STUDIES ON RIPENING CHARACTERISTICS OF PEAR (*Pyrus communis* L.) FRUIT WITH SPECIAL REFERENCE TO CELL WALL POLYSACCHARIDES

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Contents

General Introduction

Since pear (*Pyrus communis* L.) fruit is harvested before ripening, it requires several days or weeks after harvest to develop a buttery and juicy texture for human consumption. Such a texture is called "melting", as with peaches. Fruit is usually chilled at temperatures of -1°C to 1°C after harvest (Ness and Romani, 1980; Gerasopoulos and Richardson, 1996), which is important for three reasons. First, of course, fruit can be stored for a long time at low temperatures, although an abnormal softening occurs when fruit is transferred to 20°C after prolonged storage (Wang et al., 1985). Second, most late maturing pears do not ripen at warm temperatures until after exposure to a critical period of cold storage (Chen et al., 1993) that is specific to each cultivar (Chen et al., 1982). Third, the chilling makes it possible to synchronize the ripening of individual fruit. Thus, fruit is stored commercially at chilling temperatures after harvest even in cultivars that do not require chilling for ripening.

Pears, like many other climacteric fruit, produce ethylene during ripening. The rise in ethylene production that is accompanied by the climacteric rise in respiration appears to be an important regulatory event in pear ripening. The effectiveness of chilling for ripening of pears is thought to be due to induction of ethylene biosynthesis during storage. It was reported that the development of ripening capacity corresponded to the increase of internal ethylene to 1.5 - 2.0 ppm during cold storage (Chen and Mellenthin, 1981). However the mechanisms of ethylene biosynthesis induced by chilling are not clear in detail.

Important factors that affect the ripening of pears include harvest date, chilling treatment after harvest, temperature during ripening, and storage period and conditions. There are many studies which have examined the effects of

harvest date on the ripening or storage period of pears (Chen and Mellenthin, 1981; Elgar et al., 1997). Fruit harvested either before or after optimum harvest dates do not ripen normally, never developing the melting texture of ripe fruit (Ben-Arie and Sonego, 1979; Wang, 1982). Temperatures after harvest also affect the ripening of pears. The days required for fruit to ripen are fewer in higher temperatures, although fruit does not ripen normally in temperatures higher than 30°C. Relative humidity during ripening also appears to influence the ripening of pears, but its effects have not been studied. One reason is it is thought to be difficult to regulate relative humidity in a chamber for ripening.

Softening of fruit is a general characteristic of ripening in various fruit types. Among them, fruit softening is probably the most important consideration in ripening of pears because the texture of fruit is important for determining the fruit quality. Pears of good quality develop a buttery and juicy texture. The process of fruit softening may be due to changes in cell wall polysaccharides and to cell wall hydrolases (Fischer and Bennett, 1991; Huber, 1983). The changes in cell wall polysaccharides are often most apparent in an increase in soluble polyuronides and a decrease in insoluble polyuronides (Muda et al., 1995; Siddiqui et al., 1996). These changes are also observed during normal ripening of pears (Ben-Arie et al., 1979; Yoshioka et al., 1992). In contrast, there were less soluble polyuronides in 'Eldorado' pears ripened at 20°C after 36 weeks at 0°C, suggesting that the mechanism regulating the solubilization of cell wall polyuronides and the softening of fruit was impaired (Wang et al., 1985).

In pears, the softening of fruit has been thought to be accompanied by the development of a melting texture. In this study, cases in which fruit softened but never developed that texture were seen. One of the objectives of this study was to clarify the factors that cause to soften but never reach a melting texture.

Moreover, although there are many reports about changes in cell wall polysaccharides during softening of fruit including pears (Muda et al, 1995; Yoshioka et al., 1992), the changes were investigated at regular intervals during ripening. Then, the amount of cell wall polysaccharides was plotted against number of ripening days. In this study the amount of cell wall polysaccharides was plotted against flesh firmness to study the relationship between fruit softening and cell wall polysaccharides. Finally, the changes in molecular mass distribution of pectic and hemicellulosic polysaccharides during ripening of pears that had retained or had lost the ripening capacity were investigated to clarify the mechanisms of why fruit never reach the melting texture.

Chapter 1.

Cell wall changes in pear fruit softening on and off the tree

Introduction

Softening of fruit such as pears is related to changes in cell wall structure. These changes are often most apparent in an increase in soluble polyuronides and a decrease in insoluble polyuronides (Yoshioka et al., 1992; Muda et al., 1995; Sakurai and Nevins, 1997). Pears with good quality develop a buttery and juicy texture, associated with a reduction in extractable juice. This probably results from an increase in solubility of polyuronides in the flesh (Chen et al., 1981).

There are many studies which have examined the effects of harvest date on ripening or storage period of pears (e.g., Chen and Mellenthin, 1981; Elgar et al., 1997). Fruit harvested either before or after optimum harvest dates do not ripen normally, never developing the characteristic buttery and juicy texture of ripe fruit (Ben-Arie and Sonego, 1979; Wang, 1982). In most experiments studying harvest date effects, the sampling period is limited to about one month. Thus, little is known about how cell wall materials change in fruit harvested either before or after optimum harvest date.

In general, pears never soften appreciably on the tree, although exposure to cool temperatures can cause premature ripening of summer pears such as 'Bartlett' (Wang et al., 1971). Ben-Arie and Sonego (1979) studied compositional changes occurring in the cell wall of maturing 'Spadona' pears on the tree toward the end of the harvest season. But the question remains open as to the nature of such changes occurring after the optimum harvest date. The objectives of this chapter were to investigate changes in polyuronides of pear fruit on the tree during maturation over for a 10-week period beginning 6 weeks prior to the

optimum time for harvesting, and the relationships between the harvest date and the solubilization of polyuronides in fruit off the tree.

Materials and Methods

Plant material

Fruit of the cultivars 'Marguerite Marillat' and 'La France', from a commercial orchard near Yamagata was used in this study. Fruit was harvested in 1995 at 14-day intervals over a 10-week period beginning 6 weeks prior to the optimum time for harvesting (OTH), estimated from numbers of days after full bloom (September 13 for 'Marguerite Marillat' and October 12 for 'La France'). Total samples of 35 fruit were taken from 3 trees of each cultivar at each sampling time in the same orchard. Within 3 hours following harvest, flesh firmness of 5 individual fruit was determined on the opposite sides of each fruit using a rheometer (model CR-200D; Sun Scientific Co., Tokyo) with an 8 mm plunger.

After determining flesh firmness, each fruit was peeled, and 2 wedge-shaped sectors were cut from the fruit and diced into cubes of about 1 cm^3. Cubes then were freeze-dried and stored at -20°C until analyzed for polyuronide content.

The remaining 30 fruit were ripened in a chamber (852 X 435 X 1500 mm) in a room with the temperature maintained at 20°C, and relative humidity in the chamber near 100% by using ultrasonic humidifiers (model FT-30N; Ucan Co., Tokyo).

Ethylene determination

Ethylene production rates after harvest were measured for fruit harvested at 42 or 28 days before OTH at 5-day intervals, and for fruit harvested from 14 days before OTH to 28 days after OTH at 3-day intervals. Five individual fruit

were placed in 1.5-L glass desiccators. The desiccators were flushed with air and then sealed for 1 h. A 1 ml gas sample was withdrawn with a syringe and injected into a gas chromatograph (model GC-8A; Shimadzu Co., Tokyo) fitted with an activated alumina column and a flame ionization detector.

Extraction, fractionation, and characterization of polyuronides

Five individual fruit at harvest, or after having softened to less than 10 N, were peeled, cored and diced. The sampling times for softened fruit were determined by testing samples at irregular intervals after the rate of ethylene production had increased significantly. About 3 g of lyophilized flesh was added to 100 ml of boiling 80% (v/v) ethanol for 30 min. After being cooled to room temperature, the tissue was further homogenized in a Waring blender for 5 min and vacuum-filtered through a filter paper. The filtrate was discarded while the residue was twice resuspended in 80% ethanol, shaken, and filtered again. The residue was then washed twice with 100% ethanol, once with 100% acetone, and finally dried in an oven (40°C).

One hundred mg of alcohol insoluble residue (AIR) was dissolved in 100 ml of distilled water, mechanically shaken overnight at 20°C, and vacuum-filtered through a filter paper. The residue was then suspended in 30 ml of distilled water, shaken for 2 h, and filtered again. The filtrates were combined and used as the water-soluble polyuronides (WSP). The residue was extracted with 100 ml of 50 mM ethylenediaminetetraacetic acid (EDTA) in 30 mM sodium acetate buffer (pH 5.0) at 80°C for 30 min. The suspension was filtered, and the residue was then suspended in 30 ml of EDTA solution, shaken for 2 h, and filtered again. The filtrates were combined and used as the chelator-soluble polyuronides (CSP). The residue was further extracted with 100 ml of 0.05 M HCl and heated

at 100°C for 1 h. The suspension was filtered, and the residue was then suspended in 30 ml of 0.05 M HCl solution, shaken for 2 h, and filtered again. The filtrates were combined, neutralized with Tris(hydroxymethyl)aminomethane, and used for the HCl-soluble polyuronides (HSP).

The polyuronide contents were estimated by determining the uronic acid content with meta-hydroxydiphenyl as described by Blumenkrantz and Asboe-Hansen (1973).

Results

Physiological and biochemical characteristics of fruit on the tree

In 'Marguerite Marillat', flesh firmness changed little after OTH. By contrast, firmness of 'La France' fruit decreased gradually after OTH, reaching an average of 30 N for fruit left on the tree for 28 days after OTH (Fig. 1.1A).

Ethylene production in harvested fruit did not increase until 28 days after OTH in 'Marguerite Marillat' fruit and 14 days after OTH in 'La France' fruit (Fig. 1.1B).

The WSP content of 'Marguerite Marillat' fruit increased slightly at the OTH and kept the level thereafter (Fig. 1.2). CSP and HSP contents decreased rapidly between 42 days and 14 days before OTH. In 'La France', the WSP increased gradually between 14 days before and 28 days after OTH (Fig. 1.2). The CSP did not change substantially and the HSP decreased slightly during maturation on the tree.

Physiological and biochemical characteristics of fruit off the tree

Pear fruit off the tree softened to an edible firmness (less than 10 N) independent of harvest date and cultivar. Informal tasting by three panelists

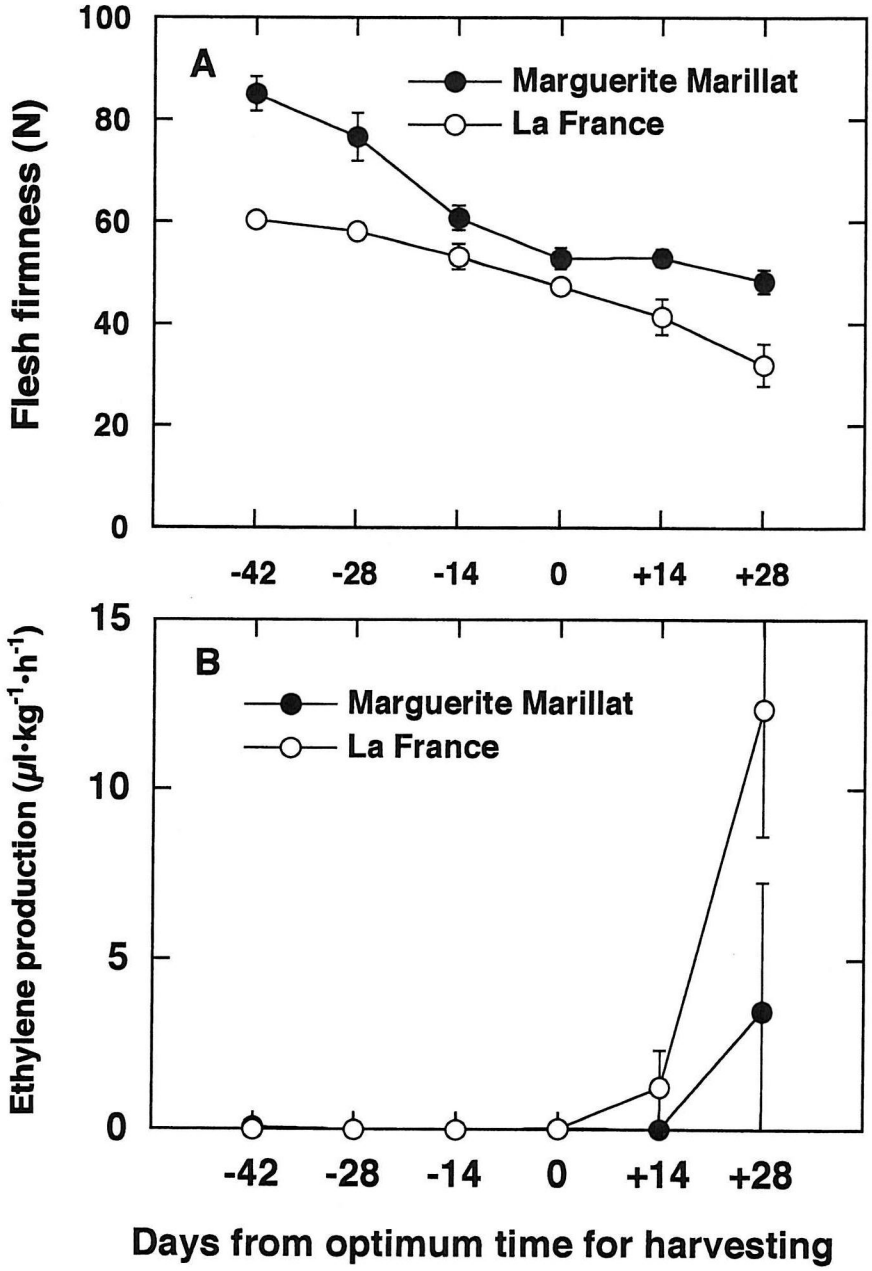

Fig. 1. 1. Flesh firmness (A) and ethylene production (B) of 'Marguerite Marillat' and 'La France' pear fruit during growth and maturation on the tree. Values are means ± S. E. (n=5).

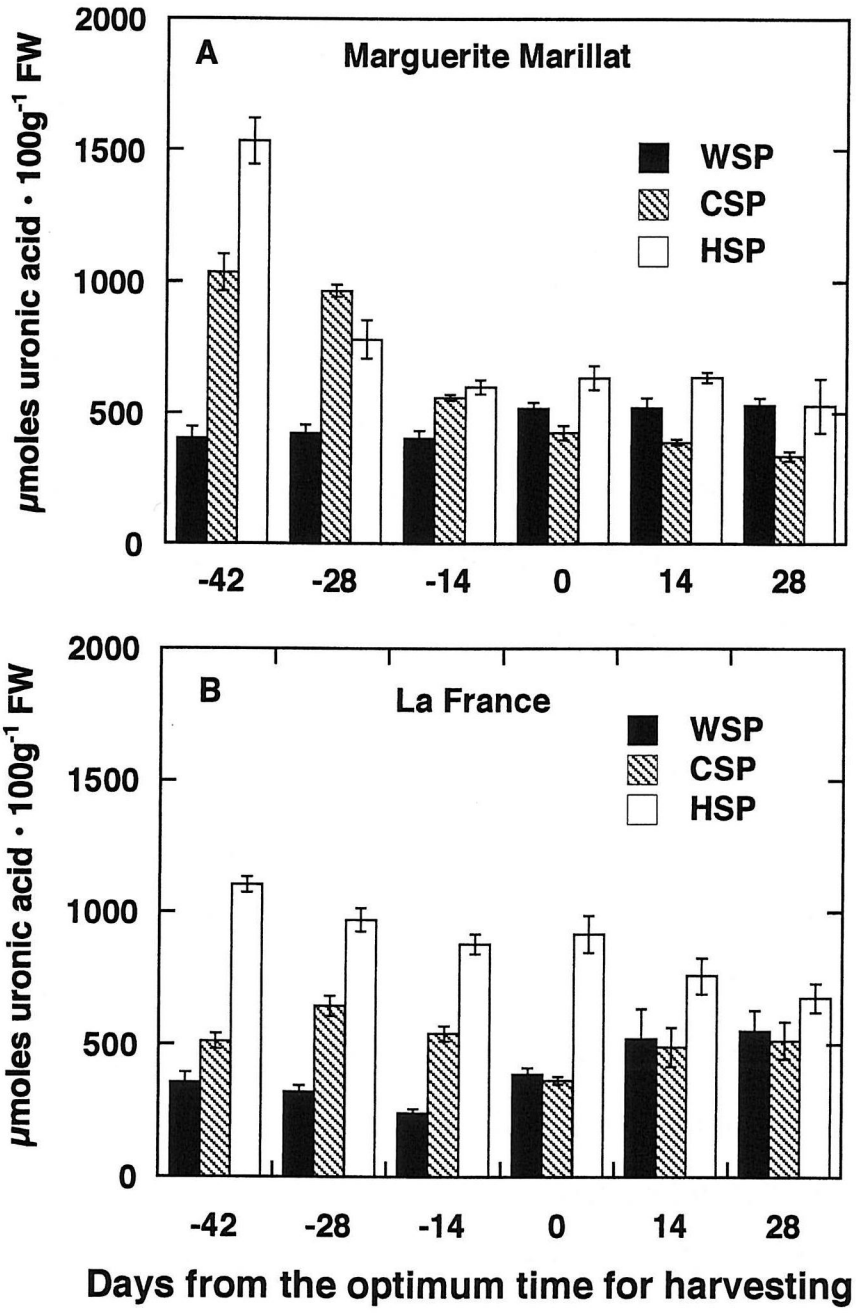

Fig. 1. 2. Polyuronide content of cell wall fractions extracted from pulp of 'Marguerite Marillat' (A) and 'La France' (B) pear fruit at harvest. Values are means ± S. E. (n=5). WSP, water-soluble polyuronides; CSP, chelator-soluble polyuronides; HSP, HCl-soluble polyuronides.

indicated that the texture of fruit harvested from 42 to 14 days before OTH became buttery and juicy during ripening off the tree. However, fruit harvested before OTH did not develop acceptable flavour quality (taste and aroma). Fruit harvested 14 to 28 days after OTH ripened but did not develop a buttery and juicy texture. Especially in 'Marguerite Marillat' fruit, the texture was coarse and dry.

The rate of softening depended on harvest date and cultivar. 'Marguerite Marillat' fruit softened faster than 'La France' fruit. In both cultivars, the later the harvest date, the faster the fruit softened. When fruit was harvested 42 days before OTH, about 70 days were required to soften for 'Marguerite Marillat' and about 90 days for 'La France' fruit. When fruit was harvested 28 days after OTH, times were 12 days for 'Marguerite Marillat' fruit and 14 days for 'La France' fruit.

'Marguerite Marillat' fruit held at 20°C after harvest produced ethylene independent of harvest date (Fig. 1.3). As with the rate of softening, the rate of increase in ethylene production was greater in late-harvested than in early-harvested fruit. In fruit harvested 42 days before OTH and 28 days after OTH, ethylene production peaked at 65 days and 6 days after harvest, respectively. Ethylene production in 'La France' fruit off the tree showed the same pattern as 'Marguerite Marillat' fruit (Fig. 1.3).

WSP in fruit which had softened to below 10 N after harvest, were at much higher levels (Fig. 1.4) than in fruit at harvest (Fig. 1.2). In both cultivars, late-harvested fruit had less WSP than early-harvested fruit. In fruit harvested at 28 days after OTH and ripened, the WSP was at a minimum, 748 and 884 μmoles uronic acid 100 g^{-1}FW in 'Marguerite Marillat' and 'La France' fruit, respectively (Fig. 1.4). The CSP and HSP levels in softened fruit were lower than in fruit at

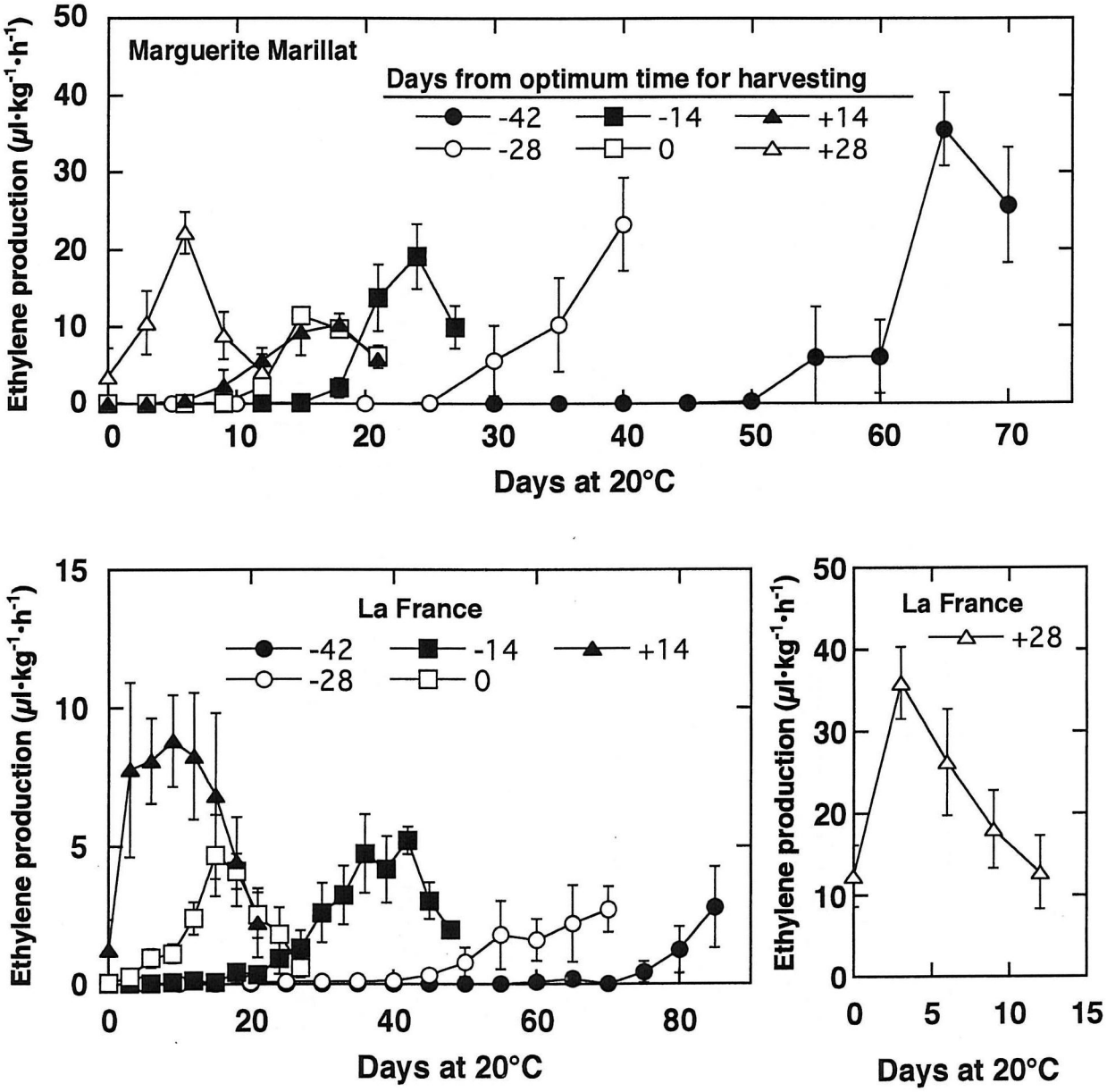

Fig. 1. 3. Effect of harvest time (numbers of days from the optimum time for harvesting) on ethylene production of 'Marguerite Marillat' and 'La France' pear fruit during ripening off the tree. Values are means ± S. E. (n=5).

