Chapter 3

Physiochemical changes of mango fruit during low temperature storage with different ripening stages

3.1 Introduction

Low temperature storage is a common technique for prolonging shelf life of many kinds of fruits but this method sometimes induces chilling injury to tropical and subtropical fruits. Chilling injury is related with alteration of the membrane structure, and it is proposed that the primary process causing chilling injury is a phase transition of membrane lipid. The membrane phase transition has many effects on tissue, including increases in membrane permeability and alteration of the activity of membrane protein. Depending on chilling sensitivity, fruits show different symptoms at different stages (Serrano et al., 1995; Lin et al., 1993). The symptoms of chilling injury in mango fruits often appear as skin browning or blackening, high susceptibility to decay, off flavor and internal breakdown.

Various physiological and biochemical changes occur in response to chilling stress such as an increase in polyamine levels. Polyamines, spermidine (Spd), spermine(Spm) and their diamine, putrescine (Put), are small aliphatic amines that are ubiquitous in

plant cells. They have been shown to bind strongly in vitro to negatively charged nucleic acid, acidic phospholipids and many types of proteins, including numerous enzymes. Recent researches indicate that polyamine actions relate with rapidly changing environmental stress such as light, temperature, water and nutrient availability (Bouchereau et al., 1999). It has been proposed that polyamine may be involved in reducing CI, especially showing the accumulation of Put during exposure to chilling stress, such as in Chinese cabbage (Wang and Ji, 1989) and apple (Kramer et al., 1989). Some data clearly demonstrate that several plants responded to low temperature acclimation with a uniform and substantial increase in Put and decrease in Spm and Spd such as in citrus (Mcdonald and Kushad, 1986), cherimoya (Escribano and Merodio, 1994) and zucchini squash (Wang and Ji, 1989). However, controversial results have been found, varying with species (Kakkar and Rai 1993). In peach it has been suggested that an increase of Spd may be a consequence of chilling injury (Valero et al, 1997). Thus, the role of polyamines in CI is still not clear. The objectives of this study were to investigate the chilling sensitivity of mango fruits with three ripening stages under two different temperatures (5 and 13 ℃) and to survey the change of polyamine level in the tissue after harvest, as well as physiological changes such as electrolyte leakage, sugar content, ACC content and polyamines level during cold storage and after transferring to 25 °C.

3.2 Materials and Methods

Fruits

Mango fruits were harvested from the Prapat orchard (a commercial fruit grower), Chiang Mai, Thailand and divided into 3 groups as follows: stage 1, green mango; stage 2, treated by acetylene gas after harvest for 2 days (more green than yellow); stage 3, treated by acetylene gas after harvest for 4 days (more yellow than green) (Fig. 3-1, upper). Acetylene treatment was used to advance the ripening stage at the time of cold storage.

Storage conditions

Fruits of each group were kept at 5 and 13 ℃. At 15-day intervals fruits of each ripening stage were sampled and placed in 25 ℃ storage for ripening, and 4 fruits were sampled and determined for CI symptom, electrolyte leakage (EL), total sugar, ACC and polyamines.

Assessing chilling injury

CI was evaluated by means of an arbitrary scale of visual symptoms, based on skin blackening, pulp browning and intensity of ripening and decay: 1= normal, 2 = slight, 3= medium and 4 = severe CI symptom. 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.

Determination of membrane permeability

Membrane permeability was determined based on electrolyte leakage. Ten discs, 2 mm thickness and 10 mm diameter, were excised from the pulp by a cork borer, washed with distilled water then placed in 50 ml of distilled water. After incubation on a shaking water bath with a frequency of 150 strokes/min at 20℃ for 3 hr, electric conductivity was measured in a suspending solution with a conductivity meter (CM-30ET). These samples were then frozen at –30℃ and boiled, then measured again for electric conductivity, the value of which was defined as 100 % leakage. EL was expressed by the following equation : % total ion leakage = (initial reading / final reading) x 100.

Sugar analysis

A sample of one gram from each fruit was extracted with distilled water and the sample extracts were passed through a cellulose nitrate membrane filter (pore size: 45μm). Then, the samples were injected into a HPLC equipment with the following conditions: column, TSK gel amide 80; detector, RI; solvent, acetonitrile 75: water25 (v/v) at a flow rate of 1 ml/min.

ACC content

ACC was determined according to the method of Lizada and Yang (1979) with slight modification. For improving the sensitivity of ACC determination, the concentration of $HgCl_2$ was increased to 50 mmol to facilitate ACC degradation to ethylene (Coleman and Hodges, 1991). For extraction of ACC, 1 g of fresh pulp tissue was homogenized with 5 ml of 80% ethanol, using mortar and pestle for 4 hr with occasional mixing. 1 nm ACC standard with 10 μl and 0.5 ml extract solution was determined in 20 ml test tubes, adding 0.01M $HgCl_4$ 0.1 ml to the extracts and ACC solution, and the volumes were then made up to 900 μl with water. The reaction vessels were sealed with a rubber serum stopper and kept in ice. The reactions were furthered by the addition of approximately 100 μ l of a cold mixture of 5% NaOCl and saturated NaOH (2:1,v/v) with successive agitation for 3 min, then 1-ml samples were withdrawn for ethylene determination using a GLC equipped with FID.

