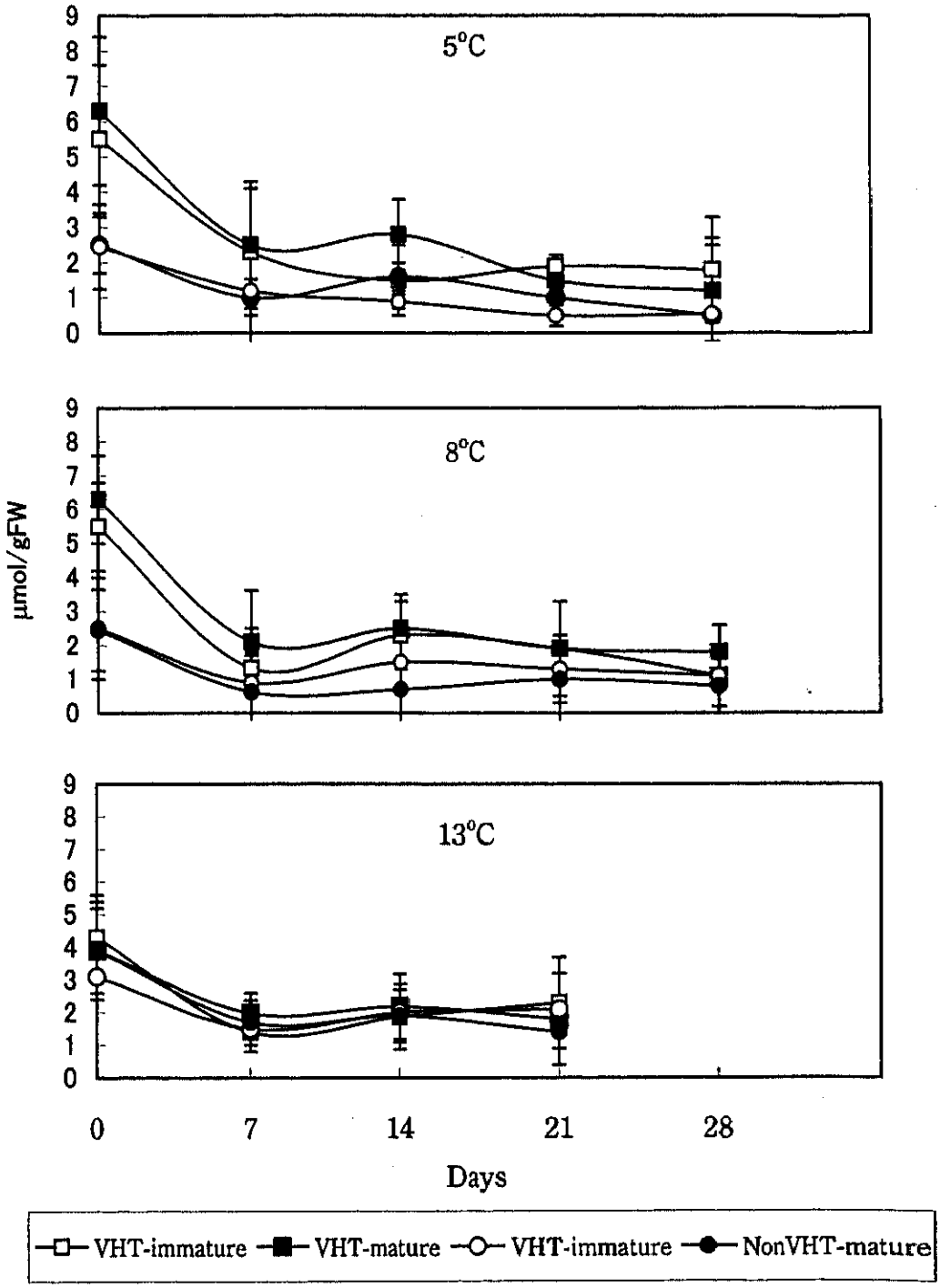

Fig. 4-14. Spermine levels in mango fruits after VHT-treated with different maturities when stored at 5, 8 and 13°C.