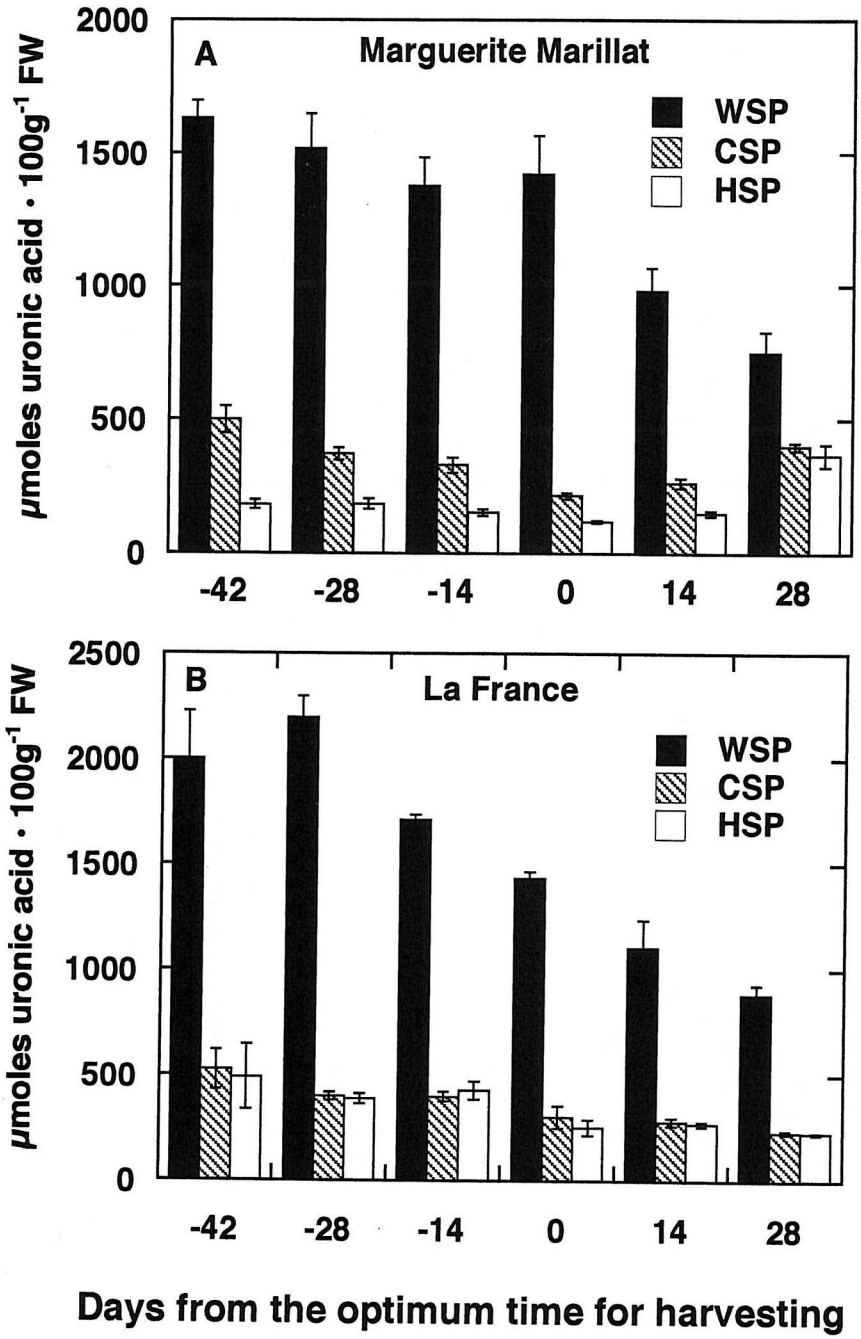

Fig. 1. 4. Polyuronide content of cell wall fractions extracted from pulp of softened 'Marguerite Marillat' (A) and 'La France' (B) pear fruit off the tree. Values are means ± S. E. (n=5). WSP, water-soluble polyuronides; CSP, chelator-soluble polyuronides; HSP, HCl-soluble polyuronides.

harvest, independent of harvest date and cultivar.

Discussion

'Marguerite Marillat' and 'La France' fruit both softened gradually on the tree, but neither softened to an edible firmness (approximately 10 N). This confirms the results of Ning et al. (1991) with 'La France' fruit. The sampling time of 28 days after OTH was when most fruit of both cultivars abscised. These results raise the question of why pears do not soften to an edible firmness on the tree.

In the two pear cultivars investigated in this study, it was found that pear fruit produced ethylene on the harvest day at the late harvests, 28 days in 'Marguerite Marillat' and 14 days after OTH in 'La France' fruit. This difference was probably in part a reflection of climate conditions during maturation. The temperature when 'La France' fruit was harvested at commercial maturity (OTH) was lower than that for 'Marguerite Marillat' fruit. It has been reported that chilling temperature enhances ethylene biosynthesis of pears, as well as for fruit such as apples and cucumbers (Knee et al., 1985; Larrigaudiere et al., 1997; Lelièvre et al., 1997). The rate of ethylene production of 'La France' fruit at 28 days after OTH was more than 10 μl kg^{-1} h^{-1} which was as high as the maximum produced in fruit harvested at OTH and ripened off the tree. These data indicate that softening of pears is apparently not induced by ethylene on the tree.

The WSP increased slightly on the tree in both cultivars. However, the amount in fruit harvested 28 days after OTH was less than one third of that in fruit harvested at OTH and ripened off the tree. Redgwell and Percy (1992) have shown that the sequence and degree of change in relation to the softening process are quite different in kiwifruit softened on the vine compared to those softened by a postharvest ethylene treatment, although the nature of the changes are the

same. Ben-Arie and Sonego (1979) have reported that the decrease in fruit firmness during ripening off and on the tree corresponded to an increase in WSP. It is not clear why WSP does not increase significantly in fruit on the tree. Two hypotheses may be proposed. First, cell wall decomposition in fruit may be inhibited by unknown substances in the tree. Abu-Goukh and Labavitch (1983) reported that purified pear polygalacturonase (PG) inhibitor proteins inhibited different fungal PGs, although they did not affect endogenous pear fruit PG activity. Second, cell wall synthesis may continue on the tree. We observed that pears continued to increase in volume after OTH, so it is likely that both cell wall synthesis and decomposition occur after OTH.

Pears off the tree softened to less than 10 N and softening was accompanied by an increase in WSP and a decrease in HSP, independent of harvest date and cultivar. Those changes in polyuronides during ripening are in agreement with results from other work on pears (Ben-Arie and Sonego, 1979; Yoshioka et al., 1993). The texture of fruit harvested between 42 days before OTH and at OTH became buttery and juicy during ripening off the tree. Wang et al. (1972) reported that 'Beurre d'Anjou' fruit ripened when harvested at 57% of its total growth period and treated with ethylene, but that no changes in firmness occurred without applied ethylene, except in fully mature fruit. It is interesting to note that fruit harvested at 42 days before OTH softened appreciably without ethylene treatment or chilling in this study. Differences in results may be related to cultivar. Both 'Marguerite Marillat' and 'La France' fruit do not require chilling for normal ripening although chilling synchronize ripening in individual fruit. Another explanation may be the difference in ripening period. They measured the flesh firmness over 20 days after harvest. In our experiment, about 70 days were required to soften for 'Marguerite Marillat' and about 90 days for 'La France'

fruit, when fruit were harvested at 42 days before OTH.

Fruit harvested at 14 days and 28 days after OTH and ripened softened to less than 10 N independently of cultivar, but the texture of such fruit did not become buttery and juicy. Especially in 'Marguerite Marillat', the texture of fruit became coarse. In peach fruit, mealiness has been attributed to impaired solubilization of polyuronides with accumulation of insoluble low methoxyl polyuronides of high molecular weight (Ben-Arie and Lavee, 1971). By contrast, in mealy nectarine fruit, soluble polyuronides increased to the same extent at the expense of insoluble polyuronides, independent of cold treatment (von Mollendorff et al., 1993). In this study, late-harvested fruit had less WSP than early-harvested fruit. In the study of Chen and Borgic (1985), fruit did not ripen normally, never developing a buttery and juicy texture of ripe fruit, and the WSP of the fruit did not change appreciably, during 9 days of ripening after fruit was transferred to 20°C after prolonged storage at -1°C or 0°C in pears. Together, these results suggest that the content of WSP is related to the texture of pear fruit.

In conclusion, the WSP increased slightly on the tree in both pear cultivars but the amount in fruit harvested 28 days after OTH was less than one third of that in fruit harvested at OTH and ripened off the tree. Pears off the tree softened to less than 10 N even in fruit harvested 42 days before OTH. The texture of fruit harvested at 14 days and 28 days after OTH and ripened did not become buttery and juicy. Those fruit had less WSP than fruit developed a buttery and juicy texture.

Summary

Changes in ethylene production and polyuronides in pear fruit on and off the tree were investigated. In 'Marguerite Marillat', flesh firmness changed little

after the optimum time for harvesting (OTH). By contrast, firmness of 'La France' fruit decreased gradually after OTH, reaching an average of 30 N for fruit left on the tree for 28 days after OTH. The amount of water-soluble polyuronides increased slightly during ripening on the tree. In both cultivars, the amount 28 days after OTH was less than one third of that in fruit harvested at OTH and ripened off the tree. Ethylene production did not increase substantially until 28 days and 14 days after OTH in 'Marguerite Marillat' and 'La France' fruit, respectively. Pears off the tree softened to less than 10 N independently of harvest date and cultivar. The amount of water-soluble polyuronides in fruit softened after harvest increased significantly from that at harvest. In both cultivars, the texture of fruit harvested at 14 days and 28 days after OTH and ripened did not become buttery and juicy. Those fruit had less WSP than fruit developed buttery and juicy texture.

Chapter 2.

Effects of chilling on pear ripening

Section 1. Physiological characteristics of pear fruit during storage and ripening

Introduction

Pears require several days or weeks after harvest to reach a buttery and juicy texture and, like many other climacteric fruit, produce ethylene during ripening. In fruit both attached to and detached from the tree, the temperature during maturation and ripening influences the rates of softening and the production of ethylene (Maxie et al., 1974; Wang et al., 1971).

Pears are usually chilled at temperatures of -1°C to 1°C after harvest (Ness and Romani, 1980; Gerasopoulos and Richardson, 1996), which is important for three reasons. First, of course, fruit can be stored for a long time at low temperatures, although an abnormal pattern of softening occurs when fruit is transferred to 20°C after prolonged storage (Wang et al., 1985). Second, most late maturing pears do not ripen at warm temperatures until after exposure to a critical period of cold storage (Chen et al., 1993) that is specific to each cultivar (Chen et al., 1982). Third, the chilling makes it possible to synchronize the ripening of individual fruit. Thus, fruit is stored commercially at chilling temperatures after harvest even in cultivars that do not require chilling for ripening.

The storage characteristics of pears was also studied. 'Bartlett' pears are stored in air for up to 80 days (Westwood, 1993). For 'Beurre Bosc' and 'Beurre d'Anjou' pears, fruit is stored in air at -1°C for 3 and 5 months respectively (Chen et al., 1983a, 1983b). An abnormal softening occurred, however, when fruit was

transferred to 20°C after prolonged storage (Mellenthin and Wang, 1976). Wang et al. (1985) reported that 'Eldorado' pears usually remained firm and dry and never reached a buttery and juicy texture, when fruit was transferred to 20°C after 36 weeks of storage at 0°C. These phenomena in pears are called the loss of ripening capacity.

In Japan, twenty seven thousand tons of pears were produced in 2000. Among them, 'La France' was the most popular cultivar because its aroma and texture are excellent. The aroma of 'La France' is characterized by common esters and hex-5-enyl acetate, while that of 'Bartlett' is associated with uncommon esters such as methyl and ethyl deca-2,4-dienoates (Jennings et al., 1964; Shiota, 1990). The texture of fruit is also important for determining the fruit quality. Pears having a melting texture, similar to peaches, are preferred. So an inferior texture after prolonged storage of pears is a serious problem for market distribution and one of the obstacles to the expansion of pear consumption. There are many reports studying the effect of chilling or storage period on pear ripening as described above. However these are limited to specific cultivars including 'Bartlett', 'Beurre Bosc' and 'Beurre d'Anjou'. In this section, we investigated physiological characteristics of 8 cultivars of pears cultivated in Japan during storage and ripening.

Materials and Methods

Plant material and treatments

Fruit was used in the study from 8 cultivars of pear. 'Bartlett' and 'Silver Bell' fruit, 'Winter Nelis' and 'Passe Crassane' fruit, and 'Marguerite Marillat', 'General Leclerc', 'La France' and 'Le Lectier' fruit were grown in the orchards of Yamagata Univ., Tsuruoka, Yamagata; Yamagata Prefectural Horticultural

Experiment Station, Sagae, Yamagata; and a commercial orchard, Tendo, Yamagata, respectively and harvested at commercial maturity (OTH). Table 2.1 shows the harvest dates of 8 cultivars. After selection for uniformity of size and free of defects, fruit was ripened at 20°C. Relative humidity was maintained near 100% by using ultrasonic humidifiers (model FT-30N; Ucan Co., Tokyo). The remainder was stored at 1°C for up to 5 months. Every month fruit was transferred to 20°C for ripening. Flesh firmness and 1-aminocyclopropane-1-carboxylic acid (ACC) content were determined during storage, and ethylene production rates were measured during storage and ripening. Tasting by three panelists was conducted using fruit softened to less than 8 N. They evaluated whether fruit developed a melting texture or not. Five replications were used for all determinations.

Determination of flesh firmness, ethylene production and ACC content

Flesh firmness and the rates of ethylene production were determined as described in chapter 1. ACC in the flesh samples was extracted in 80% (v/v) ethanol and partially purified by adsorption onto an Amberlite CG-120 column (H^+ form) as described by Mathooko et al. (1993). The ACC content in the extracts was then estimated by the method of Lizada and Yang (1979).

Results

Changes in flesh firmness, ethylene production rates and ACC concentration during storage.

There were dramatic differences in the changes in flesh firmness of different pear cultivars during storage at 1°C (Fig. 2.1). Flesh firmness of 'Marguerite Marillat' and 'General Leclerc' showed little softening during storage, even after

Table 2. 1. Harvest dates of 8 pear cultivars.

Bartlett	Sept. 1st
Marguerite Marillat	Sept. 13th
General Leclerc	Oct. 3rd
La France	Oct. 13th
Silver Bell	Oct. 19th
Le Lectier	Oct. 28th
Winter Nelis	Oct. 31st
Passe Crassane	Nov. 1st

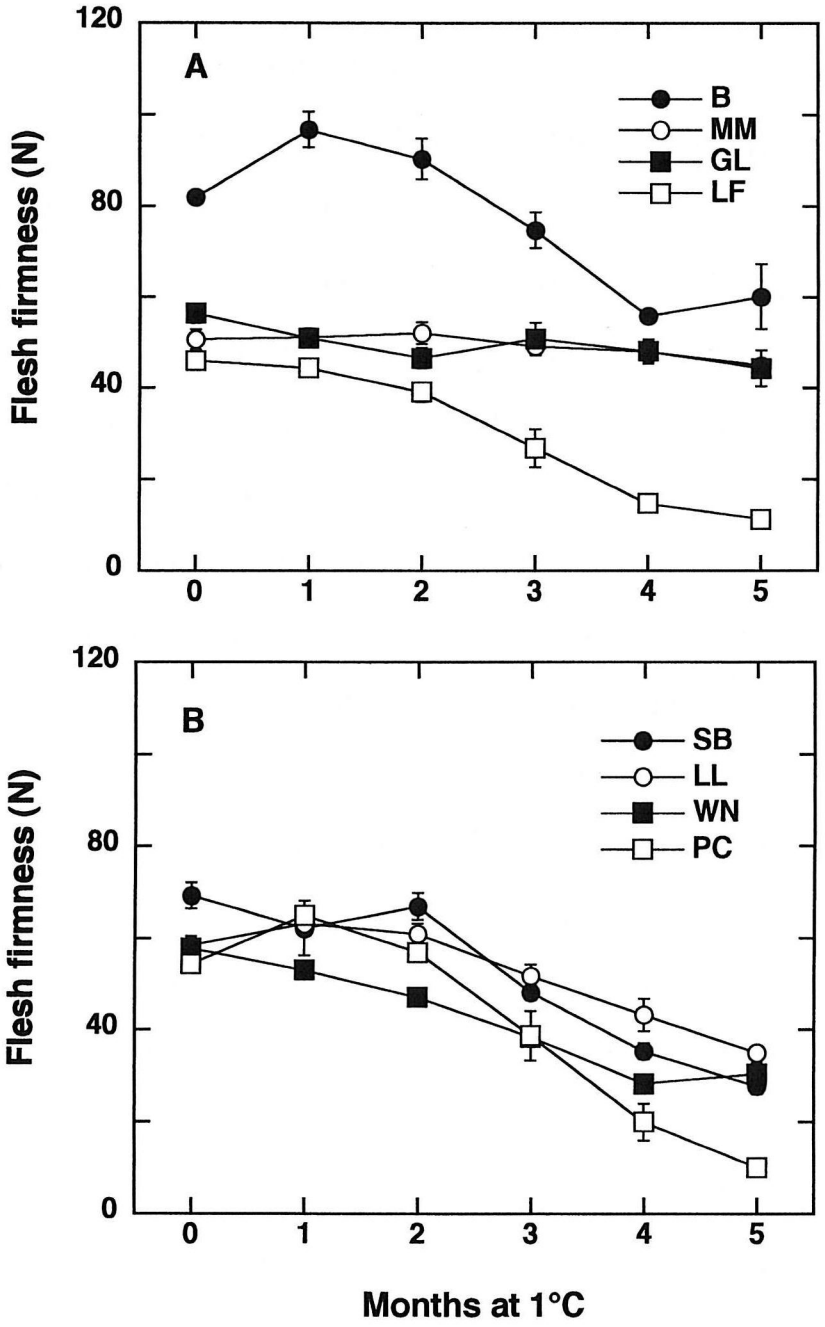

Fig. 2. 1. Changes in flesh firmness during storage at 1°C of 8 pear cultivars. Values are means ± S. E. (n=5). B; Bartlett, MM; Marguerite Marillat, GL; General Leclerc, LF; La France, SB; Silver Bell, LL; Le Lectier, WN; Winter Nelis, PC; Passe Crassane.

5 months at 1°C. The other cultivars showed a gradual softening during storage. Especially in 'Passe Crassane', fruit softened and developed a melting texture during storage.

The weight of fruit decreased linearly during storage, independent of cultivar. The weight loss of 'La France' and 'Passe Crassane' increased at a higher rate than that of other cultivars and reached 14% and 15% after 5 months, respectively (Fig. 2.2). In contrast, that of 'Le Lectier' changed at the lowest rate among the 8 cultivars, and was 7.5% even after 5 months.

'Bartlett' fruit produced ethylene significantly larger amount 2 weeks after storage at 1°C and reached a plateau at the highest level among the 8 cultivars, and then it decreased drastically 4 months after storage (Fig. 2.3). In contrast, the rates of ethylene production of 'Passe Crassane' fruit changed at the lowest rate, and was less than 10 $\mu l \cdot kg^{-1} \cdot h^{-1}$ 5 months after storage. In other cultivars, the rates of ethylene production increased gradually during storage, and reached a maximum of 10 - 20 $\mu l \cdot kg^{-1} \cdot h^{-1}$, although only 'Le Lectier' fruit produced considerable ethylene two weeks after storage.

There were dramatic differences in the changes in ACC content of different pear cultivars during storage at 1°C (Fig. 2.4). ACC in 'Le Lectier' accumulated rapidly during storage, and reached a peak of 15 $nmol \cdot g^{-1}FW$. In 'Bartlett' and 'Marguerite Marillat', ACC content increased significantly from 2 weeks to one month after storage, and then decreased rapidly. ACC content in 'Winter Nelis' increased like 'Bartlett' and 'Marguerite Marillat', but then deceased gradually. The accumulations of ACC in the other 4 cultivars, 'General Leclerc', 'La France', 'Silver Bell' and 'Passe Crassane' were comparatively low, especially in 'Passe Crassane' fruit.