Polyamine analysis

A sample of one gram from each fruit was extracted in 10 ml of 5% cold $HClO_4$, pelleted at 20,000 x g for 20 min, and the supernatant phase, containing the free polyamine fraction was benzoylated as previously described by Serrano et al. (1995). Benzoyl-polyamines were extracted in cold ether after

centrifugation (1500xg, 10 min). Each polyamine was separated by HPLC system with SIL C_{18} T5 reverse column and detected at 254 nm (UV). The mobile phase was acetonitrile:water, 1:1(v/v) at a flow rate of 1 ml/min.

3.3 Results

Chilling injury index of fruits

External CI symptoms were skin blackening and pitting, while internal CI symptoms were pulp browning and decay (Fig. 3-1,lower). The severity of CI symptoms increased with storage time. During the first 15 days of storage at 5 °C fruits at stage 2 and stage 3 showed no CI symptom and ripened normally when transferred to 25 ℃, but stage 1 fruits showed distinct CI symptoms as skin and pulp browning and decay (Figs. 3-2, 3-3). On the 45th day of storage, however, fruits of all stages were injured. At 13 ℃, stage 1 and stage 2 fruits showed similar but less pronounced CI symptoms after 15-day and 30-day storage when transferred to 25 °C for ripening, while no CI symptom was observed on stage 3 fruit (Fig. 3-3). At 45 days after storage, however, the fruit of all stages showed the same pronounced CI symptom (Fig. 3-3).

In the preliminary experiment using mango fruit imported from Thailand to Japan, it was found that full green stage and immature

mango was susceptible to chilling temperature (data not shown).

Electrolyte leakage

Electrolyte leakage increased as the fruits were stored longer at both temperatures (Fig. 3-4). When stored at 5℃ and transferred to 25 ℃, electrolyte leakage of stage 1 fruit increased to the highest level in regard to chilling injury appearance. During the first 15 days in storage, those fruits kept at 13 °C increased the percentage of electrolyte leakage faster than those kept at 5 °C but did not increase further for longer storage regardless of ripening stages.

Total sugar

Sucrose was the predominant sugar in mango fruit (Fig. 3-5). No difference in glucose contents was found among the fruits at three ripening stages at both cold temperatures. When kept at 5 ℃ the ripening process was almost inhibited resulting in no change in fructose content, whereas stage 1 and stage 2 fruits kept at 13 ℃ continued to ripen until the end of storage. At the beginning of the storage stage 3 fruits contained the higher amounts of sugar followed by stage 2 and stage 1 fruits. During the storage at both cold temperatures stage 3 fruits showed no further increase of sucrose. However, stage 2 and particularly stage 1 fruit increased sucrose during storage at 13 °C more rapidly, so that at the end of

storage there were no differences in sucrose contents among the different stages.

ACC content

ACC content was very low during storage at 5 ℃ in all stages (Fig. 3-6). In particular, stage 2 fruit had the lowest levels of ACC at 30 days in storage while an increment of ACC was evident at 13 ℃, particularly in stage 3 fruits. A considerable increase of ACC was observed, however, when stage 2 fruits kept at 5 ℃ were transferred to 25 ℃. When fruit kept at 13 ℃was transferred to 25 ℃ a similar tendency was observed only at the first 15th day.

Polyamine levels

A significant difference was found in Put (Fig. 3-7) between the fruits of different ripening stages. The Put at stage 1 fruit increased during storage at 5 ℃ and reached the maximum peak at the 15th day, when the first CI symptom of these fruits was detected as the development of skin and pulp browning and decay, then decreased throughout the storage time. In stage 2 fruit, Put increased slightly at the 15th day of storage, then gradually decreased after 15 days until the end of storage. When all fruits were taken out for ripening the Put level decreased as low as 0.107,

0.129 and 0.22 μ mol/g FW in stage1, 2 and 3 fruits, respectively. In contrast, at 13 ℃, stage 2 and stage 3 fruits showed higher levels of Put than stage 1 fruit.

Spd (Fig. 3-8) was the dominant polyamine in mango and its level in stage 3 fruit was the highest at the beginning of storage at 4.84 μ mol/g FW, but it fell sharply following the next 15 days at 5 °C. At this temperature the Spd content of stages 1 and 2 decreased during 15 days storage but the stage 1 fruits showed an increase in Spd during further storage periods. At 13 ℃ the Spd of the fruits at all stages decreased throughout the storage time, especially in stage 1 fruit Spd almost disappeared after 15-day storage.

3.4 Discussion

The observed difference in chilling injury among the fruits of different ripening stages implies that the susceptibility of mango fruits to CI depended on the degree of ripeness. When stored at 5 °C for more than 15 days and transferred to 25 ℃, stage 1 fruit showed considerable CI symptoms while no incidence was detected in the stage 2 and stage 3 fruits. These observations showed that the more ripened mango could be kept for a longer period at a lower temperature without CI. Also this result suggests that low temperature condition inhibited the development of the ripening

process. In the more ripened fruit, CO_2 accumulation had already begun before placing in low temperature storage. The fact that mango fruit can continue the ripening process again when transferred to ripen at 25 °C, maybe because the ripening process was not being totally inhibited as compared to the green mature fruits. This is similar to other kinds of fruits which showed that the more immature fruits had the higher CI, similar results were also reported in mangoes cvs. 'Amelie', 'Tommy Atkins' (Von Mollendorff et al., 1992) and 'Keitt' (Medlicott et al., 1990), that the more immature fruits had the higher of CI symptoms. Moreover Lin et al. (1993) found in greenhouse-grown pepper that more ripened fruits has more tolerance to CI than unripe ones. This finding has practical importance since it seems that stage 2 fruit can be stored longer for one more week at the lower temperature without the development of chilling injury symptoms.

