Effect of Fertilizer Application Level on Pectin Composition of Hakuho Peach (Prunus persica Batsch) During Maturation

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Abstract. Effects of fertilizer application levels on fruit texture and flesh pectin compositions of a melting peach were investigated. Hakuho trees (Prunus persica Batsch) were supplied with normal (M), high (H; M × 2), and super-high (SH; M × 4) levels of complete liquid fertilizer twice a week. Flesh firmness of the H and SH treatment fruit was lower than that of M treatment fruit at the hard-mature and firm-mature stages, although no difference was detected at the full ripe stage. Sensory scores for flesh texture at the full ripe stage were highest in the N treatment fruit and lowest in the SH treatment fruit. The content of water-soluble polyuronides (WSP) in flesh was highest in SH fruit and lowest in M fruit at the hard-mature stage, although the difference became smaller at the full ripe stage. Molecular mass analysis using a gel filtration column revealed that water-soluble polysaccharides (WSP) in flesh was highest in SH fruit and lowest in M fruit at the hard-mature stage, although the difference became smaller at the full ripe stage. The analysis of acidic fractions (pectin) in the polysaccharides using an ion exchange column, as well as juice gellation test by adding Ca and Tris buffer, also indicated that high levels of fertilizer application impairs an early degradation of flesh polyuronides resulting in the accumulation of low-molecular-weight WSP. This may ultimately cause the inferior flesh texture of overfertilized peach fruit.

The melting peach (Prunus persica Batsch), a climacteric fruit, has a smooth and juicy flesh, an attractive aroma, and a desirable taste at its fully ripe stage that continues to enjoy a large share of the market in Japan. However, it has a particularly short postharvest life because it softens to a “melting” texture within a few days at room temperature. Thus, the softening of peaches is one of the critical factors that limits storage duration and shelf life.

Fruit texture is influenced by environmental, cultural, physiological, and genetic factors (Sams, 1999). Cultivation practices such as fertilizer application and growth regulator use, irrigation, pruning, time of harvest, and cultivar selection have a major impact on fruit texture. In our previous work, Hakuho peaches grown under high nitrogen fertilization conditions had deterioration of flesh texture and flavor compared with those harvested from moderately or lightly fertilized trees as well as a delayed harvest date (Jia et al., 1999, 2000; Okamoto et al., 2001). This undesirable decrease in flesh firmness resulting from excessive fertilization is well documented. However, the effect of field practices such as fertilizer application level on the fruit pectin composition has been poorly documented. The objective of our work is to further elucidate the softening processes in peaches by investigating the changes in the physicochemical properties of pectin substances at various fruit-ripening stages.

Materials and Methods

Plant material and treatments. Twenty-four 6-year-old trees of Hakuho peach (Prunus persica Batsch) growing in the Experimental Orchard of Okayama University, Japan, were used for this study in 2003. They were trained to a central leader system and planted in buried pots (120 L) made of rootproof but water-permeable cloth. Three levels of complete liquid fertilizers (OHTUSKA EKIH #1 and #2) containing 60, 120, and 240 ppm N (medium; M, high; H, super high, SH, respectively) were applied 15 L per tree (seven trees per fertilizer treatment) and twice a week from bud burst (14 Mar.) until harvest. The complete liquid fertilizers inclusive all essential elements as shown in our previous paper (Jia et al., 1999). The fertilizer application levels in each treatment were reduced half at the end of the final harvesting stage in late May. Fruit thinning were practiced to adjust the leaf–fruit ratio 30 to 40 leaves/fruit during late May as often practiced in Okayama Prefecture and covered with orange paper bags to avoid insect and fungal attack.