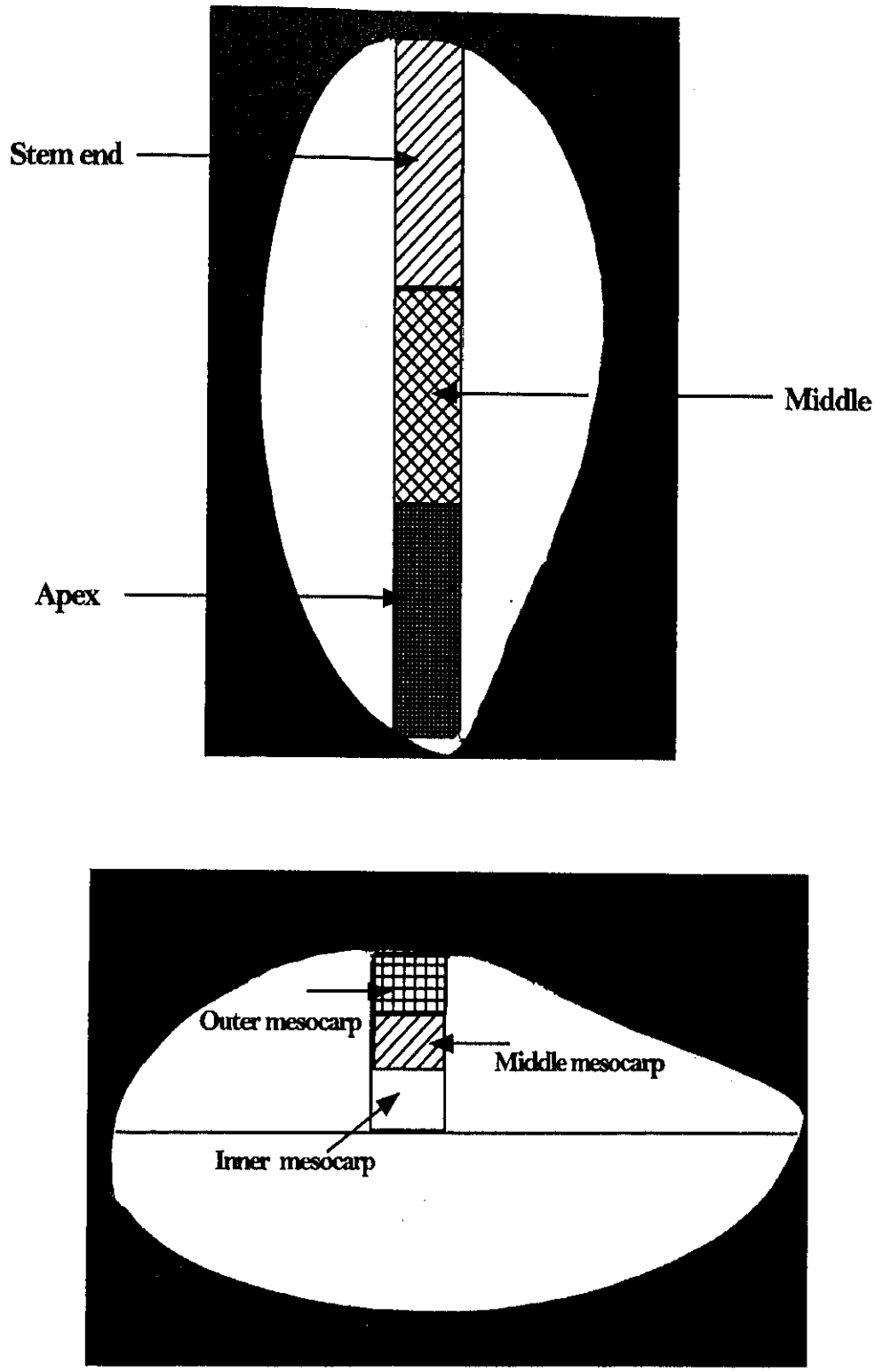

Fig. 4-15. The sampling parts of mango fruit

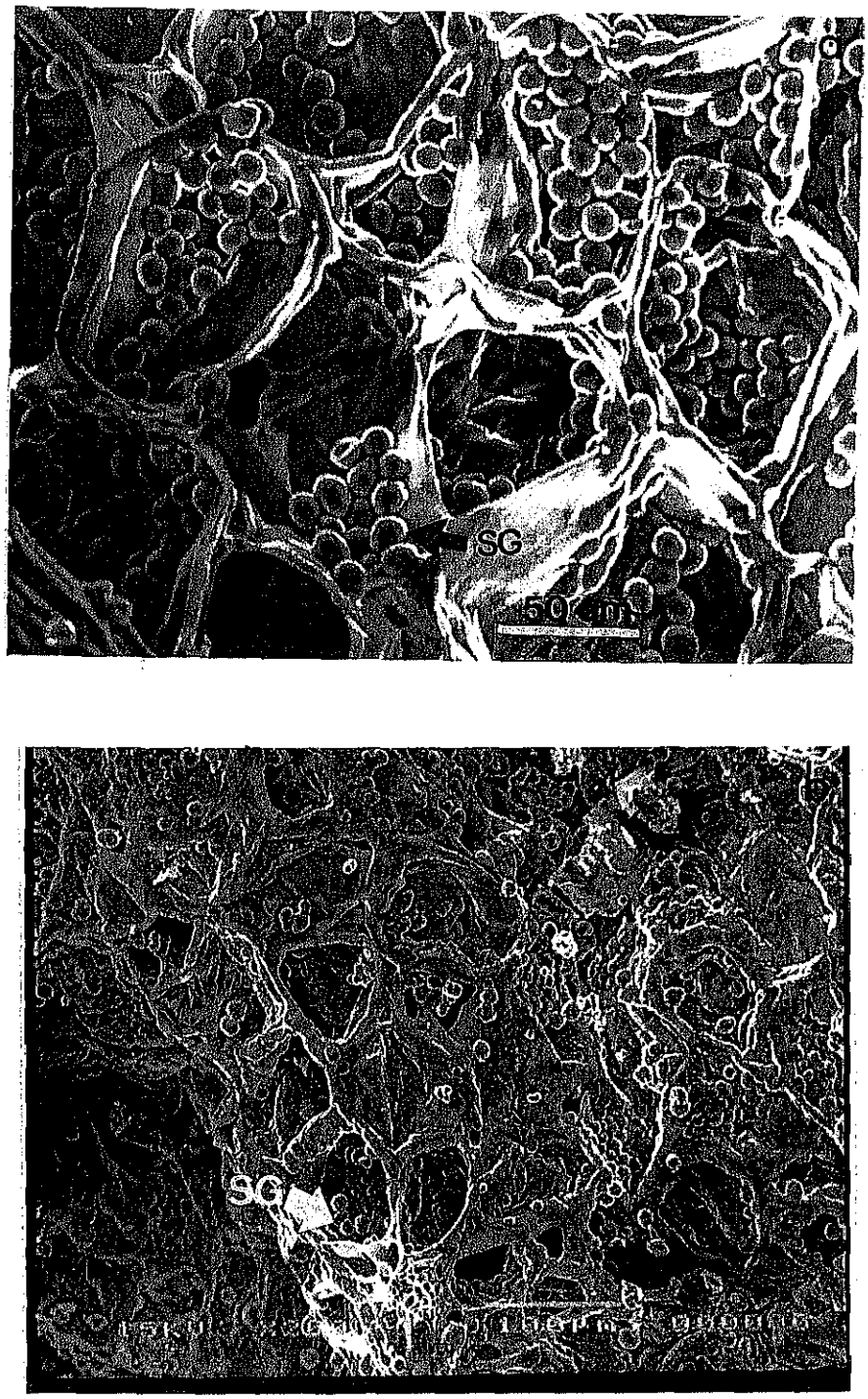

Fig. 4-16. Scanning electron micrograph of 'Nam Dok Mai' before storage showing whole cells containing starch grains (a) and after VHT when stored at 8°C showing starch grains loosely attached to cells (b). (SG=starch grains)