When stored at 5°C and transferred to ripen at 25 °C, stage 2 fruit showed the lowest percentage of electrolyte leakage consistent with chilling injury index. However, after 30-day storage, electrolyte leakage did not change significantly. Thus, electrolyte leakage may be not only a chilling injury indicator but could indicate the state of ripening of fruit (Cote et al., 1993). It was suggested that electrolyte leakage might be related to ripening processes due to changes in cellular permeability.

Low temperature and ripening stages also exerted an effect on

ACC level. During low temperature storage at 5 °C, ACC content was very low but 4 days after being transferred to 25 °C, it increased rapidly in stage 2 fruit with an increase in CI. The accumulation of ACC content when transferred from chilling to non-chilling temperature was also observed in cucumber (Wang and Adams, 1982). However, the increase in ACC content was found after 15-day storage in stage 1 accompanied by the CI occurrence, similar to the results in pepper where the elevated ACC was found to relate with CI after 2 weeks in storage (Lin et al., 1993). On the other hand, when stored at 13 °C fruits of all stages showed an enhancement of ACC content without any CI symptoms, suggesting that an increase in ACC content was responsible not only for chilling but also for ripening of fruit. The effect of ethylene on the plant responses to chilling stress is also quite variable. ACC content paralleled with ethylene production in this experiment. As ACC content is converted to ethylene under stress conditions, after removal from temperature stress, ACC content increased rapidly as a common feature of all climacteric fruit which can recover ethylene production (Ketsa et al., 1999). An increase in ethylene is related with an increase in chilling injury and the severity of chilling injury in ripened avocados was more severe in fruit treated with exogenous ethylene than those stored in an ethylene free atmosphere (Chaplin et al., 1983). These results were different from those observed for mature honeydew melons when exposed to 1000 μl/l C_2H_4 for 24 hr

which induced ripening and eliminated chilling injury (Lipton et al., 1979). In contrast, the chilling sensitivity of citrus fruit is generally increased by exposure to C_2H_4, but C_2H_4 treatments seem to enhance tolerance in some fruit (Forney and Lipton, 1990).

High polyamines levels have been correlated with increased chilling resistance (Faust and Wang, 1993) and many reports showed an increase in Put level in several fruits such as lemon, grapefruit (Mcdonald and Kushad, 1986) and zucchini squash (Kramer and Wang, 1989; Gonzales-Aguilar et al., 1997; Wang, 1994), suggesting that Put accumulation may be a protective response to various kinds of stresses that cause physiological injury of tissues. It has been hypothesized that polyamines protect the integrity of membranes by retarding lipid peroxidation and the antioxidant properties of these compounds, in turn alleviate chilling injury (Kramer and Wang, 1990; Bouchereau et al., 1999). In the present study Put level in stage 1 fruit kept at 5 ℃ decreased after 15-days storage when these fruit showed the development of CI symptom. It was suggested that the polyamines may increase cell viability during CI occurrence by retarding membrane senescence (Guye et al., 1987). An accumulation of Put in tissue has been related with the general response of plant to chilling temperature. Faust and Wang (1993) found that conditions which reduced chilling injury in lemons increased Put levels. Put level in stage 2 fruit increased during the first 15-day storage remained constant

thereafter until the end of storage. The results supported the hypothesis that the fruit maintained Put level in tissues and may prevent chilling injury. However these changes may be correlated to the ability of each plant species and variety to withstand low temperature, and may also be based on the fact that only plant tissue organs susceptible to CI had increased Put and/or decreased Spd levels. Based on this study, the information about correlation of polyamines and CI in mango fruit will be useful for further research on pressure infiltration of these compounds into the fruits to increase fruit firmness and also inhibit the development of chilling injury.

Fig. 3-1. 'Nam Dok Mai' mango at different ripening stages (upper) and severe symptom of chilling injury (lower).