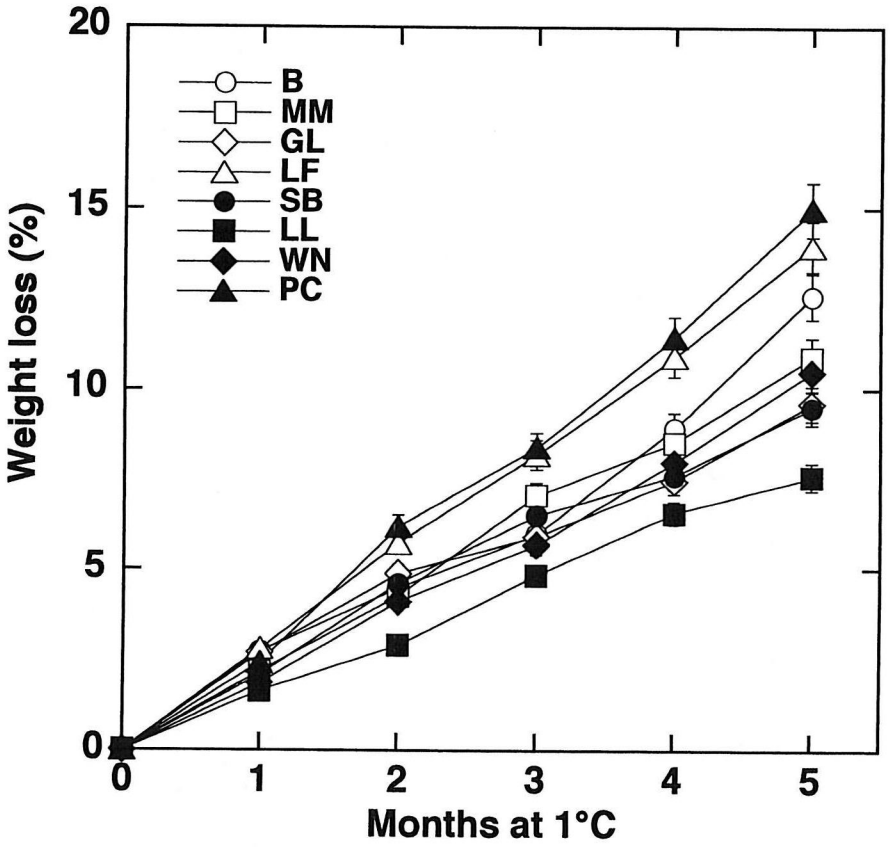

Fig. 2. 2. Changes in weight loss during storage at 1°C of 8 pear cultivars. Values are means ± S. E. (n=5). B; Bartlett, MM; Marguerite Marillat, GL; General Leclerc, LF; La France, SB; Silver Bell, LL; Le Lectier, WN; Winter Nelis, PC; Passe Crassane.

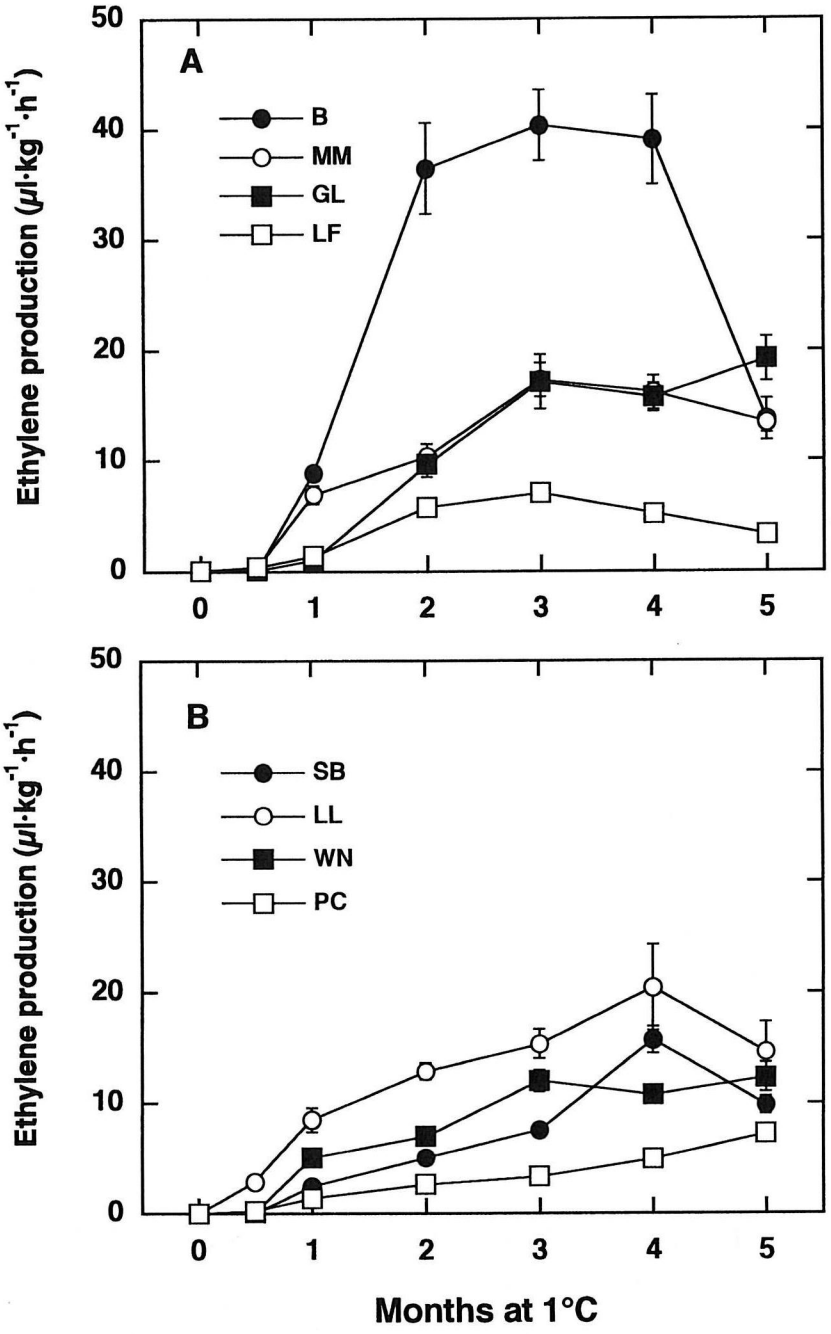

Fig. 2. 3. Changes in ethylene production during storage at 1°C of 8 pear cultivars. Values are means ± S. E. (n=5). B; Bartlett, MM; Marguerite Marillat, GL; General Leclerc, LF; La France, SB; Silver Bell, LL; Le Lectier, WN; Winter Nelis, PC; Passe Crassane.

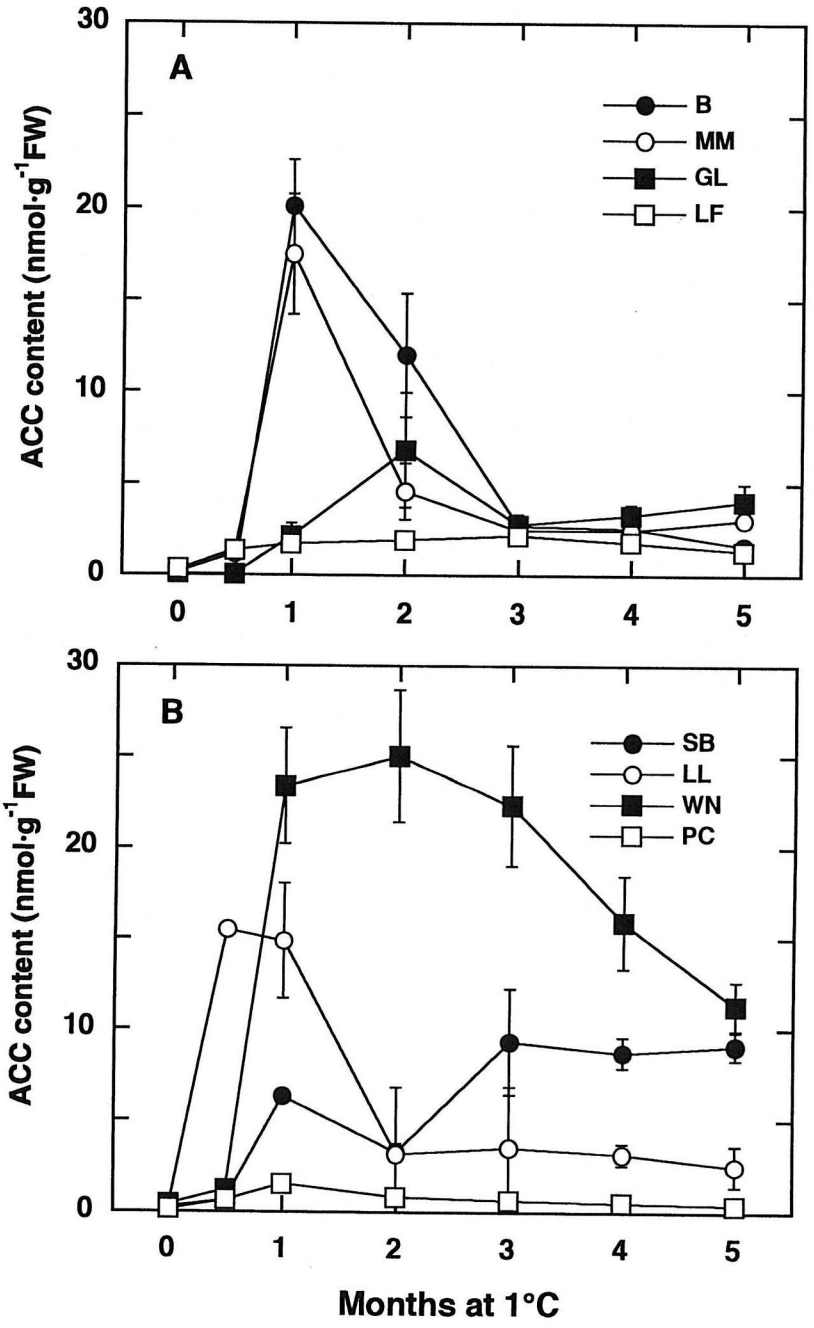

Fig. 2. 4. Changes in ACC content during storage at 1°C of 8 pear cultivars. Values are means ± S. E. (n=5). B; Bartlett, MM; Marguerite Marillat, GL; General Leclerc, LF; La France, SB; Silver Bell, LL; Le Lectier, WN; Winter Nelis, PC; Passe Crassane.

Changes in ethylene production rates during ripening.

Except for 'Passe Crassane', fruit ripened immediately after harvest produced ethylene during ripening at 20°C, although there was a cultivar difference in the time of the increase in ethylene production rates (Fig. 2.5). For the ethylene production of 'Bartlett' fruit, the time of the increase was the earliest (6 days at 20°C), and the maximum rate during ripening was the greatest (56 $\mu l \cdot kg^{-1} \cdot h^{-1}$) among the 8 cultivars. In contrast, 'Passe Crassane' fruit ripened immediately after harvest did not produce ethylene during the experimental period.

The rates of ethylene production were stimulated by storage at 1°C independent of cultivar. A longer storage period correlated with an earlier maximum ethylene production during ripening. However, the peak values decreased in fruit after prolonged storage. The tendency was clear in 'Bartlett', 'Marguerite Marillat' , 'La France' and 'Winter Nelis'.

Fruit texture after storage

Fruit, except for 'Passe Crassane', softened and developed a melting texture during ripening without storage at low temperatures (Table 2.2). In contrast, 'Passe Crassane' fruit without storage or one month storage at 1°C never reached that texture.

'Bartlett' and 'Marguerite Marillat' fruit after storage for 2 months at 1°C softened but never developed a melting texture during ripening at 20°C. Similarly, 'General Leclerc', 'La France', 'Le Lectier' and 'Winter Nelis' fruit after storage for 3 months did not develop that texture. In contrast 'Silver Bell' and 'Passe Crassane' fruit softened and developed a melting texture even after storage for 5 months.

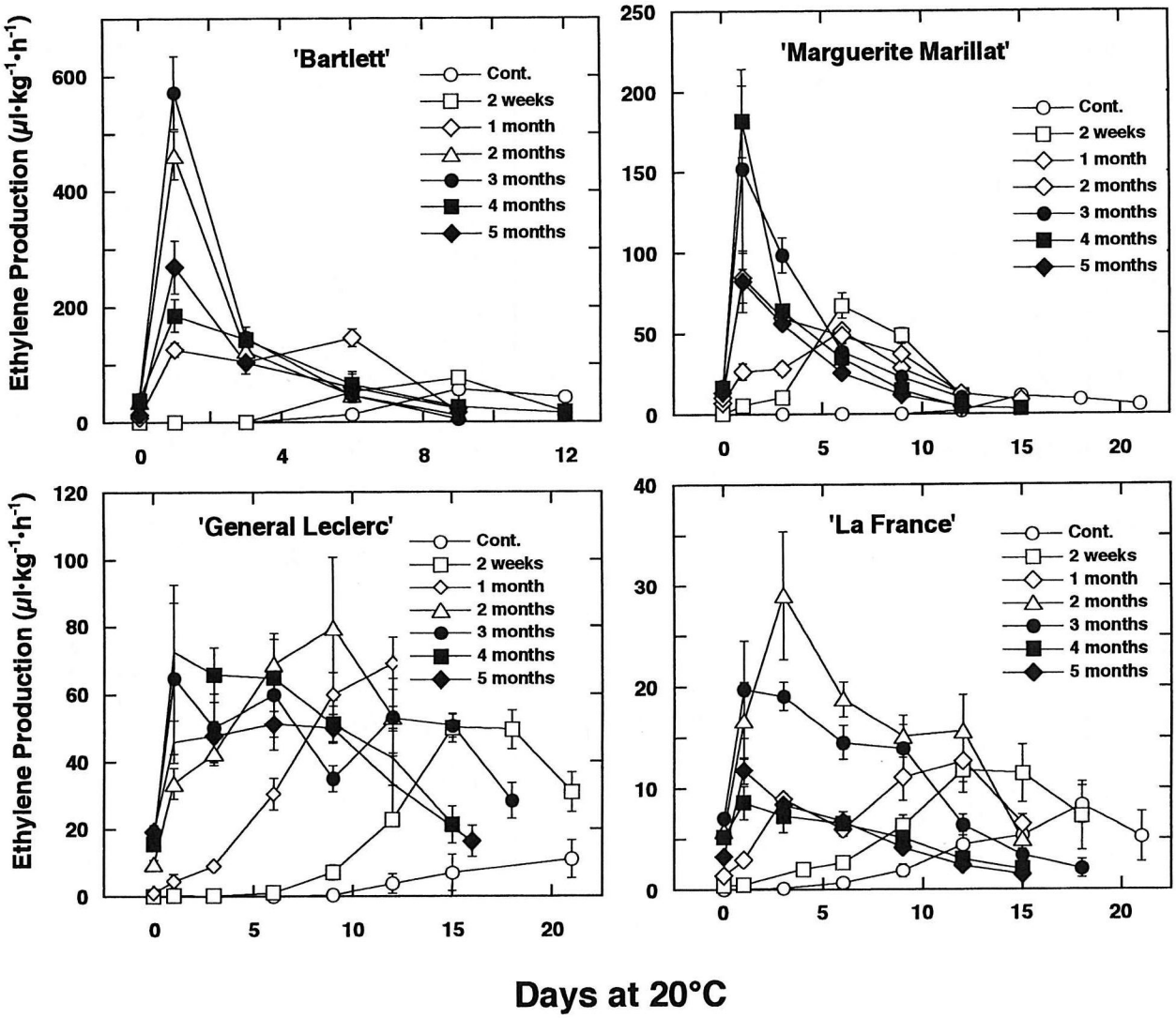

Fig. 2. 5. Changes in ethylene production during ripening at 20°C of pear cultivars. Values are means ± S. E. (n=5). B; Bartlett, MM; Marguerite Marillat, GL; General Leclerc, LF; La France.

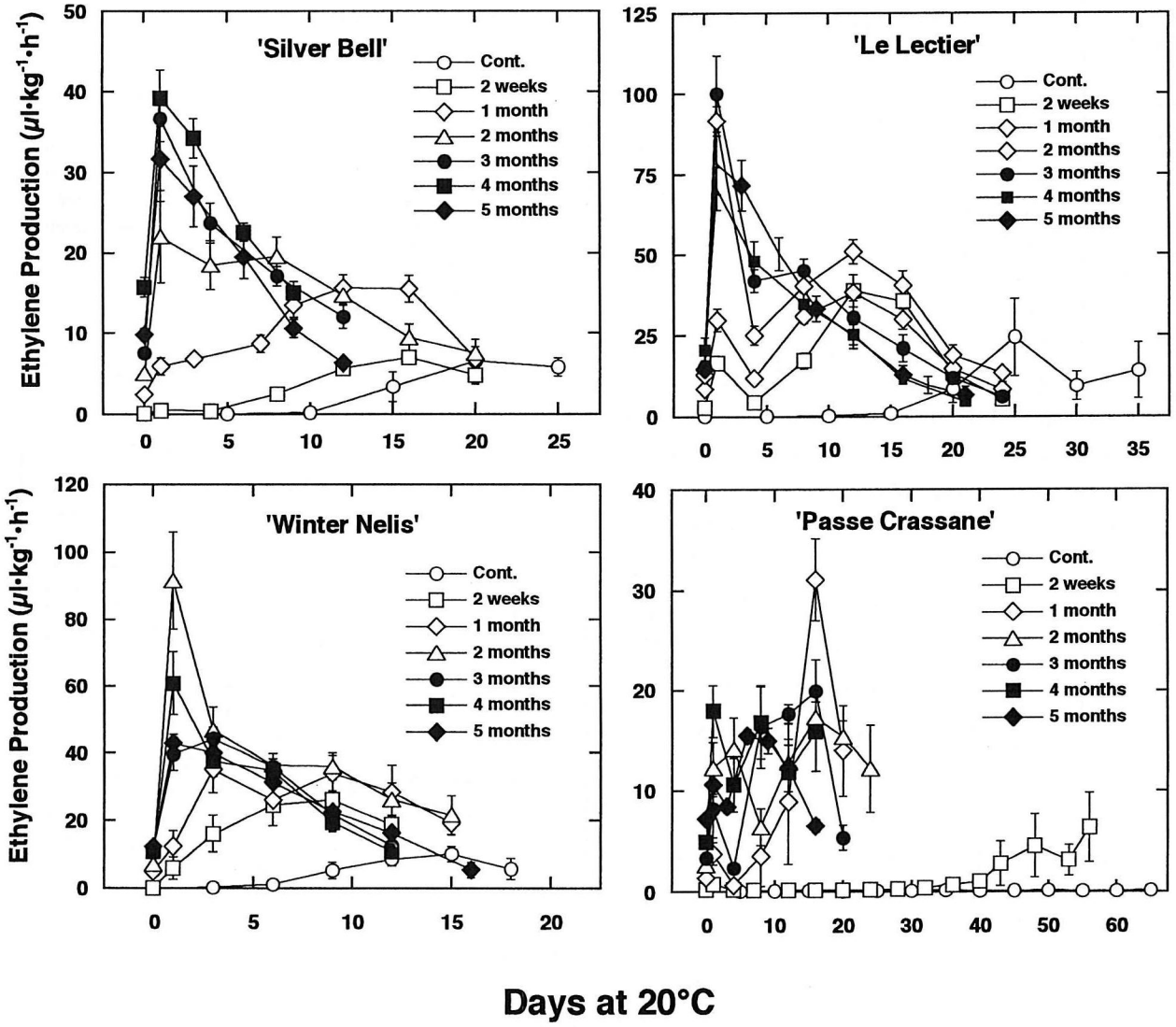

Fig. 2. 5. Continued. SB; Silver Bell, LL; Le Lectier, WN; Winter Nelis, PC; Passe Crassane.

Table 2. 2. Sensory assessment of textural quality of ripened pear fruit after monthly storage intervals. + ; buttery (melting), – ; not buttery.

	Months at 1°C					
	0	1	2	3	4	5
Bartlett	+	+	—	—	—	—
Marguerite Marillat	+	+	—	—	—	—
General Leclerc	+	+	+	—	—	—
La France	+	+	+	—	—	—
Silver Bell	+	+	+	+	+	+
Le Lectier	+	+	+	—	—	—
Winter Nelis	+	+	+	—	—	—
Passe Crassane	—	—	+	+	+	+

Discussion

Flesh firmness of 'Marguerite Marillat' and 'General Leclerc' showed little softening during storage. That of 'Bartlett' decreased slightly, but was more than 50 N even after 5 months of storage. These suggest that fruit softening in earlier maturing cultivars does not progress extensively at 1°C. Other cultivars showed a gradual softening during storage. Especially in 'La France' and 'Passe Crassane', fruit softened to less than 8 N during storage.

All cultivars produced ethylene during storage at 1°C. Similar results have also been reported in a number of pear cultivars (Chen et al., 1993; Elgar et al., 1997). In this study, the rates of ethylene production of 'Le Lectier' fruit increased significantly 2 weeks after storage, while the time of the increase in the other cultivars was one month after storage. Like ethylene production, ACC accumulated significantly 14 days after storage at 1°C only in 'Le Lectier' fruit. These results show that there is a cultivar difference in sensitivity to chilling, and that 'Le Lectier' fruit is the most sensitive to chilling among the 8 cultivars used in this study.

