Sugar Accumulation and Development of Loquat Fruit

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Summary

Development of loquat fruit was divided into two phases. The first was growth phase characterized by a growth of seed. The second was maturation phase characterized by decreasing acid content, color development and softening of the pulp tissue. In addition to these phenomena, sugar accumulation and a rapid increase in the fresh weight of the pulp tissue were also observed during maturation. This pattern of maturation is similar to that of fig fruit. The loquat fruit began to evolve ethylene at the beginning of the maturation phase.

Sorbitol was a predominant component in the young fruits of loquat. Although sorbitol content increased during the fruit development, its percentage relative to total sugar decreased. Sugar accumulation was accelerated at the beginning of the maturation phase. Sucrose was accumulated faster than any other sugar during this phase, and was a major sugar in the ripe fruit, while sorbitol became a minor component. Ninety percent of the sugar present in the ripe fruit was accumulated within two weeks of maturation. This sugar is thought to be supplied by other parts of the plant.

Introduction

Soluble sugar content is an important factor in evaluating fruit quality, and various attempts have been made to increase sugar content, which is influenced by many factors in the course of fruit development. Thus, studies on sugar accumulation in fruit will provide fundamental informations about fruit quality. The present paper reports the time courses of sugar accumulation in loquat fruit, in relation to fruit development.

Materials and Methods

Mature loquat trees (Eriobotrya japonica Lindl. cv. Tanaka) planted at the research station, Okitsu, Shizuoka, were used as materials for this study. Since loquat blooms over a long period in winter, the developmental stage of young fruit differs from fruit to fruit even in the same inflorescence. Consequently it is difficult to sample young fruit of the same stage. After the spring flush fruits develop synchronously. Therefore, the present study was started after the flush.

From the trees, 15 to 20 fruits were harvested on March 28, April 15, 28, May 12, 22, 27, 30, June 3, 9 and 14 in 1978, and April 26, May 4, 11, 17, 22, 26, 31, June 6 and 12 in 1979. From the harvest, 9 fruits having medium weights were chosen then grouped into 3 samples. Therefore, each sample consists of 3 fruits. Results on sugar, starch, and polysaccharide are means of the 3 samples. Seed and inner non-edible portions were removed and weighed. The flesh and peel of 1979 crops, or the flesh only of 1978 crops, were weighed then homogenized with ethanol at a final concentration of 80%. The homogenate was boiled for 30 min and then filtered. The resulting filtrate was used for the assay of sugar and acid. Soluble sugars were assayed enzymat-
Sorbitol was also determined enzymatically, using sorbitol dehydrogenase, glucose–6-phosphate dehydrogenase, phosphoglucose isomerase, hexokinase, and alcohol dehydrogenase (2). To determine the acid content of the pulp tissue, the filtrate was passed through a column of cation exchange resin, then titrated. Acid content was expressed as malic acid.

The residue from the homogenate was used for the assay of starch and other polysaccharides. Starch was extracted with 0.5 N NaOH, then hydrolyzed by amyloglucosidase (5). Glucose in the hydrolyzate was assayed enzymatically (3). From the residue of the homogenate, polysaccharide was extracted successively with hot water, hot 0.05 M EDTA containing 0.05 M sodium phosphate buffer (pH 6.8), and hot 5% NaOH. With each solvent, two 2 hr extractions were made, using a boiling-water bath. The cellulosic fraction was extracted from the resulting residue with 72% (w/w) H₂SO₄ at room temperature. Pectin and neutral sugar were determined in each fraction.

Using galacturonic acid as a standard, pectic substance was determined by the carbazole method (14). Neutral sugar was assayed by a phenol method, as follows: to 0.5 ml of sugar solution, 3 ml of concentrated H₂SO₄ was added, followed within 10 sec by 0.5 ml of 5% (w/v) aqueous phenol solution. The resulting solution was mixed thoroughly, and the absorbance at 480 nm was determined after 30 min. Using authentic samples, the increases in A at 480 nm due to xylose and galacturonic acid were respectively 107% and 54% of that of glucose. The content of neutral sugar in each fraction was calculated by subtracting the pectin content.

Carotenoid was extracted from the pulp with acetone and then transferred to ether, and the absorbance at 451 nm was determined. Carotenoid content was expressed as that of β-carotene.