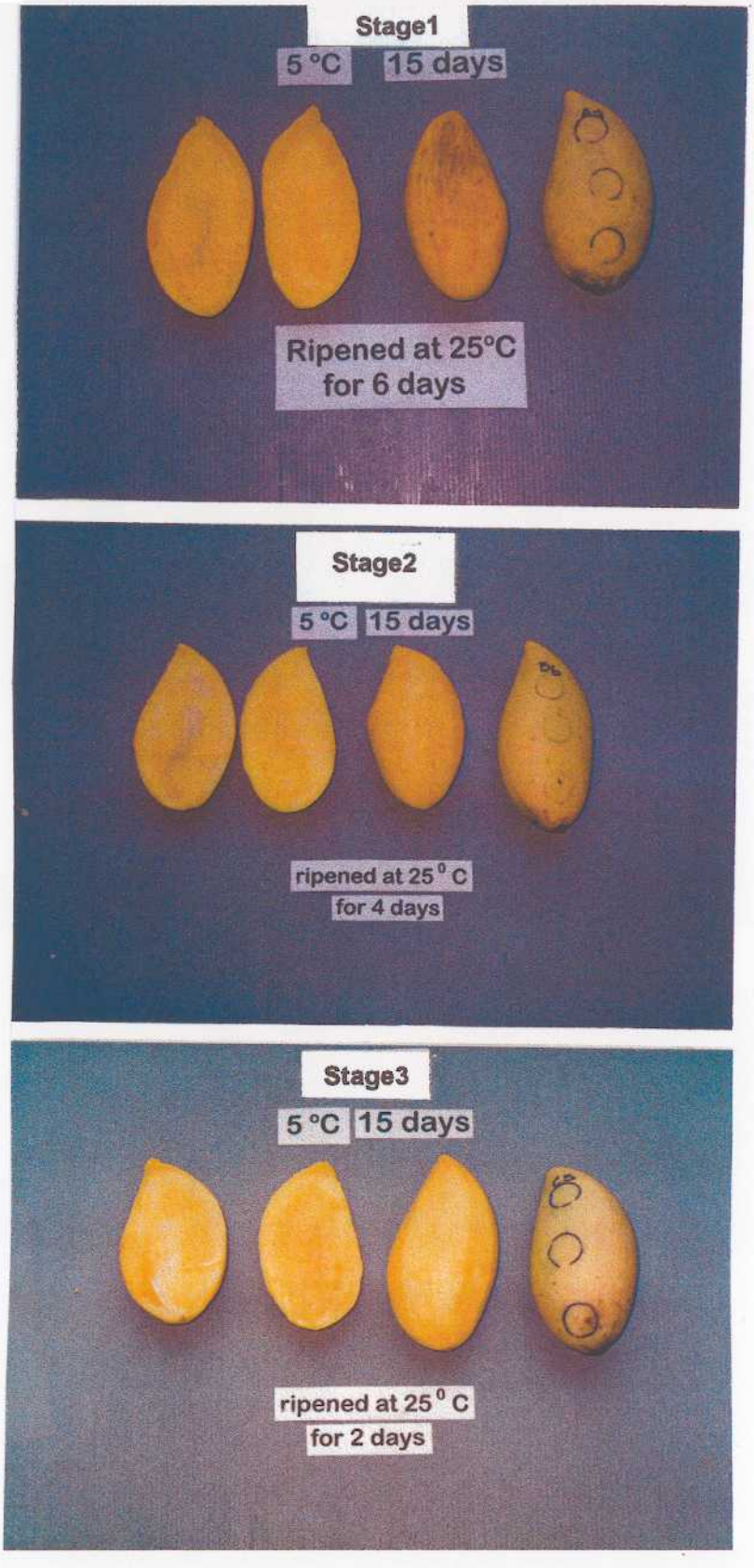

Fig. 3-2. The 'Nam Dok Mai' fruits with stage 1 showing chilling injury (CI) by skin browning (top) but stage 2 (middle) and stage 3 (bottom) showed no CI after storage at 5°C for 15 days and then transferred to 25 °C.