Fruit sampling, flesh firmness, and sensory evaluation. To limit the effects of the maturation stage on results, 60 fruits were harvested at different dates depending on the skin color from each treatment. Fruits were sampled at three stages: immature stage (green, 103 d after full bloom), firm-mature stage (fruit just changed from green to white, 108 d after full bloom), and fully ripe (harvested at the firm-mature stage and stored for 3 d at 25 °C). Thirty flesh firmness of each sample was measured according to the method of Okamoto et al. (2001). Two square pillars (1.5 × 1.5 mm) with the entire thickness of the mesocarp were excised from both cheeks of each fruit and pressed with a penetrometer (TOYO BOLDWIN, STM-T) having a plunger (diameter 0.8 mm) at the rate of 30 mm·min⁻¹. The internal stress was recorded when the height of the pillar was decreased by 5%.

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After determining flesh firmness, each fruit was peeled, and two wedge-shaped sectors were cut from the fruit, diced into 1.5 × 1.5 cm, and placed in coded containers for tasting. The taste evaluations were made by a 15-member trained sensory panel who rated on fine texture as described previously by Jia et al. (2000). Final taste ratings were: 1 = unacceptable, 2 = poor, 3 = fine, or 4 = excellent. The rank test method was used for evaluating. Other cubes were homogenized and centrifuged at 10,000 × g for 15 min and then filtered. The supernatants were weighed to calculate the percentage of extracted juice from flesh. Total soluble solids, titratable acidity, and nitrogen were measured using previously reported method (Jia et al., 1999).

Cell wall isolation and pectin polysaccharide extraction. Cell wall polysaccharides were prepared from alcohol-insoluble residues of flesh and portioned into three fractions based on solubility in different solvents according to the procedure of Murayama et al. (2002) with some modification. Twenty-gram homogenized flesh samples were extracted three times with 80% EtOH at 70 °C and centrifuged to obtain alcohol insoluble solids (AIS). They were then lyophilized and pulverized. The residue was suspended in 100 mL of distilled water, stirred overnight at 25 °C, and filtered. The filtrates were combined and designated as the WSP. The residue was resuspended in 100 mL of 50 mm ethylenediaminetetraacetic acid (EDTA) in 50 mm sodium phosphate (pH 4.5) at 25 °C for 30 min. The filtrates were combined and designated as the chelator-soluble polyuronides (CSP). The residue was further extracted twice with 50 mL of 50 mm Na2CO3 containing 20 mm NaBH4 at 25 °C. The filtrates were combined, neutralized with acetic acid, and designated as the alkali-soluble polyuronides (ASP). The uronic acid content in each fraction was determined by the carbazole–H2SO4 method. The residue after the extraction of pectic polysaccharides, hemicellulosic polysaccharides, was further extracted using 1 m KOH and 4 m KOH. The final residue was collected as cellulose (Murayama et al., 2002). Both hemicellulose and cellulose were hydrolyzed with HCl (0.5 N) at 100 °C for 2.5 h to reduce sugars that were determined by the phenol–H2SO4 method.

Size exclusion chromatography and ion exchange chromatography. Water-soluble polysaccharides extracted from AIS were chromatographed on a size-exclusion column (TSK-GEI-G 5000PD + G3000PW, 7.5 mm ID × 60 cm × 2, TOOSOH) and eluted with 0.025 M phosphate buffer (pH 7.5) plus 0.2 m NaCl at 25 °C. The molecular weight distribution of the cell wall polyuronides was estimated by gel filtration with high-performance liquid chromatography (HPLC) equipped with an RI detector (RI-1530; JASCO) using pullulan fragments (Shodex standard P-82; Showa Denko K.K. Japan) as molecular markers. Samples (200 μL) were injected and eluted at a flow rate of 0.5 mL·min⁻¹. Peaks were detected using a refractive index detector (model R401; Waters Associates, Inc. Amherst, Mass.); 1.5 mL of fractions were collected at 1-min intervals and pooled on the elution time of peaks of interest. Neutral and uronic acid polysaccharides of the cell wall in the water-soluble polysaccharides were fractionated by ion-exchange chromatography with HPLC equipped with a quaternary amine column (POROS HQ/M, 4.6 mm×100 mm ¹-²; PerSeptive Biosystems) equilibrated with 50 mm Tris-HCl buffer (pH 8.5) as described (Hegde and Maness, 1998). Neutral polysaccharides were first eluted out through the column. The polyuronides that were retained in the column were then eluted with a linear gradient of 0 to 0.5 m NaCl solution. Column fractions were quantified for contents of uronic acid content was using the assay of Blumenkrantz and Asboe-Hansen, (1973) with galacturonic acid as the standard.