It has been reported that chilling enhances ethylene biosynthesis of pears (Knee 1987, Wang et al. 1985, Lelièvre et al. 1997). There are two types in pears with respect to the chilling requirement for normal ripening. One is the cultivars, including 'Bartlett', that do not absolutely require chilling treatment (Looney, 1972). The other is the cultivars, including 'Beurre d'Anjou' and 'Passe Crassane', that absolutely do require such treatment (Chen et al., 1982; Lelievre et al., 1997). In the present study, all pears except 'Passe Crassane' softened and developed a melting texture without chilling. So these 7 cultivars do not absolutely require chilling for normal ripening, although it is done commercially to ensure or synchronize fruit ripening, except for 'Bartlett' and 'Marguerite Marillat'. Among

the 8 pear cultivars used in this study, only 'Passe Crassane' fruit required chilling for normal ripening.

The rates of ethylene production during ripening were also stimulated by chilling. This agrees with previous reports obtained for other pear cultivars, as well as for different fruits such as apple or cucumber (Jobling et al., 1991; Lelièvre et al., 1997; Wang and Adams, 1982). In general, earlier maturing cultivars produce more ethylene than later maturing ones (Abeles et al., 1992). Similar observations have been made for apples (Hansen, 1945) and Japanese pears (Itai et al., 1999). In pears, these relationships were not clearly observed in fruit ripened immediately after harvest, although 'Bartlett', an early maturing cultivar, produced more ethylene.

The ideal eating firmness for 'Bartlett' pears is reported to be 13 N in New Zealand (Agar et al., 2000). In contrast, the firmness considered suitable for eating in Japan is lower, that is, the range between 5 N and 8 N depending on cultivar. In this study, fruit softened during ripening, independent of cultivar and storage period. Wang et al. (1985) reported that 'Eldorado' pears usually remained firm and dry when fruit was transferred to 20°C after 36 weeks of storage at 0°C. In this study, the period of storage was 5 months. If the fruit is stored longer, they may remain firm during ripening.

The texture of fruit is important for determining the fruit quality. Pears of good quality develop a melting texture. An interesting observation was the fact that the texture of fruit excluding 'Silver Bell' and 'Passe Crassane' did not develop a melting texture when fruit ripened at 20°C after long-term storage. 'Bartlett' and 'Marguerite Marillat' fruit after storage at 1°C for 2 months did not develop a melting texture during ripening at 20°C. Similarly, 'General Leclerc', 'La France' and 'Le Lectier' fruit after storage for 3 months did not develop that texture. In

contrast 'Silver Bell' and 'Passe Crassane' fruit developed a melting texture even after a 5-month storage. These data showed that there is a cultivar difference in possible storage period considering fruit texture at eating firmness.

It was also reported that the possible storage period of pears depended on cultivar. Pears stored in air at -1°C for a proper period of time (usually 3 and 5 months for 'Beurre Bosc' and 'Beurre d'Anjou' pears, respectively) are capable of ripening at 20°C to desirable dessert quality on removal from cold storage (Chen et al., 1983a; Chen et al., 1983b). In pears, the ripening capacity has to be maintained during storage as well as fruit appearance. Yet it is not clear that pears lose the ripening capacity during storage. In this study, fruit that never developed a melting texture after prolonged storage at 1°C also produced ethylene during ripening, although the peak values of ethylene production decreased in fruit after prolonged storage. Thus, ethylene might be not related to the inferior texture after prolonged storage.

The weight loss of 'Silver Bell' and 'Passe Crassane' fruit reached 9% and 15% after 5 months, respectively, although both fruit developed a melting texture during ripening. Even in these two cultivars, the weight loss during storage has to be prevented to extend storage life. Modified atmosphere (MA) storage using plastic films serves the purpose. In addition, the storage in low-O_2 atmospheres at or near 0°C is a common practice that delays ripening of many horticultural crops (Kader, 1986). In pears also, the storage period could be extended by storage in low-O_2 atmospheres (Chen et al., 1983a; Chen et al., 1983b; Chen and Borgic, 1985). Research on MA or controlled atmosphere (CA) storage of pears cultivated in Japan is necessary to expand those consumption.

Summary

The physiological characteristics of 8 cultivars of pears during storage and ripening were investigated. Flesh firmness of 'Marguerite Marillat' and 'General Leclerc' showed little softening during storage, even after 5 months at 1°C. The other cultivars showed a gradual softening during storage. Especially in 'Passe Crassane', fruit softened and developed a buttery and juicy, that is, melting, texture during storage. In the rates of ethylene production, 'Le Lectier' fruit showed a significant increase 2 weeks after storage, while the time in the other cultivars was one month after storage. Like ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) accumulated significantly 2 weeks after storage only in 'Le Lectier' fruit. These results show that the time of ethylene biosynthesis of 'Le Lectier' fruit during storage is the earliest among the eight cultivars we used in this study. Excluding 'Passe Crassane', fruit did not absolutely require chilling treatment for normal ripening, so fruit softened and developed a melting texture at 20°C without chilling treatment. On the other hand, 'Passe Crassane' fruit requires chilling at 1°C more than one month for ripening. 'Bartlett' and 'Marguerite Marillat' fruit softened but never developed a melting texture during ripening at 20°C after storage for 2 months at 1°C. Similarly, 'General Leclerc', 'La France', 'Le Lectier' and 'Winter Nelis' fruit did not develop that texture after storage for 3 months. In contrast 'Silver Bell' and 'Passe Crassane' fruit softened and developed a melting texture even after storage for 5 months.

Section 2. Stimulation of ethylene biosynthesis in 'Le Lectier' fruit by chilling

Introduction

Chilling requirements for ripening of pears are well documented. 'Beurre Bosc' pears were capable of ripening after less than 20 days of chilling at -1.1°C, while 'Beurre d'Anjou' pears required at least 50 days of chilling to develop the ripening capacity (Chen et al., 1982).

Recently, the effect of chilling has been studied at the molecular level in 'Passe Crassane', a cultivar that requires chilling for normal ripening, by Lelièvre et al. (1997). They showed that chilling dramatically stimulated the levels of mRNAs hybridizing to ACC synthase and ACC oxidase probes. However it is not yet known how it is related to the ripening of pears that do not absolutely require such treatment. 'Le Lectier' fruit is found to be the most sensitive to chilling among the 8 cultivars used in the study of the previous section. In this section, the effects of chilling temperatures on ethylene biosynthesis were investigated during storage and ripening in 'Le Lectier' fruit that do not require chilling for ripening.

Materials and Methods

Plant material and treatments

Fruit of the cultivar 'Le Lectier' was used in this study. Fruit was harvested at commercial maturity (OTH) in a commercial orchard near Yamagata in 1996. Within 3 hours following harvest, 5 fruit was sampled and 50 fruit was ripened at 20°C. Relative humidity during ripening was maintained near 100% by using ultrasonic humidifiers (model FT-30N; Ucan Co., Tokyo). The remainder was

stored at 1°C or 5°C for up to 60 days. On day 15 at 1°C or 5°C, some fruit were transferred to 20°C for ripening. The rate of ethylene production, ACC content, ACC synthase activity, ACC oxidase activity, and flesh firmness were determined during storage and ripening. The rate of ethylene production, ACC content and flesh firmness were determined as described in the previous section.

Extraction and assays of ACC synthase and ACC oxidase

Five grams of cortical tissue were homogenized with 10 ml of 100 mM K-phosphate buffer (pH 8.5), containing 5 μM pyridoxal phosphate, 5 mM dithiothreitol, 30 mM sodium ascorbate, 10% (v/v) glycerol, and 2% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 28000 g for 20 min. The supernatant was desalted on a Sephadex G-25 column that had been previously equilibrated with extraction buffer. The column was eluted with the same buffer, and the resulting desalted protein fraction was collected and used as the enzyme preparation in this study. All procedures were carried out at 5°C.

ACC synthase activity was assayed in a reaction mixture containing the enzyme preparation and 500 μM S-adenosylmethionine (AdoMet) in a total volume of 3 ml. After incubation at 30°C for 30 min, the ACC formed from AdoMet was measured as described above. ACC synthase activity was expressed as the amount of ACC produced (nmol) per g fresh weight per hour.

ACC oxidase activity was assayed in a reaction mixture containing 1 ml of the enzyme preparation and 0.1 ml of 20 mM ACC and 10 μl of 2 mM $FeSO_4$. After incubation at 30°C for 30 min, a 1 ml gas sample was withdrawn with a syringe and injected into a gas chromatograph (model GC-8A; Shimadzu Co., Tokyo) fitted with an activated alumina column and a flame ionization detector. ACC oxidase activity was expressed as the amount of ethylene produced (nmol)

per g fresh weight per hour.

Five replications were used for all determinations.

Results

Effect of chilling on flesh firmness

Flesh firmness of 'Le Lectier' fruit changed little during storage for 60 days at 1°C (Fig. 2.6). At 5°C, flesh firmness decreased gradually from 20 to 45 days after harvest and then changed little. At 20°C, it decreased gradually for the initial 20 days, and a dramatic decrease occurred between day 21 and day 28. After day 28, flesh firmness continued to decrease gradually. In fruit transferred from 1°C or 5°C to 20°C on day 15, flesh firmness decreased markedly.

Effect of chilling on ethylene biosynthesis during storage

In fruit held at 20°C, the rate of ethylene production increased on day 28 and reached its maximum on day 35 (Fig. 2.7A). The time of the increase of ethylene production was hastened by chilling. At 1°C, the rate of ethylene production increased on day 15, reached its maximum on day 45, and then changed little. At 5°C, it continued to increase from day 10 to day 60. The rates of ethylene production in chilled fruit, especially in fruit held at 5°C, were higher than that held at 20°C.

ACC content was 0.54 nmol gFW^{-1} at harvest. At 20°C, ACC content remained quite low throughout the experimental period (Fig. 2.7B). In contrast, ACC accumulation was enhanced by chilling. At 1°C, ACC content increased dramatically on day 10 and reached its maximum on day 20. Then, ACC content decreased between day 45 and day 60. At 5°C, ACC content increased markedly between day 5 and day 15 and earlier than 1°C. ACC content decreased after day

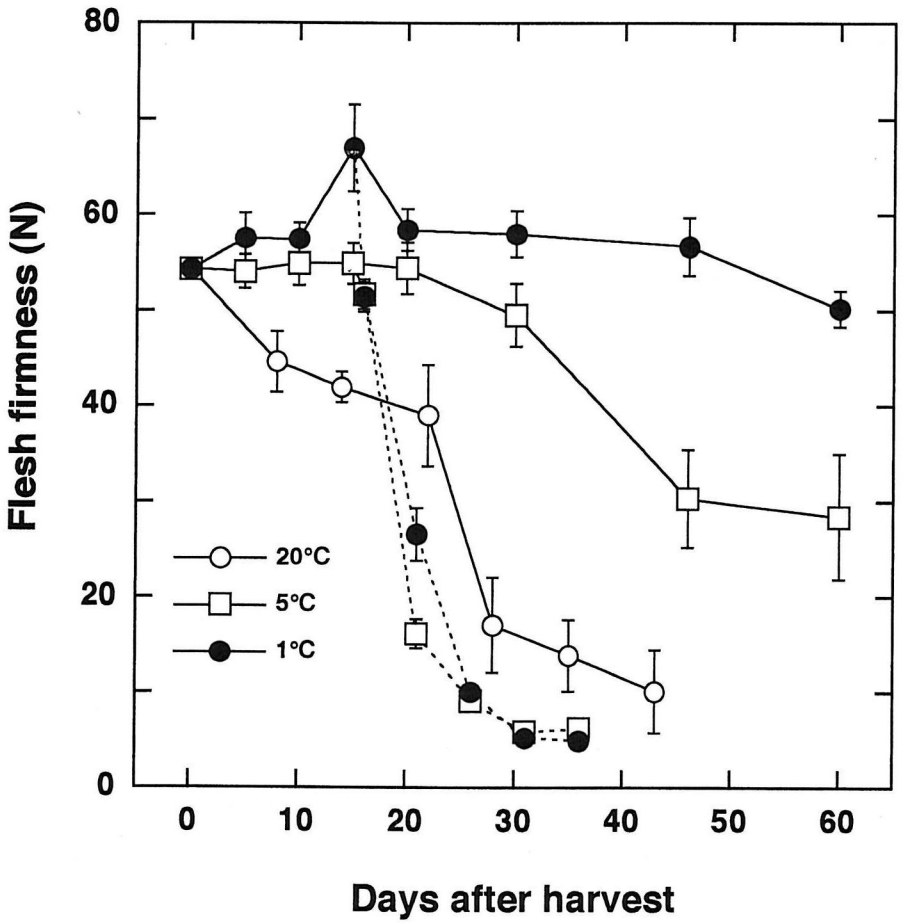

Fig. 2. 6. Changes in flesh firmness in 'Le Lectier' pear fruit. Fruit was held at 1°C, 5°C or 20°C (solid lines), or transferred to 20°C after chilling for 15 days (dotted lines). Values are means ± SE, n=5; SE bars present only when larger than symbol.

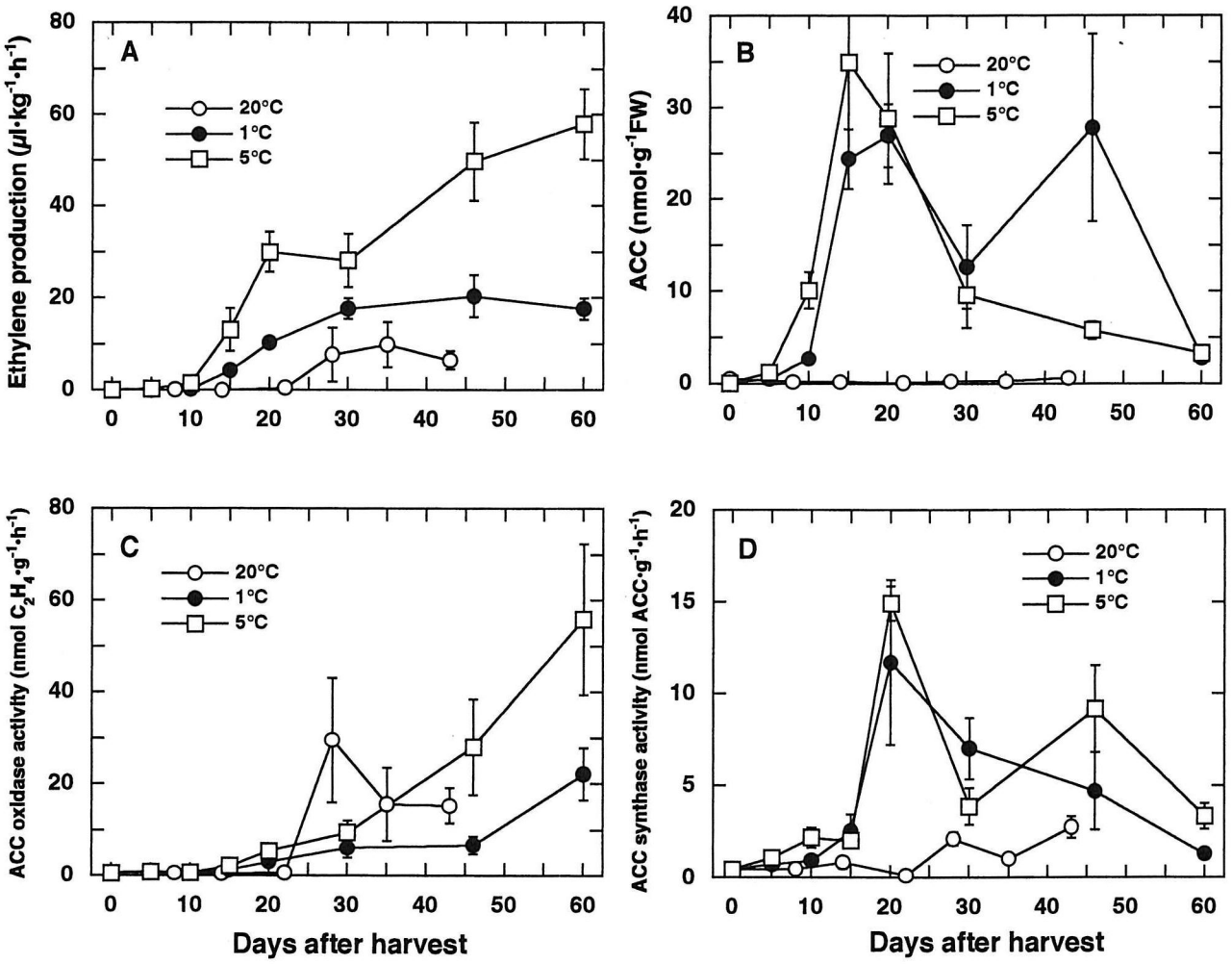

Fig. 2. 7. Changes in ethylene production (A), ACC content (B), ACC oxidase activity (C) and ACC synthase activity (D) in 'Le Lectier' pear fruit during storage at 1°C, 5°C or 20°C for 60 days. Values are means ± SE, n=5; SE bars present only when larger than symbol.

20 and was as low on day 60 as that of fruit at 1°C.

ACC oxidase activity was 0.59 nmol C_2H_4 $gFW^{-1}\cdot h^{-1}$ at harvest. In fruit held at 20°C, it did not change until 21 days after harvest (Fig. 2.7C). On day 28, ACC oxidase activity increased rapidly and reached its maximum. In fruit held at both 1°C and 5°C, the activity increased gradually after day 20. The activity in fruit held at 5°C was higher than that held at 1°C throughout the experimental period, and about 2.5 times higher on day 60.

ACC synthase activity of fruit held at 20°C was low throughout the experimental period (Fig. 2.7D). Only a slight increase was found between 28 days and 42 days. In fruit held at both 1°C and 5°C, ACC synthase activity was enhanced by chilling. It increased gradually on day 5 and day 10 in fruit chilled at 5°C and 1°C, respectively, and markedly between day 15 and day 20 in both. After day 20, the activity showed a tendency to decrease, although a slight increase was found between day 30 and day 45 in fruit held at 5°C.

Effect of chilling on ethylene biosynthesis during ripening

In fruit chilled at 1°C, the rate of ethylene production increased rapidly after transferring to 20°C and decreased between day 1 and day 6, and then increased again and reached its maximum on day 16 (Fig. 2.8A). The rate of ethylene production in fruit chilled at 5°C increased gradually after transferring to 20°C and reached its climacteric peak on day 11. ACC content decreased dramatically after transferring to 20°C and changed little after day 5 in fruit chilled at both 1°C and 5°C (Fig. 2.8B). ACC oxidase activity increased markedly between day 1 and day 6 in fruit chilled at both 1°C and 5°C (Fig. 2.8C). Then, the activity remained high in fruit chilled at 1°C, but decreased gradually in fruit chilled at 5°C. ACC synthase activity decreased for 1 and 6 days after transferring

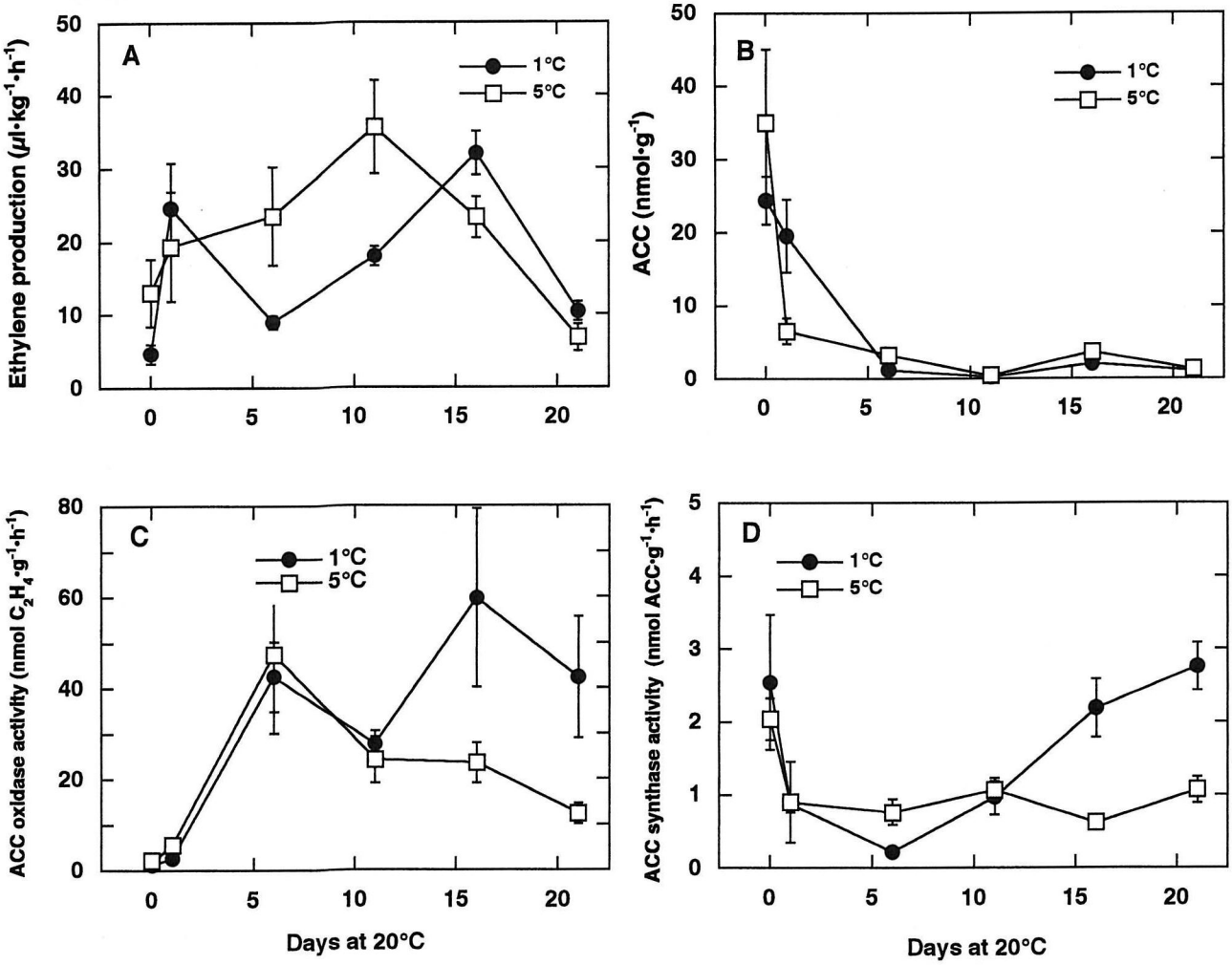

Fig. 2. 8. Changes in ethylene production (A), ACC content (B), ACC oxidase activity (C) and ACC synthase activity (D) in 'Le Lectier' pear fruit during ripening at 20°C. Fruit was chilled at 1°C or 5°C for 15 days and then transferred to 20°C. Values are means ± SE, n=5; SE bars present only when larger than symbol.

to 20°C in fruit chilled at 5°C and 1°C, respectively (Fig. 2.8D). Then the activity in fruit chilled at 5°C changed little, but it increased gradually in fruit chilled at 1°C.