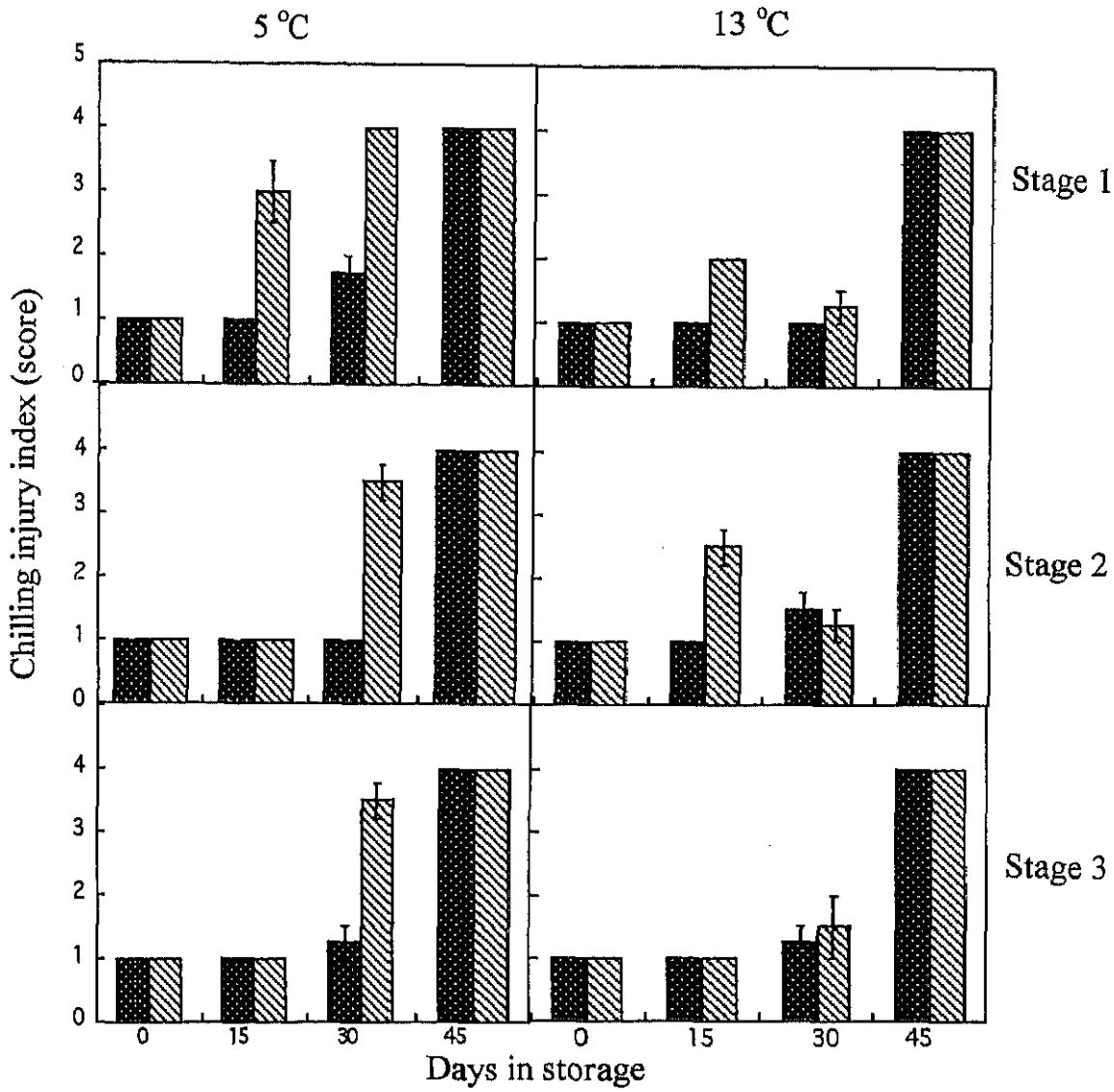

Fig. 3-3. Development of chilling injury of mango fruit with different ripening stages during storage at 5°C and 13°C (■) and after transferring to 25°C (▨) for accelerating the ripening. Vertical bars represent SE.

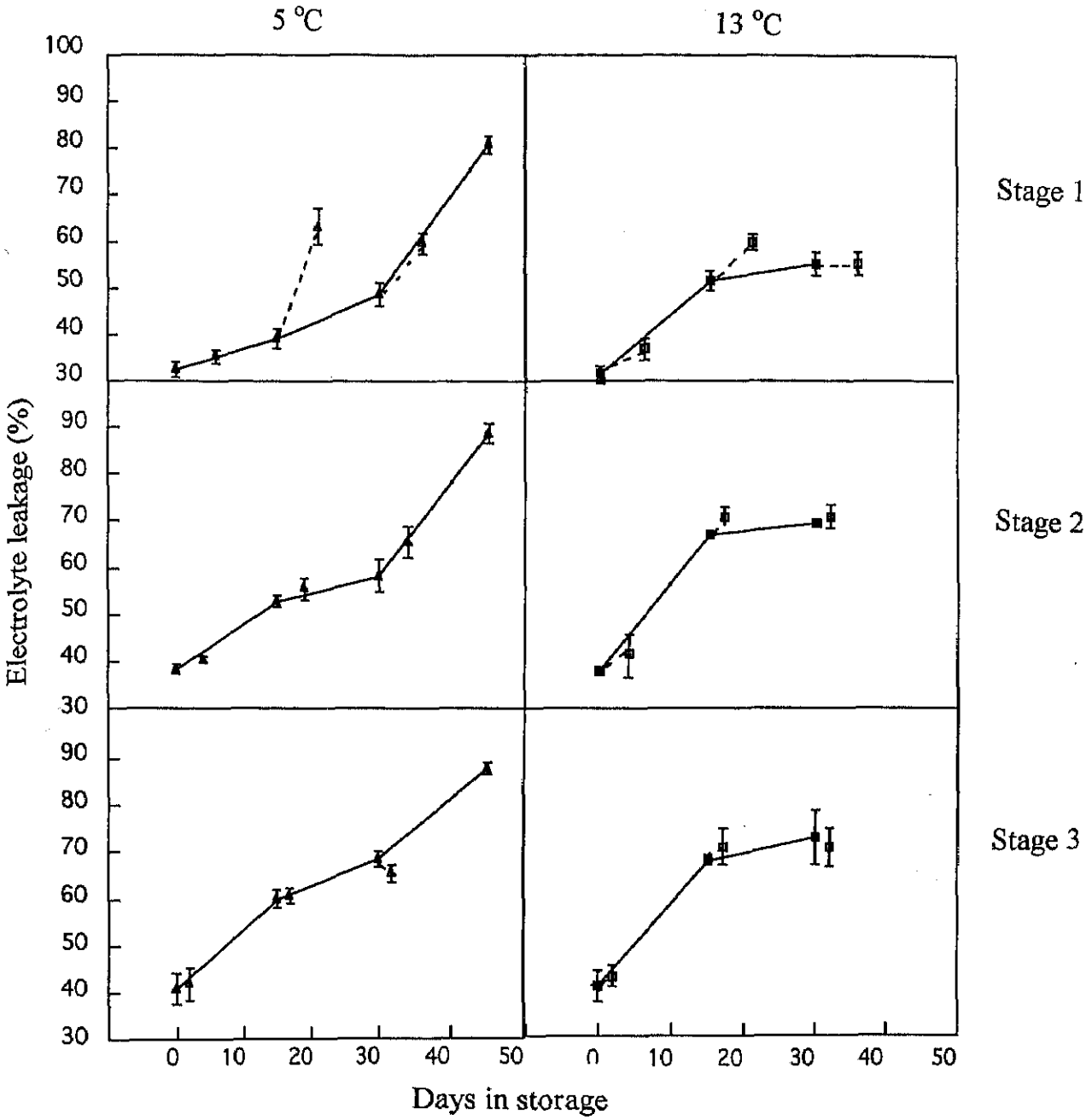

Fig. 3-4. Changes in electrolyte leakage (%) in mango fruits with different ripening stages at 5 °C (▲) and 13 °C (■) and after transferring to 25 °C (broken line) for accelerating the ripening process. Vertical bars represent SE.