Juice gelation measurement. To elucidate the physicochemical properties of juice pectin in each treatment fruit juice, gel formation after Ca2⁺ addition under different pH conditions was determined. CaCl2 was added to the three juice solutions come from 20 fruits, and the pH was adjusted to 7.8 with Tris buffer. Juice mixtures without Tris buffer and with only H2O were also evaluated. The viscosity of each mixture was determined with an Ostwald viscosimeter (1.0 mm ID and 20 cm total length) at 25 °C. The flow times were recorded for each 5-mL test solution.

Results

Effect of fertilizer application level on fruit weight, flesh N, and juice quality. The average fruit weight was increased as the fertilizer application level was increased; a significant difference was observed between M and SH treatment fruits (Table 1). A similar trend was observed in the case of flesh nitrogen content. The TSS content of juice was the lowest in the SH treatment fruits and the TA was the highest in the M treatment fruits. Sensory evaluation of flesh texture showed significant differences among the three treatments fruits at the fully ripe stage. The tasters frequently commented that the texture of the M treatment fruits was buttery and juicy, whereas that of the H and SH treatment fruits was rough (Table 1).

Effect of fertilizer application level on flesh firmness. Flesh firmness differed significantly among the three treatment fruits at the hard-mature stage (Fig. 1). At the firm-mature stage, flesh firmness was the highest in the M treatment fruits and the lowest in the SH treatment fruits. As the fertilizer application level was increased, the flesh firmness of the mature fruit was decreased; however, no significant difference was found among the three treatment fruits at the fully ripe stage. The decrease in flesh firmness was most notable in the M treatment fruits; in contrast, there was almost no change in flesh firmness in the SH treatment fruits.

Effect of fertilizer application level on yield and composition of cell wall substances. The percentage expressed juice from the flesh was similar at the hard-mature and firm-mature stages; by contrast, it was increased to some extent at the fully ripe stage (Table 2). On the other hand, no significant difference was found among the treatment fruits at each stage. The AIS content of flesh was significantly increased with fruit maturation. At the hard- and firm-mature stages, the N treatment fruits contained much lower AIS than the other treatment fruits.

The WSP content was lower in the M treatment fruits than in the H or SH treatment fruits at the three harvest stages (Table 3). The WSP content was the highest in the SH treatment fruits at the hard- and firm-mature stages. The CSP content did not change substantially in the M and H treatment fruits but decreased in the SH treatment fruits with increasing maturity. The ASP content varied slightly among the three treatment fruits at the hard- and firm-mature stages. However, a marked decrease was found in the SH treatment fruits at the fully ripe stage. Hemicellulose was detected in small quantities in all the treatment fruits, and no significant difference was observed among the three stages. Cellulose content was decreased gradually with increasing maturity, although no significant difference was found among the three treatments.

Effect of fertilizer application level on molecular mass distribution of water-soluble polyuronide fraction extracted from alcohol-insoluble solids. As fruit maturity was increased, the amount of high-molecular-mass WSP was decreased and those of low- and intermediate-molecular-mass WSPs were increased (Fig. 2). This was particularly evident in the H and SH treatment fruits at the firm-mature and fully ripe stages. At the hard-mature stage, an apparent increase in the proportion of medium-mass WSPs was evident (Fig. 2).