Discussion

It has been reported that chilling enhances ethylene biosynthesis of pear fruit (Knee, 1987; Wang et al., 1985; Lelièvre et al., 1997). There are two types in pears with respect to the chilling requirement for normal ripening. One is the cultivars, including 'Conference', that do not absolutely require chilling treatment (Wilson et al., 1990). The other is the cultivars, including 'Beurre d'Anjou' and 'Passe Crassane', that absolutely require such treatment (Chen et al., 1982; Lelièvre et al., 1997).

The rate of ethylene production in 'Le Lectier' fruit held at 1°C or 5°C increased earlier than that in fruit held at 20°C. The stimulation of ethylene production by chilling agrees with previous reports obtained for other pear cultivars as well as for different fruits and vegetables, such as apples or cucumbers (Jobling et al., 1991; Lelièvre et al., 1997; Wang and Adams, 1982). Other than pears, however, they produce less ethylene at chilling temperature than at 20°C. In such fruit, ethylene biosynthesis is stimulated upon rewarming after chilling. To my knowledge, only pear fruit is characterized by ethylene biosynthesis during storage being stimulated by chilling.

ACC synthase is a key enzyme in the biosynthesis of ethylene in higher plants (Yang and Hoffman, 1984). ACC synthase activity increased dramatically between day 10 and day 15 during storage at 1°C or 5°C. In contrast, the activity of fruit held at 20°C was low throughout the experimental period, with only a slight increase found after day 28. This agrees with a recent report concerning

'Passe Crassane' pears (Lelièvre et al., 1997). In fruit other than pears, ACC synthase activity is low during storage. Wang and Adams (1982), who carried out a detailed study using chilled cucumbers, suggested that at chilling temperatures, there may be an unmasking or stimulated production of a mRNA coding for the ACC synthase protein, but its processing and/or translation are inhibited by chilling. As ACC synthase activity has been shown to limit ethylene biosynthesis (Yang and Hoffman, 1984), it is important for ethylene biosynthesis of pear fruit that ACC synthase is induced by chilling. In addition, the ACC synthase activity of 'Le Lectier' fruit is considerably higher than that reported in 'Conference' and 'Passe Crassane' fruit (Knee, 1987; Lelièvre et al., 1997). This may be related to high sensitivity in 'Le Lectier' fruit to chilling.

Recent reports suggest that the ability to convert ACC to ethylene is induced more rapidly at chilling temperatures than at 20°C (Jobling et al., 1991). Similarly, results presented by Tian et al. (1994) showed that ACC oxidase activity, in both Japanese pears and apples, increased gradually during cold storage. In addition, Lelièvre et al. (1995) demonstrated an accumulation of ACC oxidase protein in preclimacteric apple fruit stored at 4°C, which paralleled the increase in ethylene production. ACC oxidase activity in chilled fruit increased gradually throughout the experimental period. On the other hand, the activity in fruit at 20°C increased rapidly between day 21 and day 28, and reached its maximum on day 28. The activity on day 28 in non-chilled fruit was higher than that on day 30 in chilled fruit. This suggests that chilling does not promote the ACC oxidase activity as much as ACC synthase activity in 'Le Lectier' fruit. Lelièvre et al. (1997) reported that ACC oxidase activity in 'Passe Crassane' fruit was strongly stimulated by chilling. This discrepancy may be in part a cultivar difference. In contrast to 'Passe Crassane' fruit, which require chilling for ripening, 'Le Lectier' fruit do

not absolutely require chilling.

In this study, two chilling temperatures, 1°C and 5°C, were investigated. Ethylene production, ACC content and ACC synthase activity increased earlier at 5°C than at 1°C, and the rate of ethylene production was much higher at 5°C than that at 1°C throughout the experimental period. This suggests that ethylene biosynthesis is stimulated more strongly at 5°C compared with 1°C. In fruit at 5°C, flesh firmness decreased substantially after day 28 suggesting that ripening of the fruit had progressed at this temperature. Interestingly, the decrease in flesh firmness corresponded to the second increase in the rate of ethylene production and ACC synthase activity. Tomato fruit expresses multiple ACC synthase genes that are differentially regulated during ripening, by wounding and auxin (Yip et al., 1992). In fruit at 5°C, ethylene biosynthesis may be differentially regulated during ripening and by chilling.

ACC oxidase activity increased markedly after rewarming. This accords with previous reports for other pear cultivars as well as for other fruits, such as apples (Chavez-Franco and Kader, 1993; Larrigaudiere et al., 1997). ACC synthase activity decreased immediately after rewarming to 20°C in contrast to ACC oxidase activity. As a result, ACC content decreased rapidly after rewarming. This is not in agreement with Knee (1987), who reported that ACC synthase activity and ACC content in 'Conference' pears increased rapidly after rewarming. However, fruit was stored at 1°C for 30 weeks and rewarmed at 15°C in his experiment, so I can not easily compare the results between two experiments. In apples and cucumbers, ACC synthase activity also increased immediately after rewarming (Yip et al., 1991; Wang and Adams, 1982). On the other hand, in 'Bartlett' fruit, ACC synthase activity decreased during the first 60 hours after rewarming (Chavez-Franco and Kader, 1993). No explanation can be made for these differences in

ACC synthase activity after rewarming, although it is possible that multiple ACC synthase genes are differentially regulated during ripening. These aspects need to be further studied.

In conclusion, in 'Le Lectier' fruit, chilling strongly stimulated ACC synthase activity, but did not promote the ACC oxidase activity as much as ACC synthase activity. In addition, the increase in ACC synthase activity preceded that of ACC oxidase at chilling temperatures. As a result, ACC accumulates substantially by 20 days after harvest. After rewarming to 20°C, ACC synthase activity decreased immediately in contrast with ACC oxidase activity, which was enhanced markedly.

Summary

The effects of chilling on ethylene biosynthesis of 'Le Lectier' pears were investigated during storage and ripening. The rate of ethylene production increased on day 10, day 5 and day 21 after harvest in fruit held at 1°C, 5°C and 20°C, respectively. ACC synthase activity in fruit held at 20°C was low throughout the experimental period, and only a slight increase was found after day 21. In contrast, in fruit held at both 1°C and 5°C, it increased markedly between day 15 and day 20 after harvest. An increase in ACC synthase activity preceded the increase in ACC oxidase activity. Then, ACC accumulated substantially from day 20 after harvest. ACC oxidase activity in fruit held at 20°C did not change until 21 days after harvest. On day 28, the activity increased rapidly and reached its maximum on day 35. In fruit held at both 1°C and 5°C, it increased gradually after day 15. After rewarming to 20°C, the rate of ethylene production in fruit chilled at 1°C increased immediately, decreased between day one and day 6, and then increased again and reached its maximum on day 16. Ethylene production in fruit chilled at 5°C increased gradually and reached its climacteric peak on day 11. ACC

content decreased dramatically after rewarming and changed at a low level after day one in fruit chilled at both 1°C and 5°C. ACC synthase activity decreased after rewarming for one and 6 days in fruit chilled at 5°C and 1°C, respectively. Then the activity in fruit chilled at 5°C changed little, but increased gradually in fruit chilled at 1°C. ACC oxidase activity increased markedly between day one and day 6 after rewarming in fruit chilled at both 1°C and 5°C. Then, the activity remained high in fruit chilled at 1°C, but decreased gradually in fruit chilled at 5°C. Taken together, chilling strongly stimulated ACC synthase activity, but did not promote the ACC oxidase activity as much as it did ACC synthase activity. After rewarming to 20°C, ACC synthase activity decreased immediately in contrast with ACC oxidase activity, which was enhanced markedly.

Chapter 3.

Effect of relative humidity on ripening of 'Le Lectier' pear fruit

Introduction

Pear fruit is harvested before it becomes ripe, and requires several days or weeks to soften and develop a buttery and juicy texture. Temperatures after harvest affect the ripening of pears (Kitamura, 1987; Maxie et al., 1974). The days required for fruit to ripen are fewer in higher temperatures, although fruit does not ripen normally in temperatures higher than 30°C (Maxie et al., 1974).

Fruits and vegetables are also affected by relative humidity (RH) during storage. They wilt at low RH and result in reduced commercial value. RH during ripening also appears to influence the ripening of pears, but its effects have not been studied. One reason is it is thought to be difficult to regulate relative humidity in a chamber for ripening. In this chapter, the effects of different RHs on the ripening of 'Le Lectier' pears were investigated.

Materials and Methods

Plant material and treatments

'Le Lectier' fruit was harvested at commercial maturity (OTH) from an orchard in Yamagata. On the next day, fruit without chilling was directly held at 20°C with 55%, 75%, or 95% RH. A constant temperature and humidity chamber was used for 55% and 75% RH. For 95% RH, a chamber was placed in a constant temperature room (55% RH) and the inside of the chamber was sprayed with distilled water every morning and evening to maintain the desired humidity. On day 35, the twenty fruit held at 55% RH were placed into a 79.4-liter plastic container. After the container was made airtight, fruit was treated with 200 ppm

ethylene for 48 hours. Fruit with chilling was held at 1°C for 2 weeks after harvest, and then transferred to 20°C for ripening.

Determination of ethylene production, ACC content and flesh firmness

The rate of ethylene production, ACC content, and flesh firmness were determined as described in the chapter 2.

Extraction, fractionation, and characterization of cell wall polyuronides

The cell wall polyuronides were prepared from alcohol-insoluble residues (AIR) of fruit flesh and partitioned into two fractions based on the solubility of different solvents. Fruit was peeled, cored, and diced. About 20 g of flesh was heated in 100 ml of 80% (v/ v) ethanol at 80°C for 30 min and homogenized in a Waring blender. The homogenate was filtered, and the residue was washed twice with 50 ml of 80% (v/v) ethanol. The dried residue was then suspended in 100 ml of distilled water and stirred overnight. After filtration, the residue was washed twice with 50 ml of distilled water and filtered again. The filtrates were combined and used as the water-soluble fraction. The residue was then extracted with 100 ml of 0.05M HCl and heated at 100°C for 1 h. After filtration, the residue was washed twice with 50 ml of 0.05M HCl solution. The filtrates were combined, neutralized with Tris(hydroxymethyl)aminomethane, and used as HCl-soluble fraction. The uronic acid content in each fraction was measured by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). The polyuronide content of chilled fruit was not determined.

Five replications were used for all determinations.

Results

Flesh firmness

The firmness of 'Le Lectier' fruit at harvest was 53.9 N. At 95% RH, fruit without chilling began to soften from the 3rd week after harvest, and developed a buttery and juicy texture within 5 weeks (Fig.3.1). Fruit at 55% or 75% RH did not soften as much after 8 weeks. However, fruit treated with chilling at 1°C for 2 weeks began to soften even at 55% RH from day 10 after transferring to 20°C.

Ethylene production

At 95% RH, fruit without chilling dramatically produced ethylene after 4 weeks (Fig. 3.2A). Fruit held at 55% or 75% RH after harvest did not show any increase in the rate of ethylene production. Fruit treated with chilling, however, produced ethylene (1.63 $\mu l \cdot kg^{-1} \cdot h^{-1}$) at 55% RH on day 5 after transferring from 1°C to 20°C. Then the rate of ethylene production rapidly increased and reached its maximum on day 15.

ACC content

ACC was detected (0.30 $nmol \cdot g^{-1}$) at harvest but gradually decreased during ripening in fruit held at 55% or 75% RH (Fig. 3.2B). In fruit held at 95% RH, ACC content increased from the 4th week after harvest. In fruit treated with chilling, ACC substantially accumulated on day 5 after transferring to 20°C. Thereafter ACC content reached its minimum and then increased again.

Polyuronides

The content of water-soluble polyuronides of the fruit held at 95% RH increased more than those held at 55% or 75% RH (Fig. 3.3A). On the other hand,

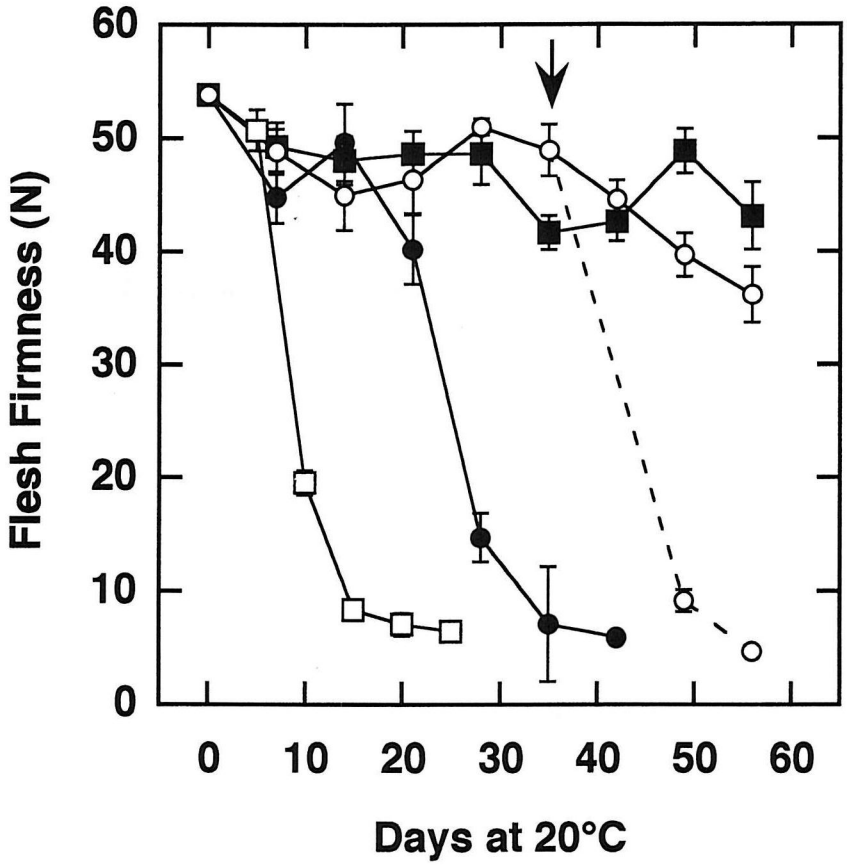

Fig. 3. 1. Changes in flesh firmness of 'Le Lectier' fruit. Fruit was held directly at 20°C with 55% RH (○), 75% RH (■), or 95% RH(●) after harvest, or held at 20°C with 55% RH after storage at 1°C for 2 weeks (□). Twenty fruit held at 55% RH after harvest directly were treated with 200ppm ethylene after 5 weeks (arrow) and held at 55% RH (broken line). Vertical bars represent the SE (n=5).

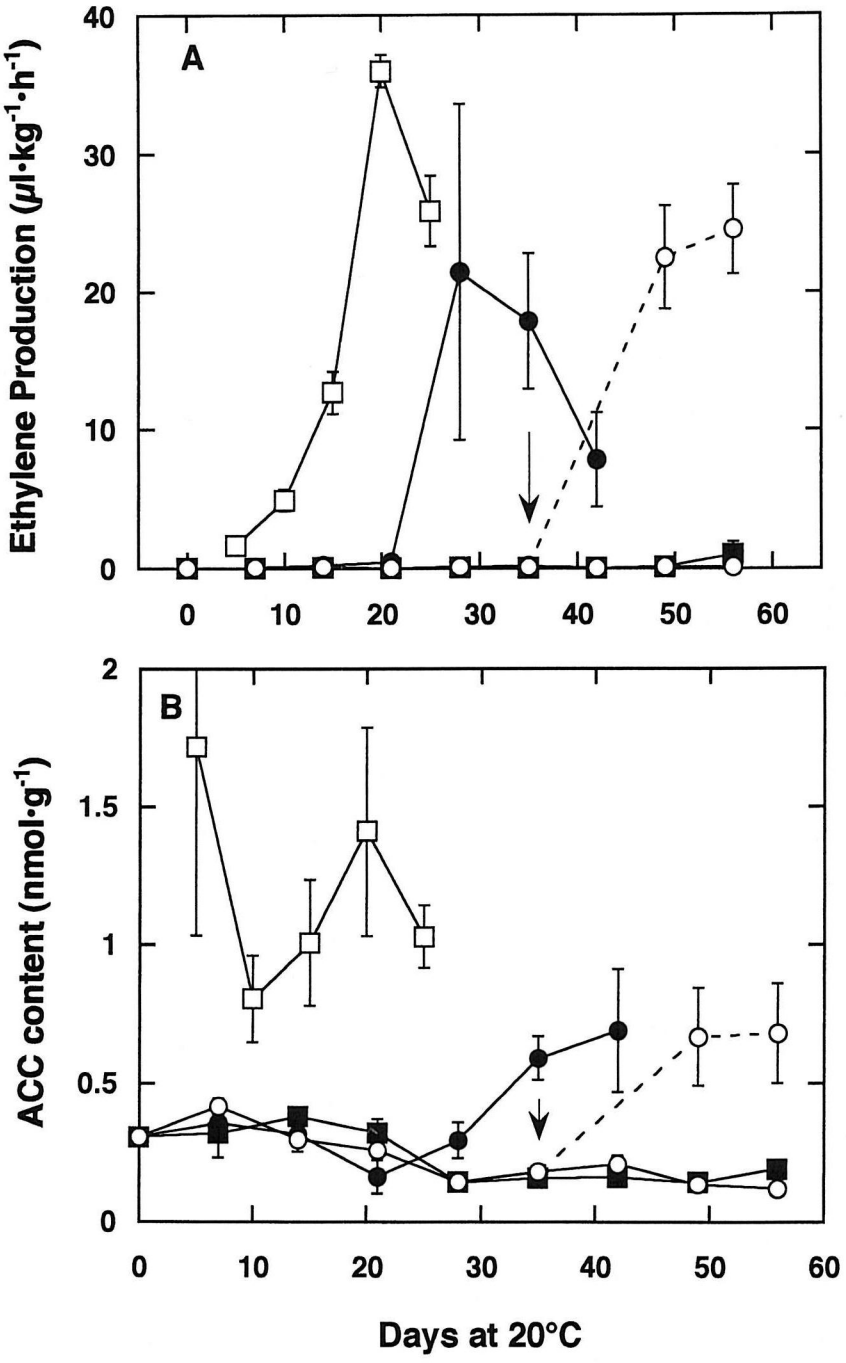

Fig. 3. 2. Changes in ethylene production (A) and ACC content (B) of 'Le Lectier' fruit. Fruit was held directly at 20°C with 55% RH (○), 75% RH (■), or 95% RH (●) after harvest, or held at 20°C with 55% RH after storage at 1°C for 2 weeks (□). Twenty fruit held at 55% RH were treated with 200ppm ethylene after 5 weeks (arrows) and held at 55% RH (broken lines). Vertical bars represent the SE (n=5).