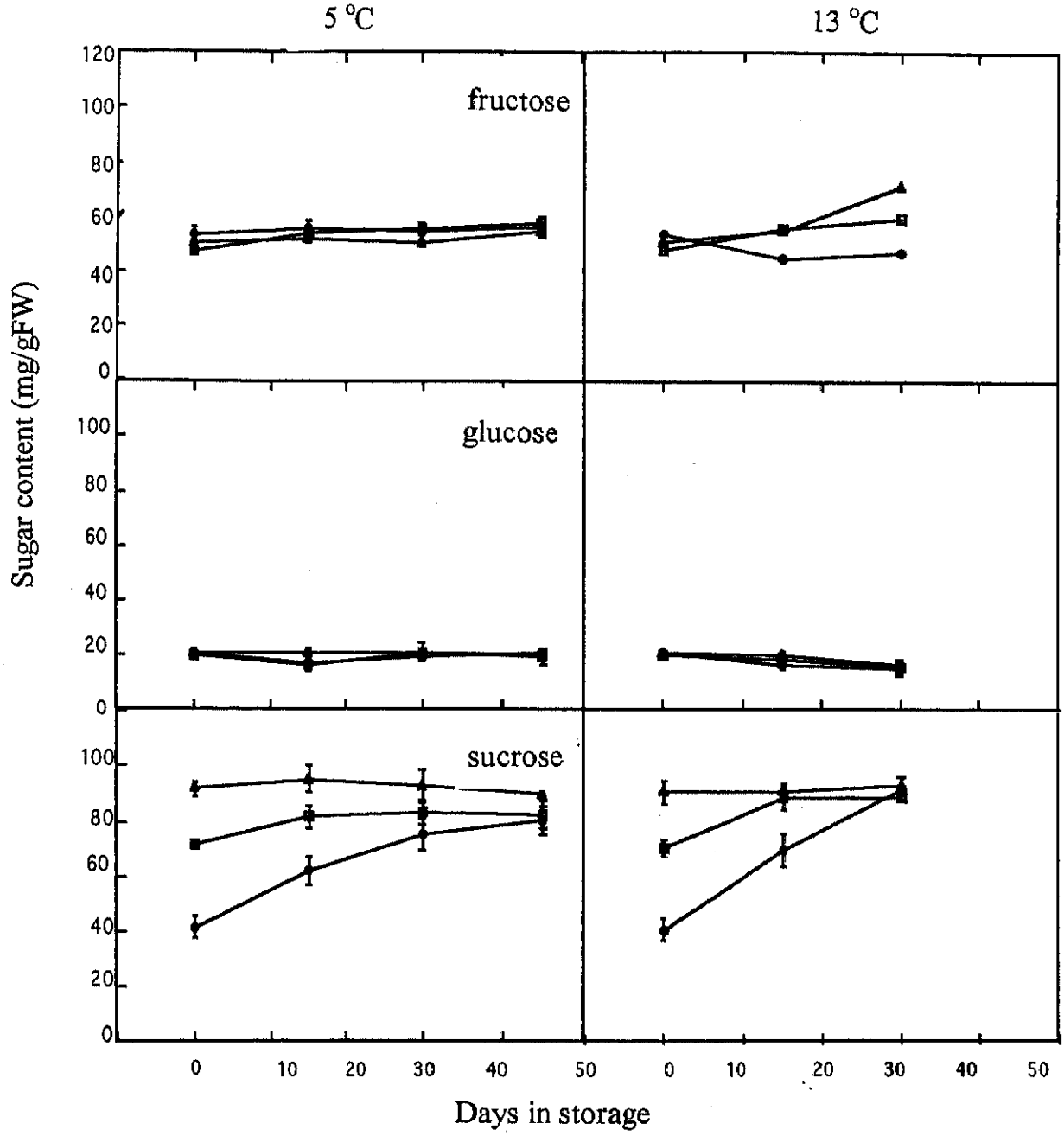

Fig. 3-5. Changes in fructose (top), glucose (middle) and sucrose (bottom) contents in mango fruits with different ripening stages at 5 °C and 13 °C. stage 1 (●); stage 2 (■) and stage 3 (▲) .Vertical bars represent SE.