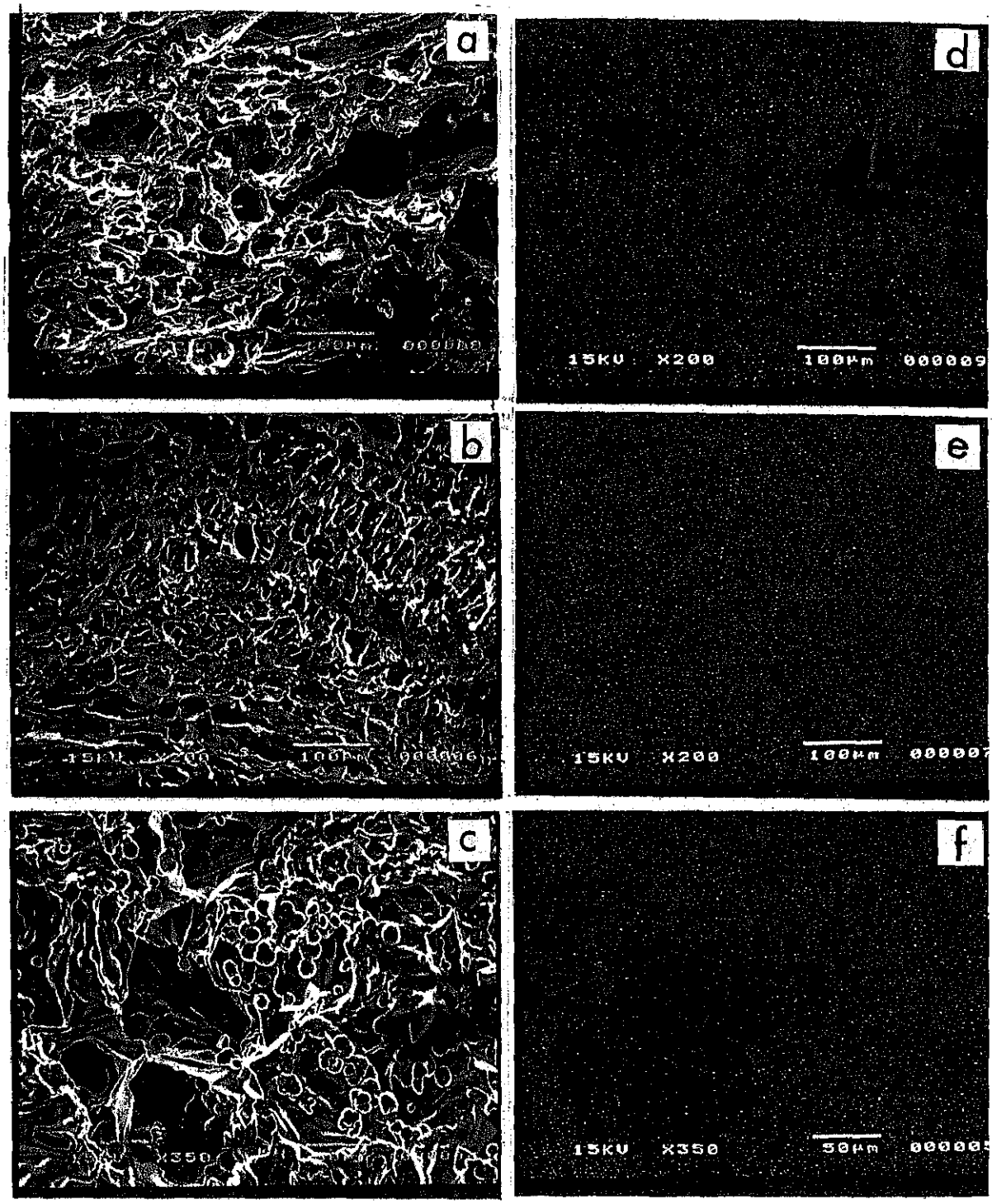

Fig. 4-17. Scanning electron micrographs (left) and digital mapping (right) of Ca from different parts of mesocarp of immature 'Nam Dok Mai' fruit after VHT and stored at 8°C; Stem end (a,d); middle (b,e) and apex (c,f).