The fruits with stems were harvested on May 23, 26, 30, June 3, 8 and 13 for respiration, and on May 27, June 1, 3, 7 and 13 for ethylene evolution. The stems were carefully cut off by a knife in order to avoid injury to the flesh tissue. Then 3 fruits were placed in a sealed container (1,000 ml) and incubated at 20°C for 1 hr or 3 hr respectively for respiration or ethylene evolution. The atmosphere in the container was sampled with a 1 ml hypodermic syringe, and the concentration of carbon dioxide and ethylene were determined by gas chromatography (9).

Results

1. Development of the loquat fruit

Figure 1 shows the patterns of fruit, pulp and seed growth in fresh weight on a logarithmic scale. In both 1978 and 1979 fresh seed weight increased until late in May, and then remained constant. On the other hand, the fresh weight of the pulp tissue was accelerated after late in May. In 1978 the rate of increase in the fresh weight of the whole fruit was constant throughout the fruit development, but in 1979 the rate was reduced after May 11.

The fruit began to evolve ethylene from the beginning of June in 1979, paralleled by an increase in respiration (Fig. 2). After ethylene evolution began, carotenoid content in the flesh tissue increased, and peel color started to change (Fig. 3). The color of the seed coat changed from white to dark brown.
and acid content began to decrease at the same time (Fig. 4). The pulp also started to soften. These changes took place simultaneously. The same trends started on May 22 in 1979. In the present study, this period is designated as a turning point. The fruit ripened around June 14 in 1978 and around June 7 in 1979.

2. Sugar accumulation

Figure 5 depicts the changes in sugar content of the flesh tissue for the experiments in 1978. Essentially similar results were obtained in 1979. Sorbitol was the major soluble sugar in young fruits. Its content reached 30 mg per fruit on May 12, then remained unchanged. After the turning point, the content increased by 10 mg per fruit. This increase during the maturation period was small but observed in both years. Sorbitol content was, however, only 1 to 2% of total sugar in the ripe fruit. Changes in the contents of glucose and fructose were
similar to each other, but different from that of sorbitol. Sucrose content was almost the same as that of glucose and fructose before the fruit reached the turning point, but increased rapidly after this point. Sucrose bacame the most abundant sugar in the ripe fruit. The contents of sucrose, fructose, and glucose were respectively 2100, 835, and 782 mg per fruit in the ripe one. Starch content was higher than that of soluble sugar on March 28, excepting the case of sorbitol. Starch content decreased until the turning point, then increased to reach 0.15 mg per g fresh weight of the pulp tissue in the ripe fruit (Fig. 6).

In order to investigate whether or not sugars are supplied by other parts of the tree, the bearing shoot (about 10 cm long) was detached from the tree on May 26 in 1979, just after the turning point. The shoot, from which the leaves were removed, was put in a vase, and the fruit was analyzed on June 5. While fruit on the tree accumulated sugar, total sugar in the detached fruit did not increase. In the detached fruit, the amount of sucrose was greatly reduced, although glucose and fructose increased. Sorbitol content also decreased (Table 1).

3. Changes in cell wall polysaccharides

The change in the content of wall polysaccharide was smaller than that in soluble sugar during the period investigated. Total polysaccharide increased until the middle of May. Increases in the cellulosic fraction and water–soluble pectin were marked in this period. The total polysaccharide content decreased around the turning point, but then increased again following the maturation period. This increase was due to the increases in pectin and neutral sugars in the EDTA-soluble fraction.

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<th>Date</th>
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Discussion

Development of loquat fruit during the period studied was divided into two distinct phases. The phase before the turning point was a growth phase characterized by a growth of seed. Next came a phase of maturation. Termination of the seed growth was followed by a rapid growth of pulp tissue, and fresh weight of the whole fruit increased in a sigmoidal fashion. This pattern differs from those of fig and stone fruits, in which a rapid growth during stage III occurs after a slow growing phase (stage II) (13, 16). The maturation of the edible portion may be triggered by the termination of seed growth in loquat fruit.

Ethylene induces maturation in many types of fruit. Loquat fruit began to evolve ethylene at the turning point. Furthermore, exogenously applied ethylene was found to accelerate the coloring of loquat fruit harvested just before the turning point (data are not shown). These findings suggest that ethylene may be a possible inducer of maturation in loquat fruit.