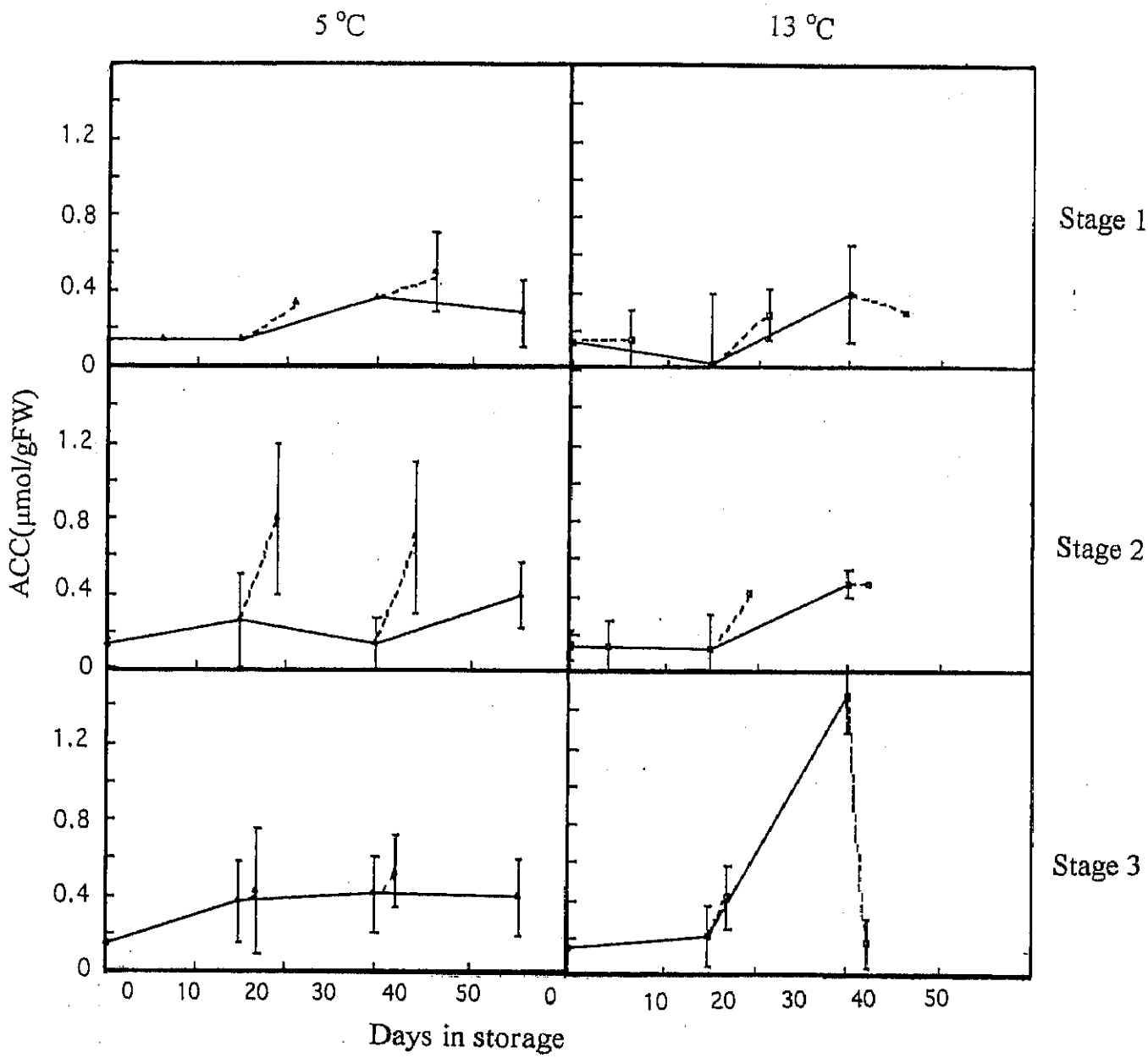

Fig. 3-6. Changes in ACC contents in mango fruits with different ripening stages at 5 °C (▲) and 13 °C (■) and after transferring to 25 °C (broken line) for accelerating the ripening process. Vertical bars represent SE.