Table 1. Effect of fertilizer application level on fruit weight, flesh N, and juice composition of Hakuto peaches.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit wt (g per fruit)</th>
<th>N (mg·g⁻¹ DW)</th>
<th>Sensory score</th>
<th>TSS (°Brix)</th>
<th>TA (g·L⁻¹)</th>
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</thead>
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<tr>
<td>M</td>
<td>232.4 b⁵</td>
<td>10.10 bc</td>
<td>38 a</td>
<td>14.4 a</td>
<td>1.59 a</td>
</tr>
<tr>
<td>H</td>
<td>249.8 ab</td>
<td>11.2 b</td>
<td>28 b</td>
<td>14.2 a</td>
<td>1.47 b</td>
</tr>
<tr>
<td>SH</td>
<td>259.1 a</td>
<td>13.8 a</td>
<td>24 c</td>
<td>14.3 b</td>
<td>1.49 b</td>
</tr>
<tr>
<td></td>
<td>Three application levels of complete liquid fertilizer containing 60 (M), 120 (H), and 240 (SH) ppm of N were supplied once a week. Fruits were harvested at the firm-mature stage and stored for 3 d.</td>
<td></td>
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<td></td>
<td>Significant difference by Duncan’s multiple range test (P ≤ 0.05) for values within each column.</td>
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<tr>
<td></td>
<td>Sensory evaluation by 15 panelists and analyzed by the rank sum test (P ≤ 0.05). Higher values indicate fine texture.</td>
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<td></td>
<td>Shown as malic acid equivalent (n = 3).</td>
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</table>
The peak of WSP was observed as the fertilizer application level was increased. Moreover, a high proportion of intermediate-molecular-mass pectin that was fractionated between 212 and 47 kDa was detected in the H and SH treatment fruits compared with that in the M treatment fruits. At the firm-mature stage, an apparent decrease in molecular mass of WSP was observed in the H and SH treatment fruits from a peak at 212 kDa to that at 27 kDa. On the other hand, the peak around 212 to 112 kDa was retained in the M treatment fruits. At the fully ripe stage, molecular mass of WPS decreased faster in the H and SH treatment fruits, indicated by a peak that corresponded to the 22.8 kDa molecular marker. In the case of the M treatment fruits, however, the molecular mass of WPS was retained at 112 kDa.

Effect of fertilizer application level on content of acidic pectic polysaccharides in water-soluble polyuronide fraction of alcohol-insoluble solids. The elution profiles of acidic pectic polysaccharides extracted from the WSP fraction of AIS are shown in Figure 3. At the hard-mature stage, the content of acidic pectic polysaccharides was increased in the SH treatment as shown by the large peak (subfraction i) eluted at 10 to 22 min. At the firm-mature stage, the content of acidic pectic polysaccharides in the H treatment fruits was also increased significantly to the level observed in the SH treatment fruits. However, the increase was only slight in the M treatment fruits. At the fully ripe stage, the content of acidic pectic polysaccharides was higher in the fruits grown under high fertilizer application levels. In addition to that, a considerable amount of polyuronides eluted with high ionic strength at the elution time of 40 to 50 min was detected in the SH treatment fruits.

The three subfractions, indicated in Figure 3, were pooled to analyze the molecular mass distribution of the acidic pectic polysaccharides (Fig. 4). Subfraction i corresponded to the major peaks of the polyuronide from the H and SH treatment fruits at the firm-mature stage, comprised low-molecular-mass pectins of \( \approx 50 \) kDa. Subfraction ii comprised intermediate-molecular-mass pectins of \( \approx 100 \) kDa. Subfraction iii comprised relatively high-molecular-mass pectins of \( \approx 100 \) to 200 kDa.

Juice gelation tests after addition with Ca. The viscosity of juice to which a known volume of \( \text{H}_2\text{O} \) was added was \( \approx 1.5 \); no significant difference in juice viscosity was observed among fruits of different maturities and fertilizer application levels (Table 4).

The viscosity of juice became a gel after Tris buffer (pH 7.8) plus \( \text{CaCl}_2 \) was added. On the other hand, all the juice became a gel after Tris buffer (pH 7.8) plus \( \text{CaCl}_2 \) was added, except the juice from...
the M treatment fruits at the hard-mature and firm-mature stages. At the fully ripe stage, however, a high level of gellation was resulted in juice from the M treatment, nearly the same degree to that in the SH treatment at the firm-mature stage.