The marked increase in respiration during fruit maturation is known as a climacteric, and is accompanied by changes in acidity, sugar, texture, color, and flavor. These changes are thought to be triggered by ethylene. The maturation of loquat was in part similar to those of climacteric fruits: the change in maturation is also distinctive and rapid in loquat. However, the maturation of loquat took place simultaneously with sugar accumulation and accelerated enlargement of the flesh tissue, which is characteristic of the growth phase in many other fruits. This pattern has also been reported in fig fruit (7, 13). The turning point is the time of transition to this unique maturation phase. The changes at the turning point are somewhat similar to those at veraison or coloring stage of grape berry; sugar accumulation, acid decrease, softening, and coloring of the berry start synchronously at this time (18, 19). The triggering substance for the veraison, however, remains unidentified, and is possibly different from that for loquat maturation (4, 6).

During the experimental periods in 1979, the fresh weight of the edible portion increased about sevenfold, but only small changes were observed in the amount of cell wall material during this period. The fruit may deposit the wall material mainly during early stage of the fruit growth. The enlargement during the experimental period seems to be mainly due to an expansion and rearrangement of the cell wall by the accumulation of water and sugar. This phenomenon has been observed in Japanese pear (21). The increase in EDTA-soluble pectin during maturation is common to the grape berry (18), but the increase in water-soluble pectin has been reported for apple and Japanese pear (11, 21).

Sorbitol is one of the soluble sugar components in fruit of the family Rosaceae. The sorbitol content in ripe loquat fruit (0.94 to 1.46 mg per g fresh weight) was lower than that in plum, pear, and Japanese pear (10, 12, 17, 20). In addition to loquat, pear and Japanese pear both accumulate mainly sorbitol in the early stage of fruit development (12, 20). Although sorbitol content increases, its percentage relative to total sugar decreases during development and maturation in pear, Japanese pear, and loquat fruit (12, 20). In loquat sorbitol accumulation paused in the middle of May and resumed after the turning point. The regulatory mechanism of the accumulation is not yet known, but interconversion between sugar and sugar alcohol in the fruit may be a factor in the carbohydrate accumulation. Since sorbitol-6-phosphate dehydrogenase has been identified in ripe fruit of loquat (8), part of the sorbitol present in ripe fruit may be synthesized in the tissue.

The change in starch content of loquat fruit is unique. During growth phase, the starch content in loquat was lower than that in apple, pear, and Japanese pear (12, 20). Starch is known to be hydrolyzed during maturation of many types of fruit (12). Starch content in loquat, however, increased during the maturation phase.

Sucrose is accumulated faster than reducing
sugars during maturation of loquat, Japanese pear, mandarin, and other fruits (20), and is a major sugar in the ripe fruits. While fructose is major in ripe fruits of apple and pear (12), which are close relatives to loquat. Ninety percent of soluble sugar present in the ripe loquat fruit accumulated within the two weeks after the turning point. Starch hydrolysis and the breakdown of cell wall materials could not account for this accumulation. In addition, detached fruit no longer accumulated sugar (Table 1). Thus, the major portion of the accumulated sugar may be transported from other parts of the plant. Consequently, carbohydrate metabolism in the tree during this period may affect fruit quality.

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ピオ果実の発育と糖の蓄積
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摘 要

ピオ果実の発育は2つの期間に分けられた。前期
は生長期であり、種子重が急速に増加した。後期は成熟
期であり、種子重の増加は止まり、種皮が着色した。ま
たこの期に果肉においてリンゴ酸の減少、カロチノイド
の増加および果肉の軟化が見られた。これらの他の果実
にも見られる一般的な成熟現象に加え、ピオの成熟期に
は果実新鮮重の増加と糖の急速な蓄積もみられた。ピオ
のこの成熟特性はイチジクのそれに類似している。

ピオ果実は成熟期の初めにエチレンを発生した。エチ
レンが成熟の引き金になっている可能性が考えられる。

ピオ幼果で最も多い可溶性の糖はソルビトールであっ
た。その含量は生長期の初期と成熟期に増加したが、全
糖に対する割合は減少した。成熟果のソルビトールは全
糖のわずか1～2％であった。

果肉のデンプン含量は生長期に減少し、成熟期に増加
した。

糖の蓄積速度は成熟期の初めに増加した。特にシュ糖
の蓄積速度の増加は著しく、シュ糖は成熟果で最も多く
存在する糖であった。成熟果に存在する糖の約90％は
この成熟期の2週間に蓄積した。この糖は主として果実
以外の組織から転流するものと考えられ、この期間にお
ける樹体の糖代謝は果実の品質に大きな影響を持つもの
と推定される。