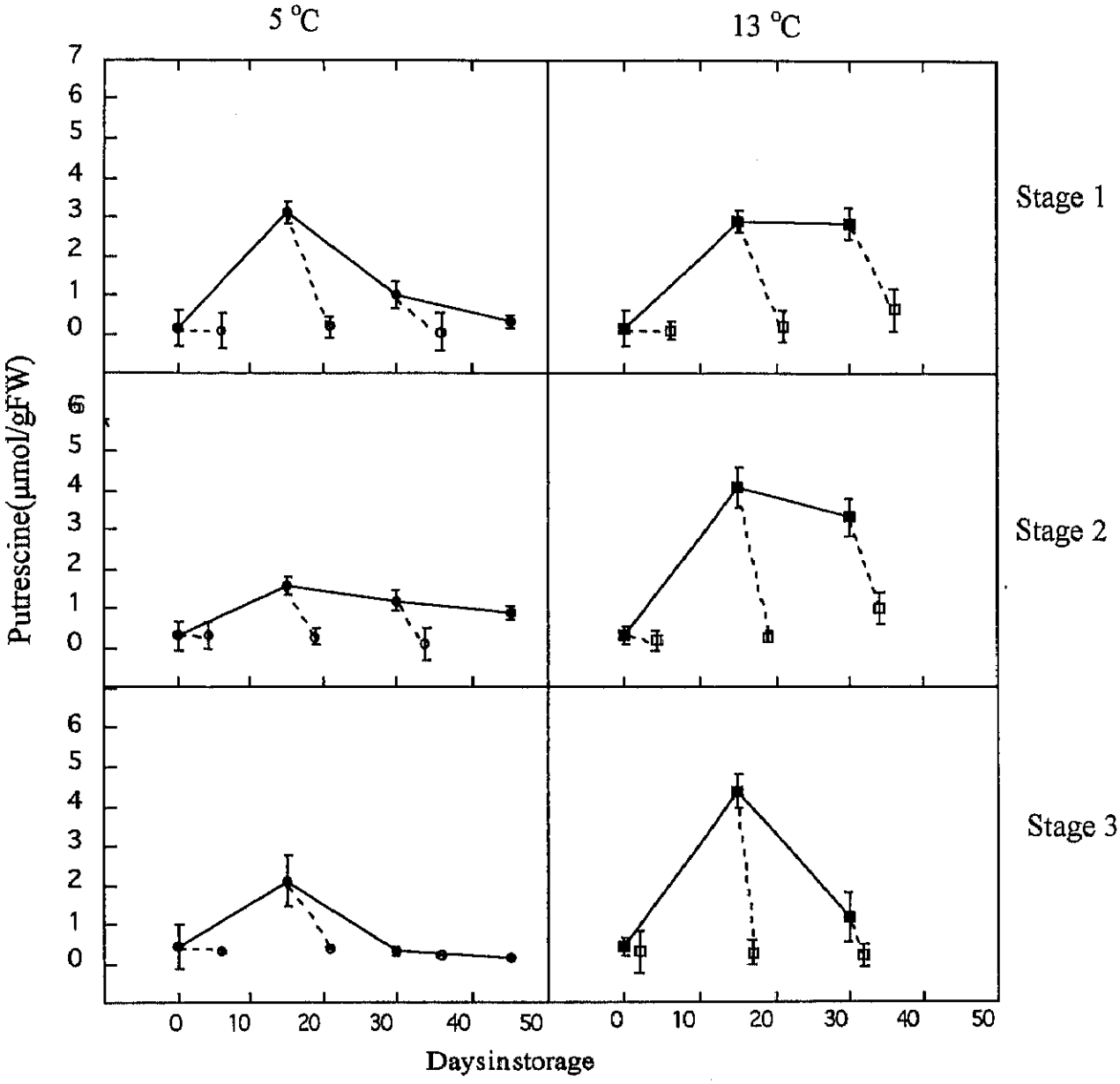

Fig. 3-7. Changes in putrescine of mango fruits with different ripening stages during storage at 5°C (▲) and 13°C (■) and after transferring to 25°C for accelerating the ripening process (broken line). Vertical bars represent SE.

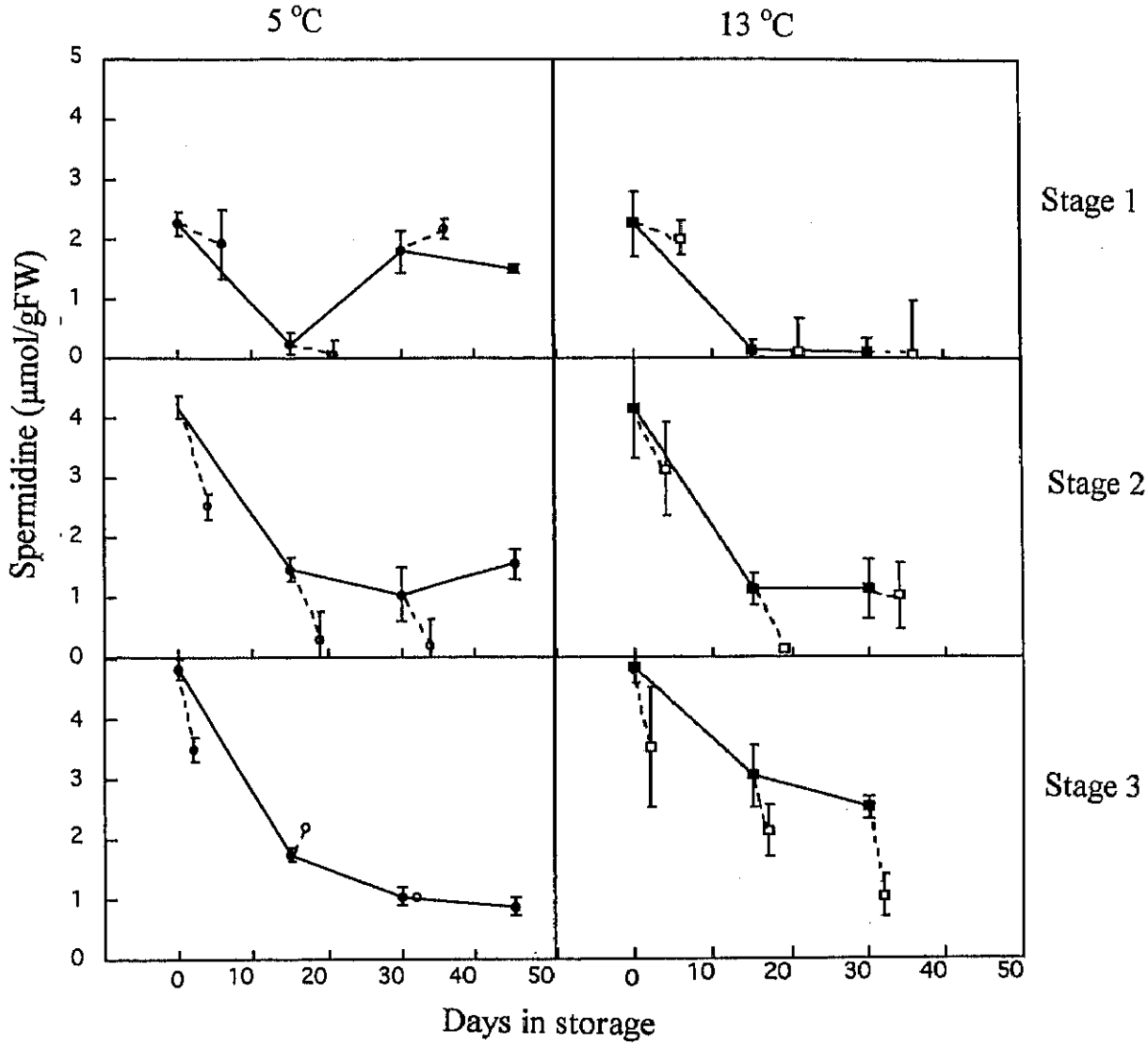

Fig. 3-8. Changes in spermidine of mango fruits with different ripening stages during storage at 5°C (▲) and 13°C (■) and after transferring to 25°C for accelerating the ripening process (broken line). Vertical bars represent SE.