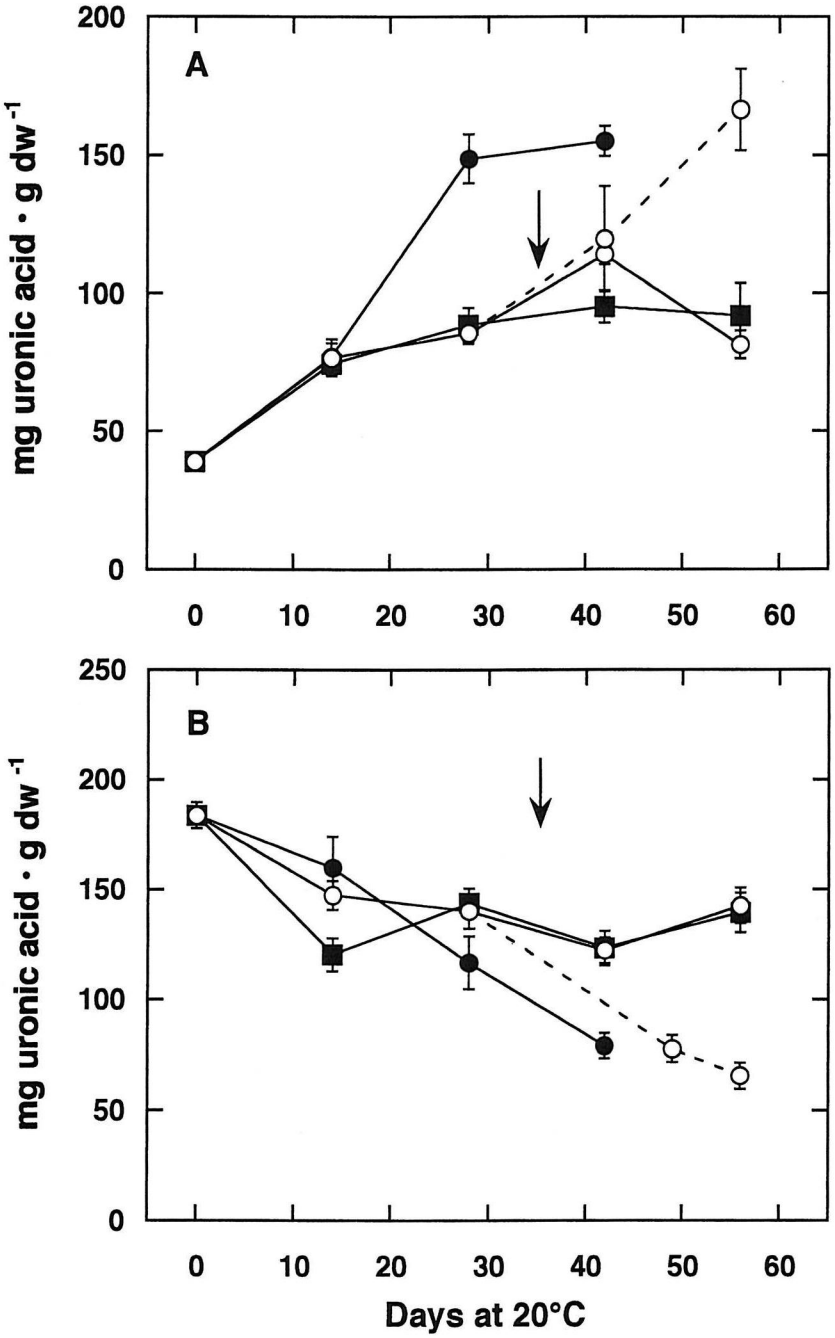

Fig. 3. 3. Changes in the content of water-soluble (A) and HCl-soluble (B) polyuronides of 'Le Lectier' fruit. Fruit was held directly at 20°C with 55% RH (○), 75% RH (■), or 95% RH (●) after harvest. Twenty fruit held at 55% RH were treated with 200ppm ethylene after 5 weeks (arrows) and held at 55% RH (broken lines). Vertical bars represent the SE (n=5).

the content of HCl-soluble polyuronides of the fruit held at 95% RH significantly decreased as compared with that of the fruit held at 55% or 75% RH (Fig. 3.3B).

Ethylene treatment

In fruit treated with 200ppm ethylene after five weeks at 55% RH, flesh firmness dramatically decreased, and ethylene production rates and ACC content substantially increased (Fig. 3.1 and 3.2). In addition, water-soluble polyuronides increased and HCl-soluble polyuronides decreased as much as those of the fruit held at 95% RH (Fig. 3.3).

Discussion

'Le Lectier' fruit held at 55% or 75% RH never softened appreciably nor produced ethylene. Yoshioka et al. (1992) reported that water-soluble polyuronides increased and HCl-soluble polyuronides decreased during ripening of pear fruit. However, in fruit held at 55% or 75% RH, water-soluble polyuronides did not increase and HCl-soluble polyuronides did not decrease as much as those held at 95% RH. The texture of the fruit held at 55% or 75% RH was "rubbery". In nectarine fruit, high temperature (46°C) treatment induced a "rubbery" texture (Lay-Yee and Rose, 1994).

The failure of fruit held at 55% or 75% RH to ripen normally seemed to be related to the capacity of the ethylene biosynthesis. This is likely for two reasons. First, those fruit did not show any increase in ethylene production for 8 weeks. Second, fruit treated with 200ppm ethylene after five weeks at 20°C with 55% RH produced significant ethylene. Ethylene plays an important role in the ripening of pear fruit, but it is not yet known why ethylene biosynthesis is suppressed by low RH. In fruit held at 55% or 75% RH, ACC did not increase throughout the

experimental period. These results suggest that ACC synthase activity may be suppressed by low RH. But the activities of ACC synthase or ACC oxidase were not measured in this study, so this remains an open question.

Fruit chilled at 1°C for 2 weeks properly softened even at 55% RH. This is not surprising because it is known that chilling promotes ethylene biosynthesis (Chen et al., 1982 ; Wang et al., 1985). In this study, fruit chilled at 1°C produced ethylene and substantially accumulated ACC on day 5 after transferring to 20°C .

These results suggest that low RH inhibits the normal ripening of 'Le Lectier' fruit due to the suppression of ethylene biosynthesis, if fruit is not chilled after harvest.

Summary

'Le Lectier' fruit was harvested at a commercial harvest. On the next day, the fruit was held at 20°C with 55%, 75%, or 95% relative humidity (RH). The flesh firmness at 95% RH rapidly decreased from the 3rd week and reached less than 10 N, appreciable firmness, after 5 weeks. In those fruit, there was a marked increase in the rate of ethylene production after 4 weeks. Moreover, ACC content gradually increased from the 4th week. Fruit at 55% or 75% RH showed greater flesh firmness than those at 95% RH after 4 weeks and never softened appreciably. Those fruit did not show any increase in the rate of ethylene production and ACC content throughout the experimental period. After 5 weeks at 55%, fruit treated with 200ppm ethylene for 48 hours, showed a significant increase in ethylene production and ACC content. The flesh firmness rapidly decreased after ethylene treatment. Moreover, the content of the water-soluble polyuronides increased and that of HCl-soluble polyuronides decreased after treatment. Fruit chilled at 1°C for 2 weeks produced ethylene and properly softened even at 55%

RH. These results suggest that low RH inhibits the normal ripening of 'Le Lectier' fruit due to the suppression of ethylene biosynthesis, if fruit is not chilled after harvest.

Chapter 4.

Effect of storage periods on cell wall polysaccharides of pear fruit during ripening

Section 1. Relationship between fruit softening and cell wall polysaccharides in pear fruit after different storage periods

Introduction

Pear fruit is usually chilled at temperatures of -1°C to 1°C after harvest for a period of time and then transferred to ambient temperature for ripening (Elgar et al., 1997; Gerasopoulos and Richardson, 1996). An abnormal softening occurs, however, when fruit is transferred to 20°C after prolonged storage (Mellenthin and Wang, 1976; Wang et al., 1985). This phenomenon is called the loss of ripening capacity. Wang et al. (1985) reported that 'Eldorado' pears usually remained firm and dry and never reached a buttery and juicy texture, when they were transferred to 20°C after 36 weeks of storage at 0°C. They also reported that there was less soluble polyuronide in fruit after 36 weeks, suggesting impairment of mechanisms regulating the solubilization of cell wall polyuronides and the softening of fruit. This is likely because the amount of water-soluble polyuronides increased and those of insoluble polyuronides decreased significantly during normal ripening in pears (Ben-Arie and Sonego, 1979). However, no report determining the cell wall polysaccharides other than soluble polyuronides in pears whose ripening capacity was lost, has been available, and thus the mechanisms by which fruit loses ripening capacity are not clear.

Moreover, although there are many reports about changes in cell wall polysaccharides during softening of fruit including pears (Muda et al, 1995;

Yoshioka et al., 1992), the changes were usually investigated at regular intervals during ripening. Then, the amount of cell wall polysaccharides was plotted against number of ripening days. I think that it should be plotted against flesh firmness to study the relationship between fruit softening and cell wall polysaccharides as provided by Ahmed and Labavitch (1980).

As described in chapter 2, 'Marguerite Marillat' and 'La France' fruit, popular cultivars in Japan, lose their ripening capacity when fruit is stored more than 2 months and 4 months, respectively. Such fruit softened but never reached a buttery and juicy texture. In this section, to clarify the mechanisms by which fruit failed to reach the buttery and juicy texture after prolonged storage, the changes in the cell wall polysaccharides were investigated during ripening of 'Marguerite Marillat' and 'La France' fruit which had retained or had lost their ripening capacity, and plotted the amount of cell wall polysaccharides against flesh firmness.

Materials and Methods

Plant material and treatments

Fruit of the cultivars 'Marguerite Marillat' and 'La France' was used in this study. Fruit was harvested at commercial maturity (OTH) in a commercial orchard near Yamagata in 1998. After selection for uniformity of size and freedom from defects, fruit was stored at 1°C. After one month (short-term storage) of both cultivars, and 4 months (long-term storage) of 'Marguerite Marillat' or 5 months (long-term storage) of 'La France', fruit was transferred to 20°C for ripening. Flesh firmness was measured every day during ripening on the opposite sides of each fruit using a rheometer (model CR-200D; Sun Scientific Co., Tokyo) with an 8-mm plunger. Based on flesh firmness, 20 fruit were selected at different stages

of softening (from hard to soft) in each storage period in both cultivars for analysis of cell wall polysaccharides.

Extraction, fractionation, and characterization of cell wall polysaccharides

Fruit was peeled, cored and diced. About 20 g of flesh from single fruit was boiled in 80 ml of 100% (v/v) ethanol for 30 min. After being cooled to room temperature, the tissue was further homogenized in a Waring blender for 5 min and vacuum-filtered through a glass fiber filter (Whatman GF/C). The filtrate was discarded, while the residue was resuspended three times in 80% ethanol, boiled for 30 min, and filtered again. The residue was then washed once with 100% ethanol, once with 100% acetone, and finally dried in an oven (40°C).

Fifty mg of alcohol-insoluble residue (AIR) was dispersed in 20 ml of distilled water, mechanically shaken overnight at 20°C, and vacuum-filtered through a GF/C filter. The residue was then suspended in 20 ml of distilled water, shaken for 1 h, and filtered again. The filtrates were combined and designated as the water-soluble polyuronides (WSP). The residue was then extracted with 20 ml of 50 mM ethylenediaminetetraacetic acid (EDTA) (pH 6.5) at 20°C twice. The filtrates were combined and designated as the chelator-soluble polyuronides (CSP). The residue was further extracted with 20 ml of 50 mM Na_2CO_3 containing 20 mM $NaBH_4$ at 20°C twice. The filtrates were combined, neutralized with acetic acid, and designated as the alkaline-soluble polyuronides (ASP). The uronic acid content in each fraction was measured by the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973).

Depectinated AIR was treated for 2 h with 100 U of α-amylase (hog pancreatic α-amylase, Sigma) in 50 mM Na-acetate buffer (pH 6.5). Then the residue was used for the subsequent extraction of hemicellulosic polysaccharides.

The residue was extracted with 20 ml of 4 M KOH containing 20 mM $NaBH_4$ for 24 h, mechanically shaken overnight at 20°C, and vacuum-filtered through a GF/C filter. The residue was then suspended in 20 ml of KOH solution, shaken for 1 h, and filtered again. The filtrates were combined, neutralized with acetic acid, and designated as hemicellulosic polysaccharides. The total sugar content in this fraction was measured by a phenol-sulfuric acid method (Dubois et al., 1956)

The residue was washed with diluted acetic acid and a mixture of ethanol:diethyl ether (1:1, v/v). After drying, the residue was designated as cellulose. This fraction was dissolved in 0.2 ml of 72% H_2SO_4, kept for 1 h at room temperature, diluted with 2.8 ml of distilled water, and then heated for 1 h at 120°C. Total sugar content of the solution was measured by a phenol-sulfuric acid method (Dubois et al., 1956).

Results

Effect of storage period on pectic polysaccharides

Fruit softened during ripening, independent of cultivar and storage period. In both cultivars, fruit after short-term storage reached a buttery and juicy texture, while fruit after long-term storage did not.

In 'Marguerite Marillat', the relationship between flesh firmness and WSP content in fruit after short-term storage showed that the amount of WSP varied convexly with decreasing flesh firmness and was highest in fruit whose flesh firmness is 23.8 N (Fig. 4.1A). As with fruit after short-term storage, WSP content varied convexly with decreasing flesh firmness in fruit after long-term storage. However, WSP levels of fruit after long-term storage tended to be lower than those of fruit after short-term storage, when compared in fruit of similar flesh

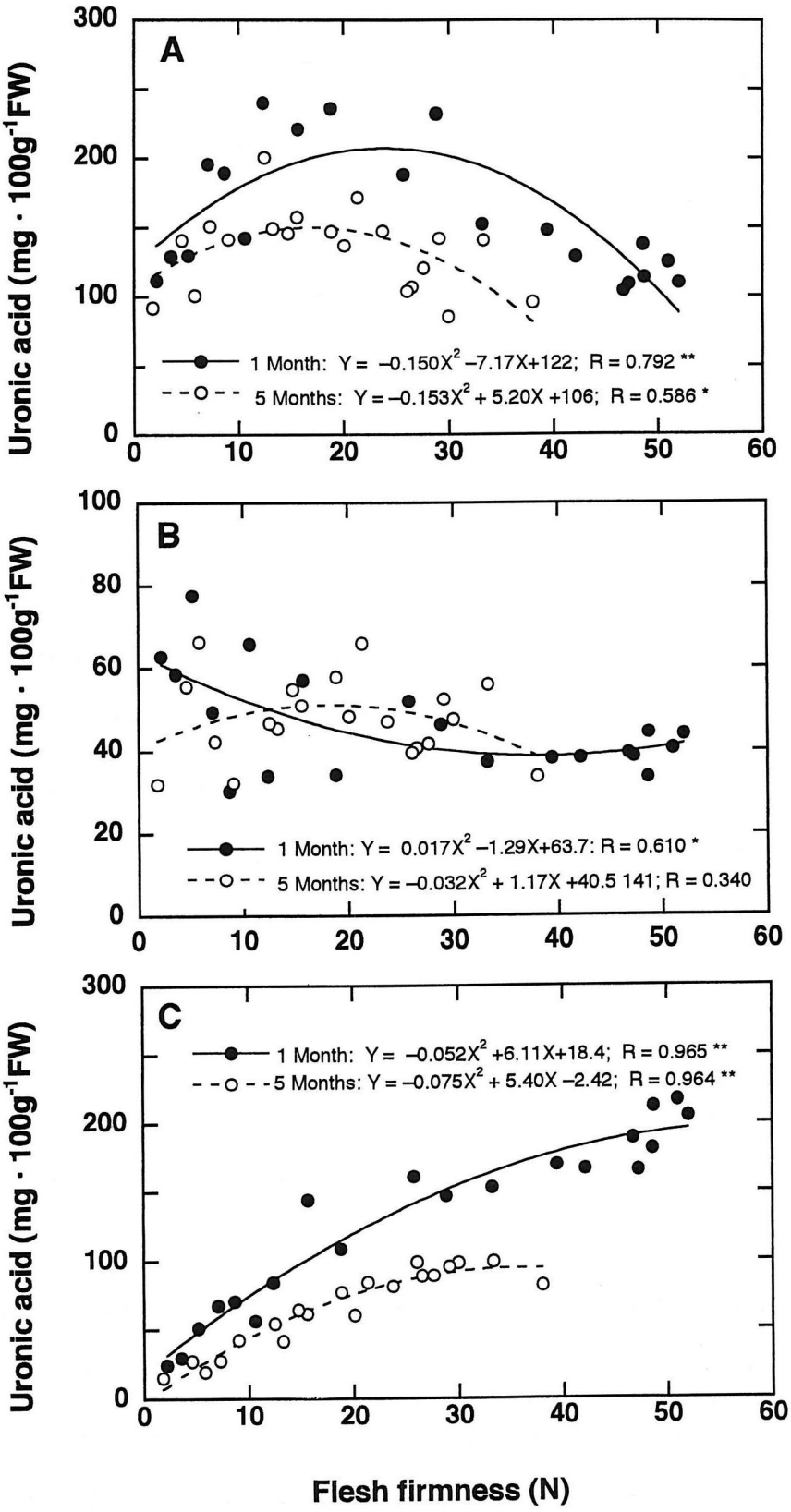

Fig. 4. 1. The relationship between flesh firmness and the amount of pectic polysaccharides in 'Marguerite Marillat' fruit after different storage periods. A: water-soluble polyuronides, B: chelator-soluble polyuronides, C: alkaline-soluble polyuronides. Statistical significance is given at $p < 0.01$ (**) and $p < 0.05$ (*).

firmness. In 'La France', the lower the flesh firmness, the higher the WSP content in fruit after short-term storage, while lower flesh firmness was correlated with lower WSP content in fruit after long-term storage (Fig. 4.2A).

CSP levels in 'Marguerite Marillat' after short-term storage were high in fruit that was low in flesh firmness, but there was no relationship between flesh firmness and CSP content in fruit after long-term storage (Fig. 4.1B). In 'La France', there were no relationship between flesh firmness and CSP content, independent of storage period (Fig. 4.2B).

The relationship between flesh firmness and ASP content showed the highest correlation in both cultivars among cell wall polysaccharides that were determined in this study ($R = 0.808 \sim 0.965$). The lower the flesh firmness, the lower the ASP content, independent of cultivar and storage period (Fig. 4.1C and 4.2C). Fruit after short-term storage contained more ASP than fruit after long-term storage in both cultivars, when compared with fruit having similar flesh firmness.

Effect of storage period on flesh firmness on hemicellulosic polysaccharides and cellulose

In 'Marguerite Marillat', lower flesh firmness correlated with higher hemicellulosic polysaccharides, independent of storage period (Figs. 4.3A). However, the differences in content between hard and soft fruit was very slight.

In 'La France' after short-term storage, the lower the flesh firmness, the lower the amount of hemicellulosic polysaccharides, but the differences in content between hard and soft fruit was slight (Fig. 4.3B). There was no relationship between flesh firmness and the amount of hemicellulosic polysaccharides in fruit after long-term storage.

For cellulose content, lower flesh firmness correlated with higher amounts of cellulose in 'Marguerite Marillat' after long-term storage (Fig. 4.4A), while

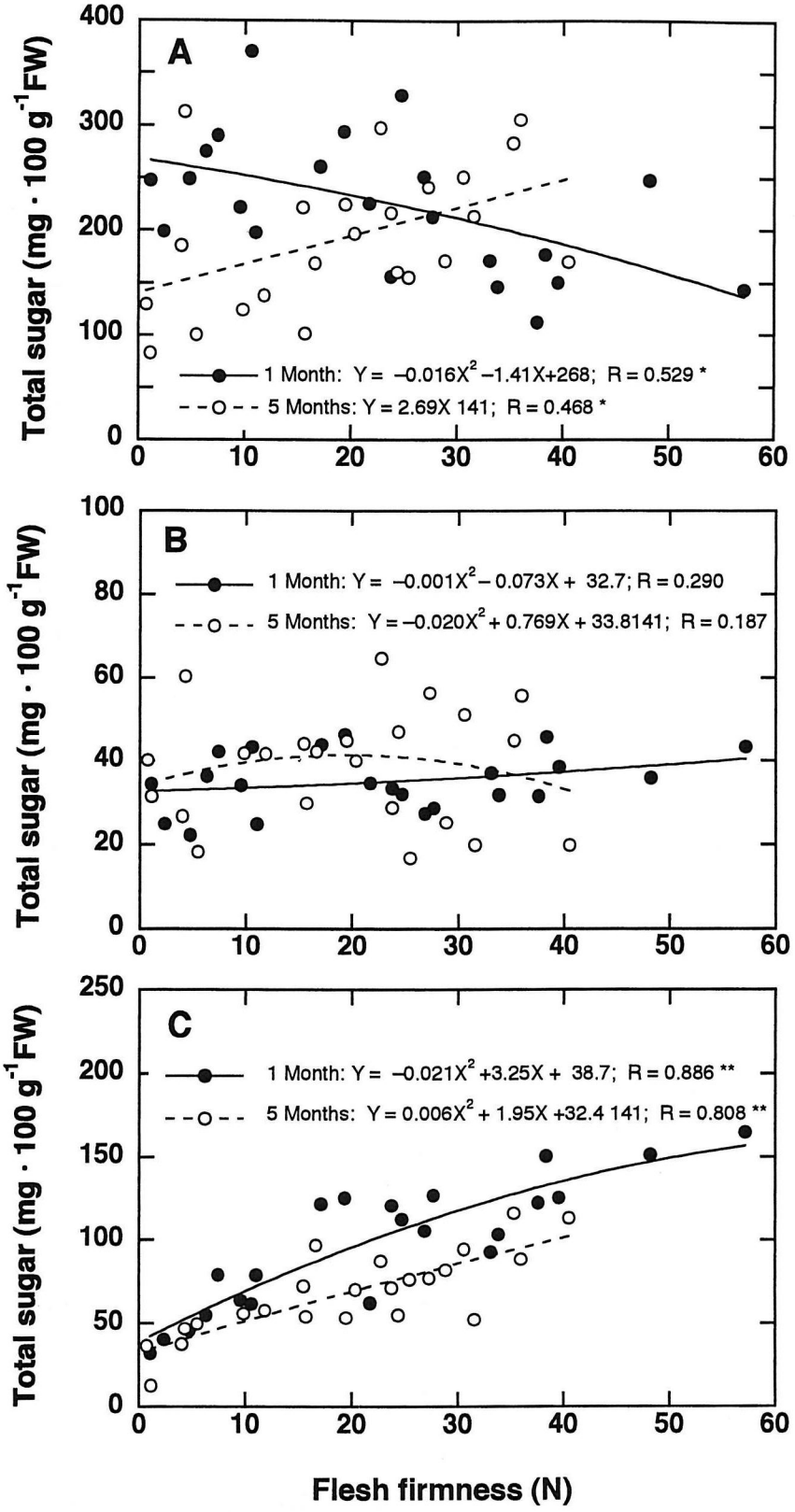

Fig. 4. 2. The relationship between flesh firmness and the amount of pectic polysaccharides in 'La France' fruit after different storage periods. A: water-soluble polyuronides, B: chelator-soluble polyuronides, C: alkaline-soluble polyuronides. Statistical significance is given at $p < 0.01$ (**) and $p < 0.05$ (*).