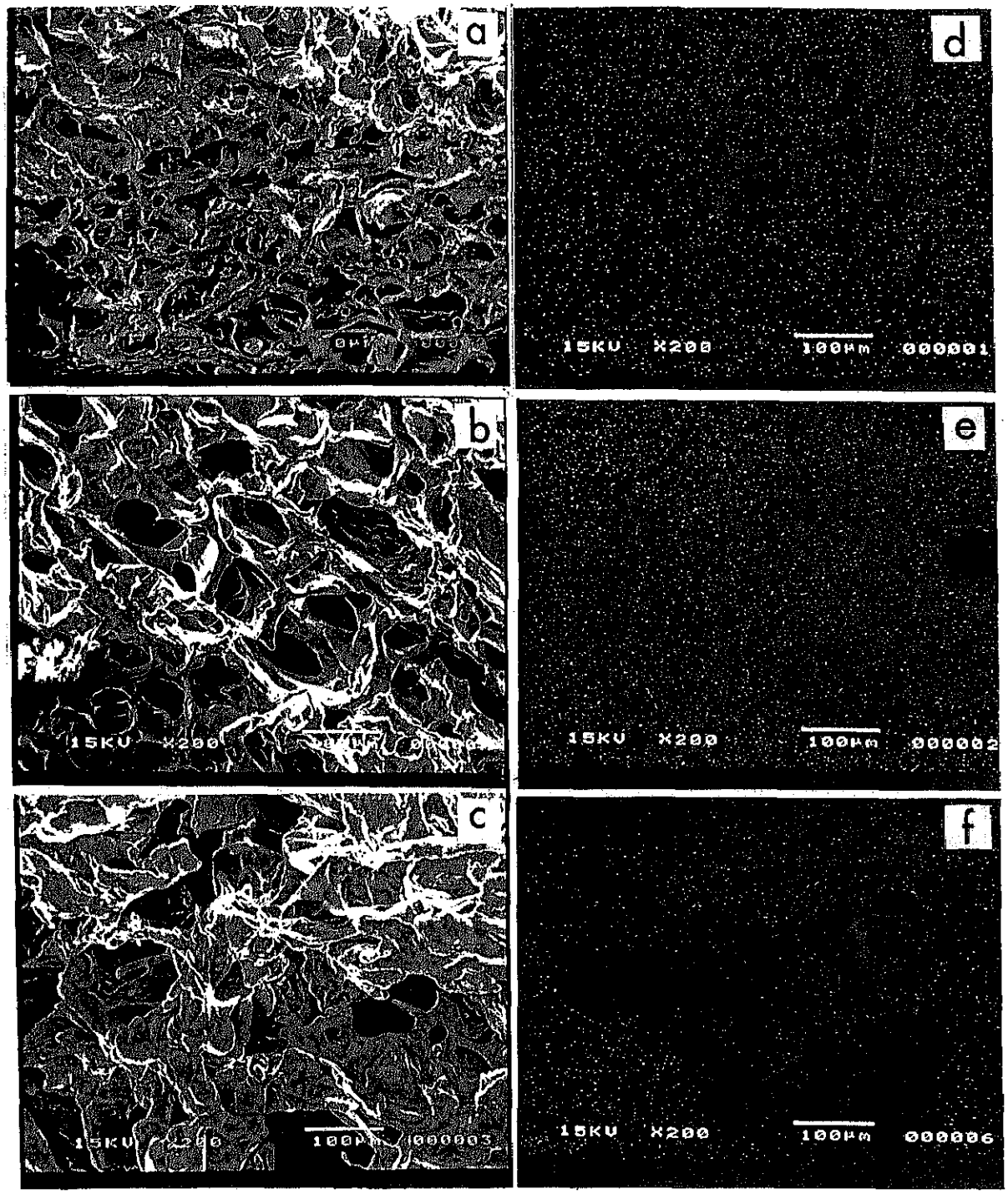

Fig. 4-18. Scanning electron micrographs (left) and digital mapping (right) of Ca from different parts of mesocarp of mature 'Nam Dok Mai' fruit after VHT-treated and stored at 8°C; Stem end (a,d); middle (b,e) and apex (c,f).