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Discussion

Pectic substances are noncellulosic constituents of the plant cell wall and play an important role not only in flesh softening, but also in changing the texture of fruit tissue. Our data indicate that peaches from overfertilized trees, like in the cases of H and SH-treated fruits, lose flesh firmness at the early stage of maturation, namely the hard- and
firm-mature stages; this is accompanied by high AIS and WSP contents and low ASP content in the flesh. Such characteristics of fleshy tissue seem to be responsible for the poor flesh texture (Table 1). On the other hand, the CSP content does not seem to affect the texture. Similar results have been reported by Chen and Borgic (1985) and Murayama et al. (1998, 2002) for pear. It is possible to assume that a part of ASP is converted into WSP during the ripening process, and this is associated with the softening of peaches. In general, most WSPs are partitioned in fruit juice and they affect the flesh texture of peach, particularly in terms of juiciness and firmness of the flesh; thus, we focused on the investigation of peach juice WSP.

The changes in the molecular mass of WSP indicate that high-molecular-mass substances exist in the H and SH treatment fruits than the M treatment fruits at the hard-mature stage (Fig. 2). Interestingly, marked decreases in WSP molecular size were observed in the H and SH treatment fruits at the firm-mature and fully ripe stages. On the other hand, at the fully ripe stage, the peak WSP molecular size of the M treatment fruits was constant and significantly larger than those of the H and SH treatment fruits. The flesh texture at the fully ripe stage differed notably; it was buttery and juicy in the M treatment fruits and coarse and poorly resilient in H and SH treatment fruits.

The analysis of pectic substances in AIS suggested that a large amount of acidic pectic polysaccharides was eluted from 14 to 40 min only in the SH treatment fruits at the hard-mature stage and the H treatment fruits at the firm-mature stage (Fig. 3). In the case of juice from the M treatment fruits, the amount of acidic pectic polysaccharides was considerably smaller even at the fully ripe stage. These pectic substances, indicated as subfractions i and ii in the upper graph of Figure 3, appeared as peaks ranging from ≈50 to 100 kDa (Fig. 4) and were considered to be low-molecular-mass pectins. Particularly in the case of SH treatment fruits, special acidic pectic polysaccharides that were eluted between 40 and 50 min were obtained at the fully ripe stage (Fig. 3). That the molecular weight was larger than 110 kDa suggested that in the SH treatment fruits, WSP was degraded in an unconventional manner, resulting in polyuronides with a wide range of molecular masses. These results suggest that the high fertilizer application level impaired the early solubilization of polyuronides resulting in the accumulation of low-molecular WSP, which ultimately causes the inferior texture of peaches.

The results in Table 4 indicate that at the hard- and firm-mature stages, the juice from the SH treatment fruits exhibited a high degree of gellation at high pH (7.8) and high Ca++ conditions, although the juice from the M treatment fruits did not show such gellation. At the fully ripe stage, however, the juice from the M treatment fruits showed the highest degree of gellation, whereas that from the SH treatment fruits showed the lowest. The juice gellation may be a result of cross-linking between Ca++ and small polygalacturonide fragments produced by the degradation of flesh WSP. The early gellation of juice from high-fertilized peach may be an indication of an earlier collapse of flesh pectin in those fruit than in the normally fertilized counterpart. At all stages of maturity, the intrinsic viscosity of the juice from the M treatment fruits was higher than those of juices from the H and SH treatment fruits according to the panel evaluation (data not shown). This indicates that the length of the polygalacturonide chain is related, at least in part, to juice viscosity. Factors other than the length of the polygalacturonide chain and the amount of water-soluble pectin may also influence juice viscosity. Peach softening during ripening has been attributed to the enzymatic degradation of pectic polymers (Callahan et al., 1992; Dowans and Brady, 1990; Pressey and Avants, 1978). Although pectin esterase is considered to be the most dominant pectic enzyme associated with peach softening, other pectic enzymes may well be involved. Further investigation is needed in this area.

**Literature Cited**