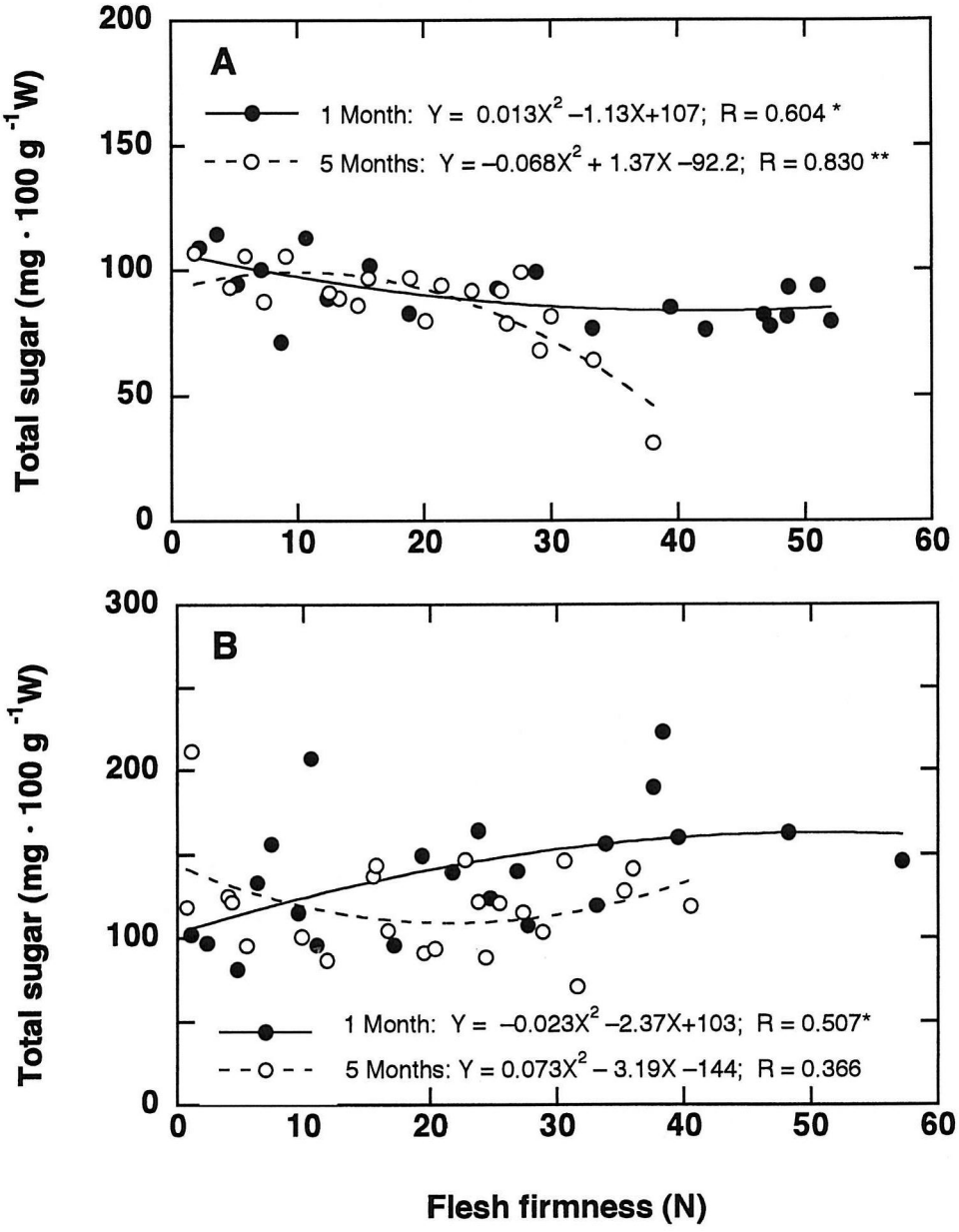

Fig. 4. 3. The relationship between flesh firmness and the amount of hemicellulosic polysaccharides in 'Marguerite Marillat' (A) and 'La France' (B) fruit after different storage periods. Statistical significance is given at $p < 0.01$ (**) and $p < 0.05$ (*).

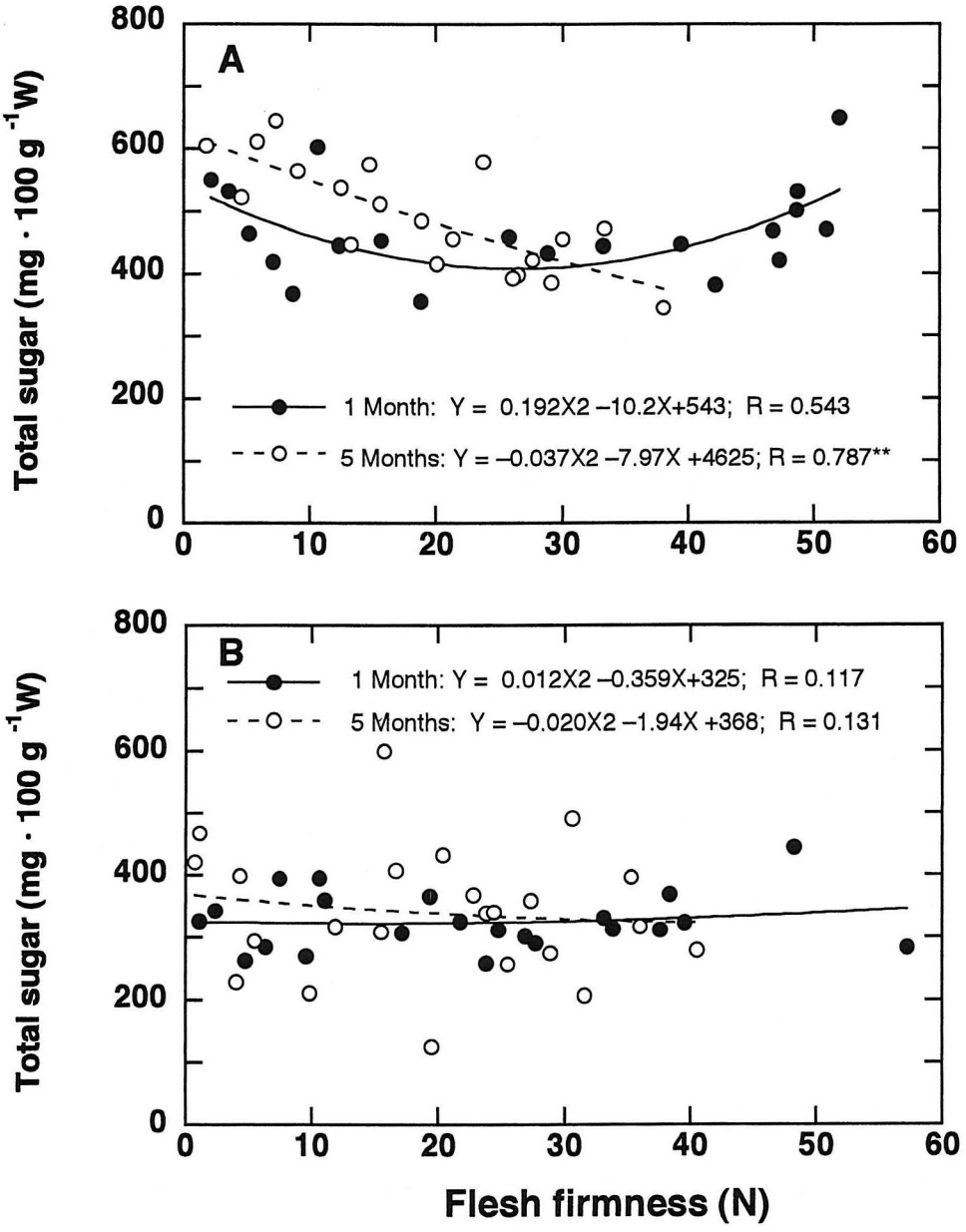

Fig. 4. 4. The relationship between flesh firmness and the amount of cellulose in 'Marguerite Marillat' (A) and 'La France' (B) fruit after different storage periods . Statistical significance is given at $p < 0.01$ (**) and $p < 0.05$ (*).

there was no relationship between flesh firmness and the amount of cellulose in 'Marguerite Marillat' after short-term storage, and 'La France' after short-term and long-term storage (Figs. 4.4A and 4.4B).

Discussion

After short-term storage, fruit softened and reached a buttery and juicy texture during ripening in both cultivars. There was a cultivar difference in the relationship between flesh firmness and WSP content. In 'La France', lower flesh firmness correlated with increasing WSP content. In pears, the amount of WSP increased during normal ripening (Yoshioka et al., 1992). On the other hand, in 'Marguerite Marillat', WSP varied convexly with decreasing flesh firmness. This suggests that some polyuronides were extracted in the late stage of softening by ethanol added to the preparation of AIR by advanced decomposition of polyuronides. Ahmed and Labavitch (1980) reported that galacturonic acid in the 80% ethanol precipitable portion of the supernatant fraction of pear fruit homogenates actually increased during fruit softening. Kim et al. (1991) also reported that a steady decline in cell wall galactosyl residues was accompanied by an increase in soluble galactose during tomato fruit ripening. In any case, WSP content increased when fruit was transferred to 20°C after short-term storage.

After long-term storage, fruit in both cultivars also softened during ripening. Wang et al. (1985) reported that 'Eldorado' pears usually remained firm and dry when fruit was transferred to 20°C after 36 weeks of storage at 0°C. In this study, the period of long-term storage was 4 months in 'Marguerite Marillat' and 5 months in 'La France'. If fruit was stored longer, they may remain firm during ripening. An interesting observation was the fact that the texture of fruit storage in both cultivars was not buttery and juicy when fruit ripened at 20°C after

long-term storage. In 'La France' after long-term storage, lower flesh firmness correlated with lower WSP content. In 'Marguerite Marillat', the relationships between flesh firmness and WSP content were similar to those of fruit after short-term storage. However, WSP levels of fruit after long-term storage tended to be lower than those of fruit after short-term storage, when compared with fruit having similar flesh firmness. In peaches, mealiness has been attributed to impaired solubilization of polyuronides with accumulation of insoluble low methoxyl polyuronides of high molecular weight (Ben-Arie and Lavee, 1971). In the study of pears by Chen and Borgic (1985), fruit did not ripen normally, never developing a buttery and juicy texture of ripe fruit, and the WSP content of the fruit did not change appreciably, during 9 days of ripening after fruit was transferred to 20°C after prolonged storage at -1°C or 0°C. Together, the lower amount of WSP seems to be related to the inferior texture of pear fruit after prolonged storage.

The CSP content was lower than WSP or ASP content, independent of cultivar and storage period. In addition, the amount of CSP after short-term storage was similar to those of fruit after long-term storage in both cultivars. Thus, it seems that this fraction is not crucial in inducing dry texture. Yoshioka et al. (1992) reported that CSP content in 'Red Bartlett' pears was higher than WSP or HCl soluble polyuronide content at harvest and decreased during ripening. However, CSP was extracted with 50 mM EDTA at 80°C in their experiment, while I extracted CSP at 20°C. The difference in extraction temperature may have affected CSP content.

As for ASP, the lower the flesh firmness, the lower the ASP content, independent of cultivar and storage period. In addition, the differences in ASP content between hard and soft fruit were significant. As described in chapter 1,

ASP content decreased during softening of pears. Thus, the amount of ASP seems to be associated with the softening of pears. In storage between one and 4 months the 'Marguerite Marillat' fruit lost 10 N of firmness but 50% of their ASP was lost. 'La France' fruit between one and 5 months decreased in firmness almost 20 N and in ASP 25%. In this section, I have directed my attention not to the quality but the quantity of cell wall polysaccharides. Quality changes in cell wall polysaccharides, such as the molecular weight of pectic and hemicellulosic polysaccharides or neutral sugar composition of pectic polysaccharides, also play an important role in fruit softening (Nara et al., 2001; Redgwell et al., 1997a, b). Changes in molecular weight of pectic and hemicellulosic polysaccharides during ripening of pears are described in the next section.

Flesh firmness in both cultivars almost never changed during one month storage. Immediately after fruit was transferred to 20°C, however, flesh firmness in fruit after long-term storage was significantly lower than that in fruit after short-term storage. It is interesting to note that fruit after short-term storage contained more ASP than fruit after long-term storage in both cultivars, when compared with fruit having similar flesh firmness. This suggests that extensive decomposition of ASP occurred during storage at 1°C in both cultivars. ASP is thought to be metabolized and to become WSP during normal ripening of pears. As mentioned above, however, WSP levels of fruit after long-term storage also tended to be lower than those of fruit after short-term storage, when compared with fruit having similar flesh firmness. This suggests that WSP was further metabolized so that it was alcohol soluble and lost from the AIR. Again, the lower content of pectic polysaccharides after prolonged storage seems to be related to inferior texture of fruit.

The differences in the amount of hemicellulosic polysaccharides between

hard and soft fruit were slight. In avocados, the net sugar content of hemicellulose also changed little during ripening (Sakurai and Nevins, 1997). Moreover, there was no negative correlation between flesh firmness and cellulose content, independent of cultivar and storage period. This suggests that cellulose is not degraded during ripening of pears. It has been reported that cellulose content is not changed during softening of pears (Ahmed and Labavitch, 1980), as well as for fruit such as avocados (Sakurai and Nevins, 1997) and persimmons (Ben-Arie et al., 1996), except for grapes, in which cellulose is degraded during veraison (Yakushiji et al., 2001). Thus, it seems that the amounts of hemicellulosic polysaccharides and cellulose are not crucial in inducing inferior texture.

In conclusion, after long-term storage, 'Marguerite Marillat' and 'La France' fruit softened, but never reached a buttery and juicy texture. Polyuronide, especially WSP and ASP, in fruit after prolonged storage were lower than those in fruit after one month storage. This seems to be one of the causes in fruit of inferior texture.

Summary

The relationship between flesh firmness and the cell wall polysaccharides of 'Marguerite Marillat' and 'La France' pears which had retained or had lost their ripening capacity was investigated. In both cultivars, fruit softened and reached a buttery and juicy texture after short-term storage (one month at 1°C), while fruit softened but never reached that texture after long-term storage (4 months in 'Marguerite Marillat' or 5 months in 'La France' at 1°C). In 'Marguerite Marillat', the water-soluble polyuronides (WSP) levels in fruit after long-term storage tended to be lower than those after short-term storage, when compared with fruit having similar flesh firmness. In 'La France', lower flesh firmness

correlated with higher WSP content in fruit after short-term storage, while the lower the flesh firmness, the lower the WSP content in fruit after long-term storage. The relationship between flesh firmness and alkaline-soluble polyuronide (ASP) content showed the highest correlations in both cultivars among cell wall polysaccharides determined in this study. Lower flesh firmness correlated with lower ASP content, independent of cultivar and storage period. Fruit after short-term storage contained more ASP than that after long-term storage in both cultivars, when compared with fruit having similar flesh firmness. The differences in the amount of hemicellulosic polysaccharides between hard and soft fruit were slight. There was no negative correlation between flesh firmness and cellulose content, independent of cultivar and storage period. These data suggest that lower polyuronide content, especially WSP and ASP, in fruit after prolonged storage is one cause of the inferior texture of pears after prolonged storage.

Section 2. Effect of storage period on molecular mass distribution of pectic and hemicellulosic polysaccharides in pear fruit

Introduction

As described in chapter 2, 'Marguerite Marillat' and 'La France' fruit lose their ripening capacity when fruit is stored more than 2 months and 4 months, respectively. Such fruit softened but never reached a buttery and juicy texture. In the previous section, it has been shown that lower polyuronide content, especially water-soluble and alkali-soluble polyuronides, in 'Marguerite Marillat' and 'La France' fruit after prolonged storage is one cause of the inferior texture of fruit after prolonged storage.

In addition to quantitative changes in cell wall polysaccharides, qualitative changes, such as the molecular weights of pectic and hemicellulosic polysaccharides or the neutral sugar composition of pectic polysaccharides, play an important role in fruit softening (Nara et al., 2001; Sakurai and Nevins, 1997). Even if the quantity of hemicellulosic polysaccharides changed little, their molecular mass decreased drastically during ripening of muskmelon (McCollum et al., 1989) and avocado (O'Donoghue and Huber, 1992; Sakurai and Nevins, 1997). In this section, the changes in molecular mass distribution of pectic and hemicellulosic polysaccharides during normal and abnormal ripening of 'Marguerite Marillat' and 'La France' fruit were investigated to clarify the mechanisms by which fruit failed to reach a melting texture after prolonged storage .

Materials and Methods

Plant material and treatments

Fruit of the cultivars 'Marguerite Marillat' and 'La France' was used. Fruit was harvested at commercial maturity (OTH) in a commercial orchard near Yamagata. After selection for uniformity of size and freedom from defects, fruit was stored at 1°C. After one month (short-term) storage in both cultivars, and 4 months (long-term) storage in 'Marguerite Marillat' or 5 months (long-term) storage in 'La France', fruit was transferred to 20°C for ripening. Flesh firmness was measured every day during ripening on opposite sides of each fruit using rheometer (model CR-200D; Sun Scientific Co., Tokyo) with an 8-mm plunger. Five fruit at different ripening stages were selected for analysis of the molecular mass distribution of pectic and hemicellulosic polysaccharides. Tables 1 and 2 show the stages of softening of fruit used for studies of molecular mass distribution of pectic and hemicellulosic polysaccharides.

Extraction of pectic and hemicellulosic polysaccharides

The alcohol insoluble residue (AIR), which was prepared as described in the previous section, was dispersed in distilled water, mechanically shaken overnight at 20°C, and vacuum-filtered through a GF/C filter. The residue was then suspended in distilled water, shaken for 1 h, and filtered again. The filtrates were combined and designated as the water soluble polyuronides (WSP).

For analysis of molecular mass distribution of hemicellulosic polysaccharides, AIR was treated for 2 h with 100 U of α-amylase (hog pancreatic α-amylase, Sigma) in 50 mM Na-acetate buffer (pH 6.5). The residue was depectinated by the method of Sakurai and Nevins (1997). Briefly, the residue was dispersed in 50 mM EDTA (pH 6.8), heated at 100°C for 15 min, and vacuum-filtered through a glass fiber filter (Whatman GF/C). The residue was then extracted

Table 4. 1. Stages of softening of 'La France' fruit used for molecular mass distribution of water soluble polyuronides (WSP).

	flesh firmness(N)	state of ripeness	WSP content (mg/100gFW)	
			uronic acid	total sugar
short-term storage				
stage 1	57	unripe	143	95
stage 2	33	unripe	171	89
stage 3	22	unripe	226	126
stage 4	7	soft and melting	290	149
stage 5	2	overripe	247	128
long-term storage				
stage 1	41	unripe	170	105
stage 2	25	unripe	155	88
stage 3	16	unripe	101	74
stage 4	5	soft but not melting	100	94
stage 5	1	overripe	83	168

Table 4. 2. Stages of softening of 'Marguerite Marillat' and 'La France' fruit used for molecular distribution of hemicellulosic polysaccharides (HP).

	flesh firmness(N)	state of ripeness	HP content (mg/100gFW)
Marguerite Marillat			
short-term storage			
stage 1	52	unripe	124
stage 2	42	unripe	149
stage 3	29	unripe	131
stage 4	16	unripe	132
stage 5	5	soft and melting	95
long-term storage			
stage 1	38	unripe	103
stage 2	28	unripe	115
stage 3	21	unripe	118
stage 4	16	unripe	90
stage 5	5	soft but not melting	106
La France			
short-term storage			
stage 1	48	unripe	151
stage 2	27	unripe	167
stage 3	19	unripe	166
stage 4	6	soft and melting	140
stage 5	1	overripe	106
long-term storage			
stage 1	36	unripe	115
stage 2	27	unripe	126
stage 3	20	unripe	107
stage 4	12	soft but not melting	109
stage 5	4	overripe	146

twice at 100°C with EDTA. Hemicellulosic polysaccharides were then extracted twice from depectinated AIR with 4 M KOH containing 20 mM $NaBH_4$. The filtrates were combined and neutralized with glacial acetic acid and dialyzed against H_2O.

Gel-filtration chromatography

WSP (1 mg of galacturonic acid equivalent) in 1 ml were applied to a Sephacryl S-300 column (93 X 1.5 cm) and eluted with 30 mM Na-acetate buffer (pH 5.0) containing 10 mM EDTA. Four-ml fractions were collected at a flow rate of 18 $ml \cdot h^{-1}$, and uronic acid content and total sugar content in each fraction were measured by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973) and the phenol-sulfuric acid method (Dubois et al., 1956), respectively.

Hemicellulosic polysaccharides (1 mg of total sugar equivalent) in 1 ml were applied to a Sephacryl S-400 column (95 X 1.5 cm) and eluted with 30 mM Na-acetate buffer (pH 5.0) containing 10 mM EDTA. Four-ml fractions were collected at a flow rate of 18 $ml \cdot h^{-1}$, and aliquots were used to determine total sugar and xyloglucan content. Total sugar content was measured as described above. The xyloglucan content in each fraction was measured by the iodine method (Wakabayashi et al., 1991).

Results and Discussion

Molecular mass distribution of water soluble polyuronides (WSP)

WSP extracted from 'La France' AIR were subjected to gel filtration chromatography. In short-term stored fruit, the major portion of uronic acid and total sugar component at stages 1 to 3 eluted near the void volume (Fig. 4.5A and 4.5B). When fruit developed a melting texture (stage 4), the WSP, especially the

uronic component, showed striking molecular mass downshifts concomitant with increases in the level of the smaller polymer. In overripe fruit (stage 5), molecular mass downshifts proceeded more extensively. The downshift in molecular mass distribution of WSP during fruit softening has been reported in pears (Yoshioka et al., 1992), avocados (Sakurai and Nevins, 1997; Wakabayashi et al., 2000), and muskmelon (McCollum et al., 1989). In this study, the downshift of molecular mass distribution of WSP from stage 3 to 4 was the largest among the softening stages. Wakabayashi et al. (2000) suggested that polygalacturonase (PG) plays a central role in polyuronide degradation in ripening avocado fruit cell walls.

The uronic acid component of WSP of long-stored fruit showed a lower molecular weight than that of short-term stored fruit (Fig. 4.5C). The sugar content around the void volume did not exceed 20%. The total sugar component of long-term stored fruit eluted slightly after the void volume and did not exceed 20%, except for stage 5 (Fig. 4.5D). Tomatoes transformed with an antisense PG gene showed a softening process similar to the control fruit from mature green to turning stages, but did not soften after the turning stage (Carrington et al., 1993). In peaches, a marked increase in both PG-related RNA and endo PG activity was associated with the melting stages (Lester et al., 1994). These results suggest that the depolymerizaion of pectic polysaccharides through PG action may be related not only to softening of fruit at a late stage, but also to textural changes in fruit, such as development of a melting texture.

The uronic acid component of WSP extracted from long-term stored fruit also showed the tendency toward molecular mass downshifts as fruit softened, but the relationship between fruit softening and changes in molecular mass distribution of the total sugar component was not clearly seen. Pectic polysaccharides of pears are considered to be a backbone containing galacturonosyl

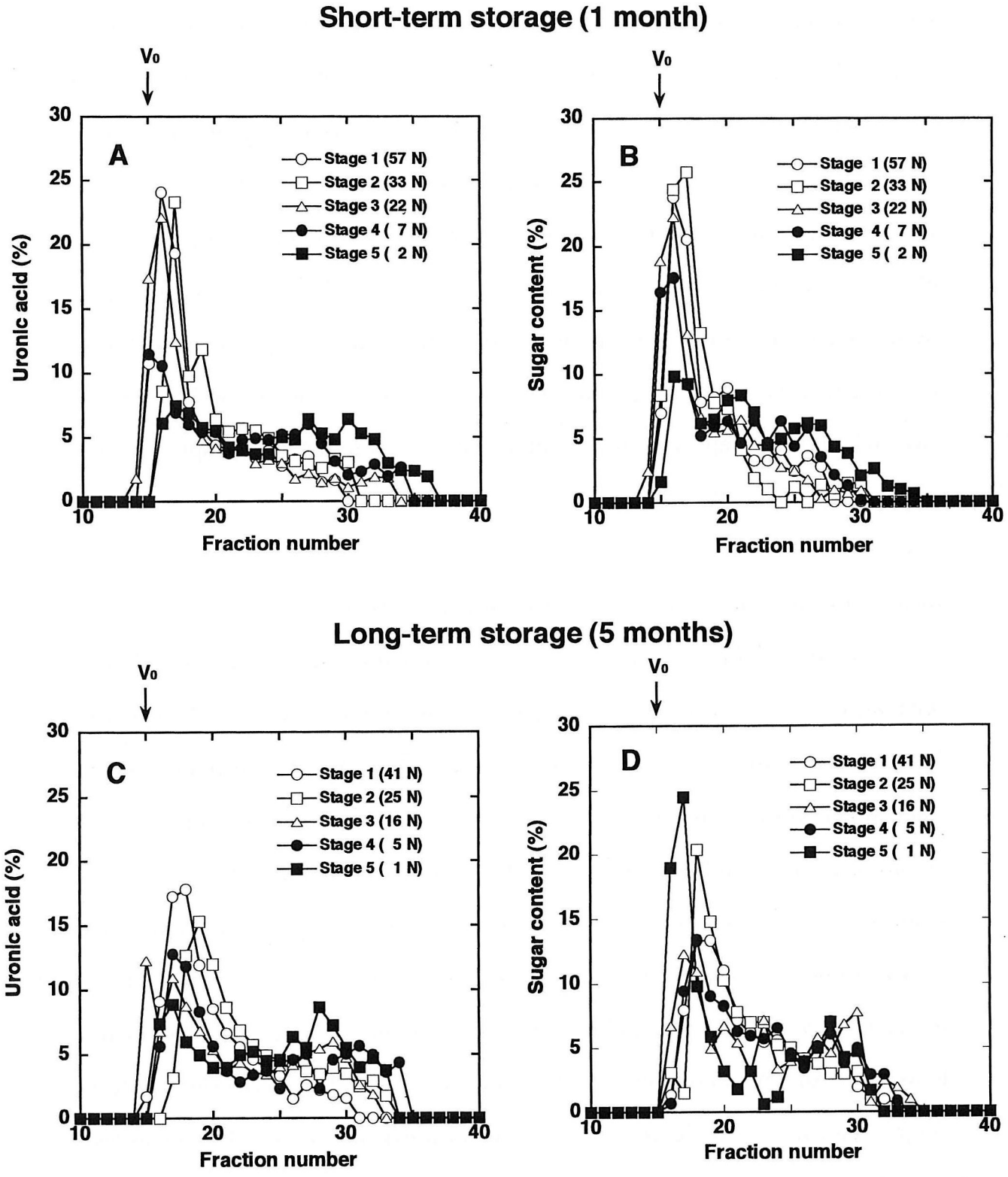

Fig. 4. 5. Molecular mass distribution of water soluble polyuronides from 'La France' fruit at different softening stages. Fractions were analyzed for uronic acid (A, C) and total sugar (B, D). Fruit was transferred to 20°C after one (A, B) or 5 months (C, D) at 1°C.

and rhamnosyl residues with a short side chain of galactose and large, highly branched arabinan side chains (Dick and Labavitch, 1980). It was reported that the net loss of non-cellulosic neutral sugar residues from cell walls during ripening paralleled losses of arabinose in pears (Gross and Sams, 1984), and that polyuronides with fewer neutral sugars were released preferentially (Yoshioka et al., 1994). These results suggest that arabinan side chains lose sensitivity to hydrolysis during long-term storage at cold temperatures. In the present study, measurement of neutral sugar composition was not determined, so it is not yet clear whether the depolymerization of a side chain component is responsible for the development of the melting texture of pear fruit.

Molecular mass distribution of hemicellulosic polysaccharides

Hemicellulosic polysaccharides extracted from 'Marguerite Marillat' and 'La France' AIR were also subjected to gel filtration chromatography. In 'Marguerite Marillat' fruit after short-term storage, the molecular mass distribution of the total sugar component of hemicellulosic polysaccharides was not changed significantly until fruit developed a melting texture (stage 5) (Fig. 4.6A). Then the peak shifted greatly toward the low molecular mass region. The molecular downshift of xyloglucan commenced after stage 1, but its molecular distributions in stages 2, 3 and 4 were similar (Fig. 4.6B). The molecular distribution of the total sugar component of long-term stored fruit showed slight downshifts only at stage 5 (Fig. 4.6C). The degree of the molecular downshift of xyloglucan was less prominent in long-term stored fruit than in short-term stored fruit (Fig. 4.6D).

In 'La France' fruit, the molecular distribution of the total sugar component of hemicellulosic polysaccharides of short-term stored fruit showed a clear molecular mass downshift after stage 4 when the fruit developed a melting texture

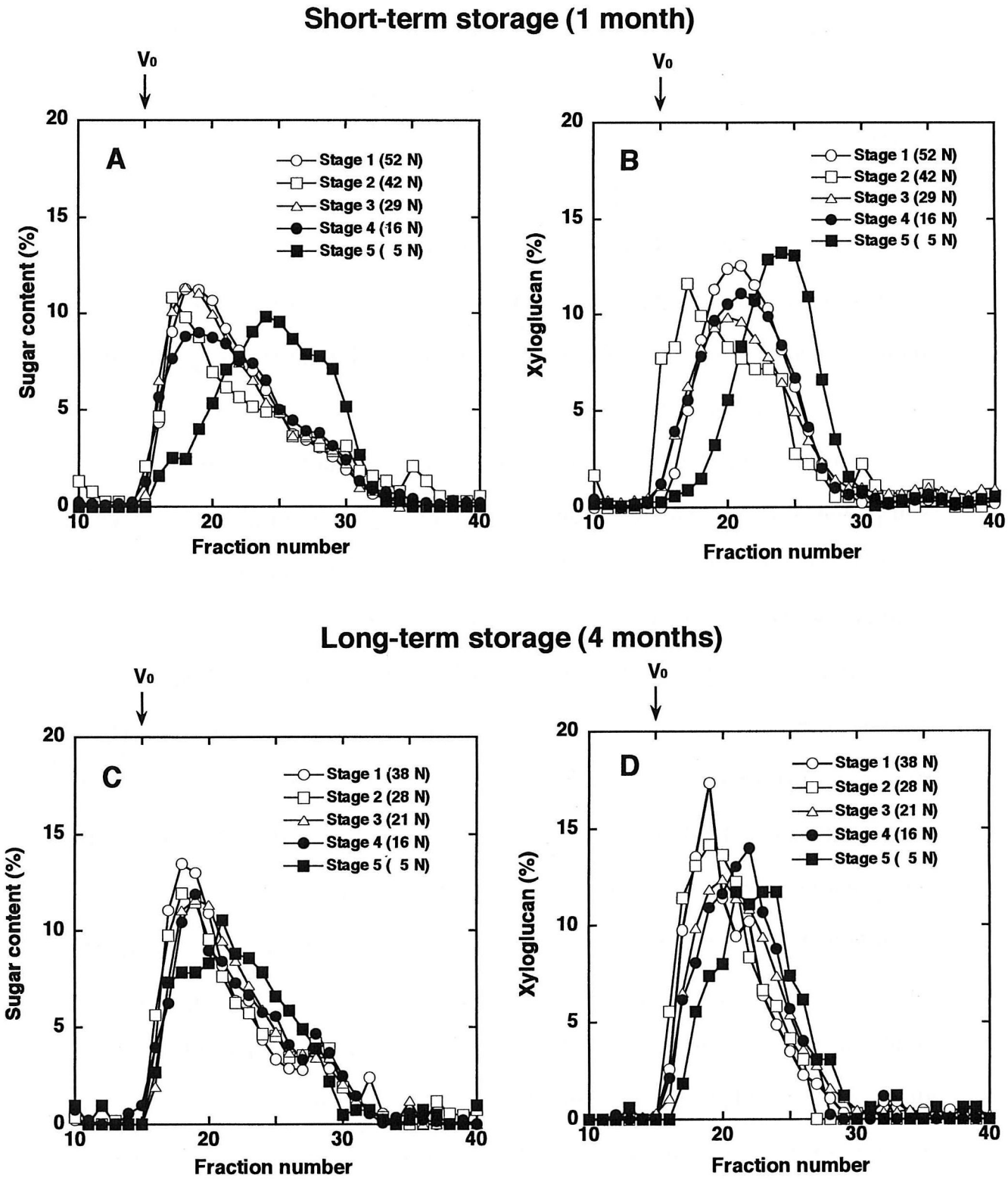

Fig. 4. 6. Molecular mass distribution of hemicellulosic polysaccharides from 'Marguerite Marillat' fruit at different softening stage. Fractions were analyzed for total sugar (A, C) and xyloglucan (B, D). Fruit was transferred to 20°C after one or 4 months at 1°C.

(Fig. 4.7A). A molecular mass downshift of xyloglucan was observed after stage 1 but not after stage 4 (Fig. 4.7B). A downshift of molecular mass distribution of hemicellulose was reported during softening of many fruit types, such as papayas (Paull et al., 1999), tomatoes (Sakurai and Nevins, 1993), and grapes (Yakushiji et al., 2000). In addition, Sakurai and Nevins (1997) showed that the molecular mass downshift of xyloglucan within the hemicellulose B fraction determined by both the presence of a specific fucose residue and iodine staining, reflects the consequences of depolymerization.

In 'La France' fruit, the molecular mass distribution of the total sugar component did not change in long-term stored fruit, although the peak levels gradually decreased from stage 1 to 5 (Fig. 4.7C). The peaks of hemicellulosic polysaccharides and xyloglucan were eluted just after the void volume in all the stages (Figs. 4.7C and 4.7D). The extent of depolymerization of hemicellulosic polysaccharides was much larger in short-term stored fruit than long-term stored fruit in both cultivars. The smaller extent of depolymerization may influence the inferior texture of fruit after prolonged storage at 1°C. As described above, the relationships between hemicellulosic polysaccharides and fruit softening are well documented. However, no reports is available as to the effect of those polymers on a melting texture. In addition, the mechanisms involved in degradation of hemicellulosic polysaccharides still remain to be studied, although endo-1,4-glucanase or xylanase are candidates. Research on the mechanisms of depolymerization of hemicellulosic polysaccharides during the development of a melting texture of pear fruit is necessary.

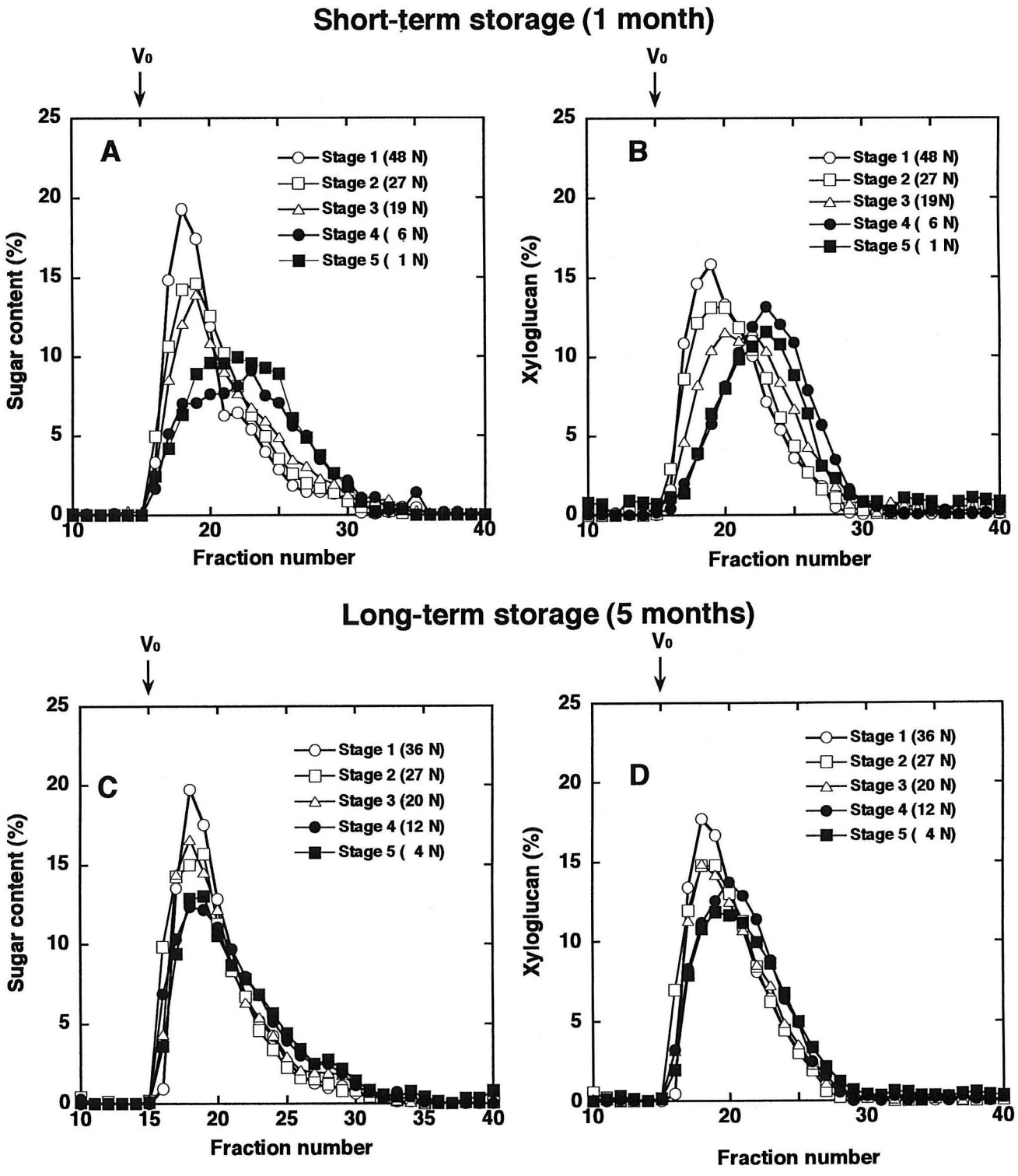

Fig. 4. 7. Molecular mass distribution of hemicellulosic polysaccharides from 'La France' fruit at different softening stages. Fractions were analyzed for total sugar (A, C) and xyloglucan (B, D). Fruit was transferred to 20°C after one or 5 months at 1°C.

Summary

Fruits of the pear cultivars 'Marguerite Marillat' and 'La France' did not reach a melting texture after long-term storage while they did after short-term storage. Short storage of both cultivars produced remarkable decreases in the contents and molecular downshifts of hemicellulosic polysaccharides and xyloglucan when they reached the melting stage, but long-term stored fruits that did not reach melting stage showed smaller changes in the content and molecular size. These results suggests that the normal ripening process of pears to a melting texture requires a regulated metabolism of hemicellulosic polysaccharides and xyloglucan.

Conclusions and Prospects

This study was designed to investigate the ripening characteristics of pear fruit with special reference to cell wall polysaccharides.

In Chapter 1, changes in polyuronides of 'Marguerite Marillat' and 'La France' pears on and off the tree were investigated. In both cultivars, the amount of water-soluble polyuronides (WSP) increased slightly during ripening on the tree, but the amount 28 days after the optimum time for harvesting (OTH) was less than one third of that in fruit harvested at OTH and ripened off the tree. Pears off the tree softened to less than 10 N independently of harvest date and cultivar. The amount of WSP in fruit softened after harvest increased significantly from that at harvest. In both cultivars, the texture of fruit harvested at 14 days and 28 days after OTH and ripened did not become buttery and juicy. Those fruit had less WSP than fruit that developed a melting texture.

In Chapter 2, the physiological characteristics of 8 cultivars of pears and the effects of chilling on ethylene biosynthesis of 'Le Lectier' pears during storage and ripening were investigated. Excluding 'Passe Crassane', pears do not absolutely require chilling for normal ripening. On the other hand, 'Passe Crassane' fruit requires chilling at 1°C more than one month for ripening. 'Bartlett' and 'Marguerite Marillat' pears softened but never developed a melting texture during ripening at 20°C after storage for 2 months at 1°C. Similarly, 'General Leclerc', 'La France', 'Le Lectier' and 'Winter Nelis' pears did not develop that texture after storage for 3 months. In contrast 'Silver Bell' and 'Passe Crassane' pears softened and developed a melting texture even after storage for 5 months. In 'Le Lectier' pears, chilling strongly stimulated 1-aminocyclopropane-1-carboxylic acid (ACC)

synthase activity, but did not promote the ACC oxidase activity as much as ACC synthase activity. In addition, the increase in ACC synthase activity preceded that of ACC oxidase at chilling temperatures. As a result, ACC accumulated substantially by 20 days after harvest. After rewarming to 20°C, ACC synthase activity decreased immediately in contrast with ACC oxidase activity, which was enhanced markedly.

In Chapter 3, the effects of differences in relative humidity (RH) on the ripening of 'Le Lectier' pears were investigated. The flesh firmness at 95% RH rapidly decreased from the 3rd week and reached less than 10 N, appreciable firmness, after 5 weeks. In those fruit, there was a marked increase in the ethylene production after 4 weeks. The fruit at 55% or 75% RH showed greater flesh firmness than those at 95% at and after 4 weeks and never softened appreciably. Those fruit did not show any increase in ethylene production for as long as 8 weeks before being discarded. After 5 weeks at 55% RH, fruit held in 200 ppm ethylene for 48 hours showed a significant decrease in flesh firmness. Moreover, the amount of WSP increased and that of HCl-soluble polyuronides decreased after treatment.

In Chapter 4, the relationship between flesh firmness and the cell wall polysaccharides, and molecular distribution profile of pectic and hemicellulosic polysaccharides of 'Marguerite Marillat' and 'La France' pears was investigated. After one month at 1°C in both cultivars (short-term storage), and 4 months in 'Marguerite Marillat' or 5 months in 'La France' (long-term storage), fruit was transferred to 20°C for ripening. Long-term stored fruit did not reach a melting texture, while that of short-term stored fruit did in both cultivars. In 'Marguerite Marillat', the WSP levels in fruit after long-term storage tended to be lower than

those after short-term storage, when compared with fruit having similar flesh firmness. In 'La France' pears, lower flesh firmness correlated with a higher amount of WSP in fruit after short-term storage, while the lower the flesh firmness, the lower amount of WSP in fruit after long-term storage. The relationship between flesh firmness and the amount of alkaline-soluble polyuronide (ASP) showed the highest correlations in both cultivars among cell wall polysaccharides determined in this study. Lower flesh firmness correlated with the lower amount of ASP, independent of cultivar and storage period. Fruit after short-term storage contained more ASP than that after long-term storage in both cultivars, when compared with fruit having similar flesh firmness. Both fruits after short-term storage exhibited a remarkable decrease in the amount and molecular downshifts of hemicellulosic polysaccharides when they reached a melting texture, but those of long-term stored fruit that did not reach that texture showed a smaller change in the amount and molecular size.

Future strategies of pear ripening could attempt to intervene at various levels from the existing technology, such as modified or controlled atmosphere storage, to the new technology. Manipulation could involve the control of the expression of ripening-related genes. The understanding of the physiological basis of pear ripening is important to develop such a new technology. In past studies of pear ripening, fruit softening was thought to be accompanied by the development of the buttery and juicy texture. As described above, it was found that there were cases in which fruit softened but never reached that texture. The texture of fruit is an important determinant of the fruit quality. Especially in pears, fruit with good quality develop a melting texture. As with the changes in the amount of cell wall polysaccharides, these changes in quality were found to

play a role in the development of the inferior texture The mechanisms of the development of a melting texture are now being investigated at a molecular level.

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