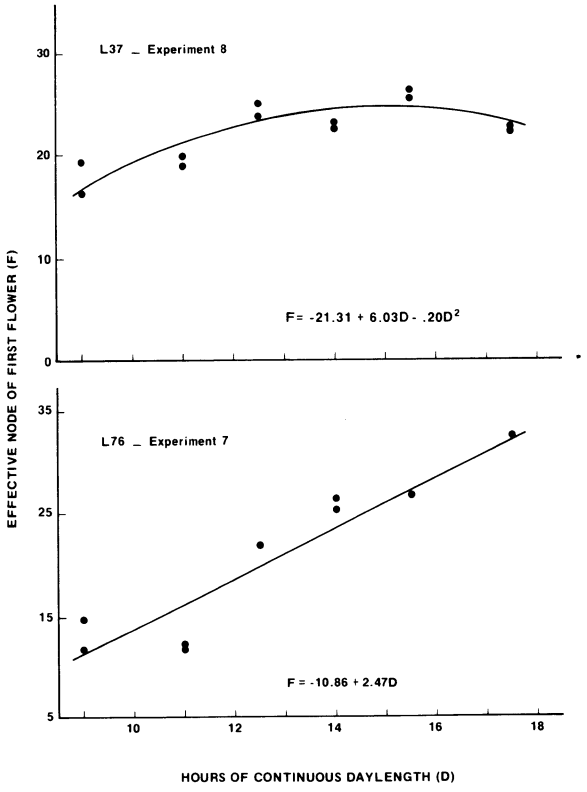

Fig. 3. Flowering responses of L37 and L76 to photoperiod in Experiments 8 and 7, respectively.

a graded series of photoperiods. This general procedure allows one to investigate polygenic systems, as well as simpler genetic systems. Hence, it may be most useful in the analysis of quantitative short- and long-day response types.

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Compositional Changes in the Developing 'Hayward' Kiwi Fruit in California[1]

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Abstract. **'Hayward' kiwi fruit (*Actinidia chinensis* Planch.) samples collected at 20-day intervals after full bloom, May 18, until harvest in late October, were analyzed for carbohydrates, protein, organic acids including ascorbic and dehydroascorbic acids, tannins and polyphenolic substances, and cytokinin-like compounds. Starch is a predominant carbohydrate stored in the carpellary tissue which becomes hydrolyzed as the fruit approach maturity. Quinic acid was the main organic acid in young fruit which disappeared concurrently with the appearance of ascorbic acid. Mature fruit has low concentrations of tannins and polyphenolic substances but many raphides. Types of cytokinins changed as the fruit developed.**

Cultivation of kiwi has spread from New Zealand, where it was commercially developed, to many parts of the world. The fruit has gained rapidly in popularity, but little has been published with respect to the physiology of the vine and crop. Few early studies have emphasized the nutritional values of the fruit (2,8) while others have evaluated the cultural practices related to the growing of the vines and harvesting of the crop (11). Seasonal changes of the following constituents were investigated: carbohydrates, organic acid including ascorbic acid (Vitamin C), protein, tannins and polyphenolic substances, and the cytokinin-like compounds. These analyses could serve as a basis for testing whether future cultural modifications or changes in growing sites alter the yield and/or nutritious value of the fruit.

Materials and Methods

Samples consisting of 30 to 40 'Hayward' fruit were collected

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[2]Visiting Scholar, Department of Horticulture, Hirosaki University, Hirosaki, Japan, and Pomologist, respectively. The authors are grateful to the Tanimoto Bros. (Cal-Kiwi) of Gridley, CA for their generous cooperation in the project and to Dr. J. van Staaden for sharing with us his soybean callus starters and timely suggestions on our cytokinin assay techniques.

at 20-day intervals from vines trained to the "T" bar trellis system near Gridley, Calif. Sampling was begun on June 7, 20 days after full bloom, (May 18), and continued until harvest on October 28. After the fruit were weighed and diced, a 500-g sub-sample was lyophilized. A 2-g sample of dry matter was extracted with 80% ethanol for sugar and organic acid determinations. Alcohol-insoluble substances (AIS) were analyzed for starch. Ten- or 20-g sub-samples of fresh pulp were blended with 80% ethanol and homogenized for cytokinin studies

Carbohydrate determinations. An aliquot of ethanolic extract was evaporated and the residue was silylated according to Sweeley et al. (13). Derivatives were injected into a SS column (3.18 1520 mm), packed with 3.8% SE 30 coated onto Chromsorb Q, which was programmed to heat from 100 to 260°C at 4°/min, in a gas chromatograph (Varian Model 1400). Amounts of glucose, fructose, inositol and sucrose were estimated by comparing peak heights with those of sugar standards and trehalose used as the internal standard.

Starch was determined on insoluble residue after digestion with amylase (refined diastase from CalBiochem) for 24 hr at 37°C. The hydrolyzate was analyzed for sugar with anthrone reagent (12).

Organic acids. Ethanolic extracts were eluted through a column of Dowex-1 (22 × 180 mm) with 200 ml water, which was discarded, and then with 200 ml 6 N formic acid. A 4-ml aliquot of the latter was evaporated and the residue was silylated and analyzed by gas chromatography as with sugar samples. Tartaric acid was used as the internal standard.

Ascorbic acid. A 5-g sample of fresh pulp was blended with metaphosphoric acid and brought to 250 ml. A 2-ml aliquot was analyzed for total and dehydroascorbic acid according to Roe et al. (8). Distribution of ascorbic acid in different parts of the fruit was determined.

Protein analysis. A 1-g lyophilized sample was homogenized repeatedly with 80% ethanol using a tissue grinder. The homogenate was centrifuged between grinding and the supernatant decanted and filtered. The insoluble residue was finally homogenized with water and the entire mixture brought to 100 ml. A portion of this mixture was centrifuged and an 1-ml aliquot of the supernatant was analyzed for protein according to Lowry et al. (3). The ethanolic extract was brought to 250 ml and an aliquot analyzed for Folin-Denis-sensitive substances as described below.

Substances sensitive to Folin-Denis reagent. A 1-ml aliquot of the ethanolic extract was reacted with 5 ml of Folin-Denis reagent and 10 ml of saturated Na_2CO_3 solution according to Smith et al. (11). Optical density of the resulting solution was converted to catechol equivalence from a standard curve. (Phosphotungstic and phosphomolybdic acids used to make the Folin-Denis reagent are known to react with alkaloids, tannins, proteins, and ascorbic acid. Since proteins are not very soluble in 80% ethanol, they are not a serious source of interference with this method. Quinic acid does not react with the reagent.)

Assays for cytokinin-like substances. Fresh fruit samples (10 to 20 g) were blended with 80% ethanol and the homogenates filtered. After evaporating the filtrate to eliminate the alcohol, the residue was passed through a column of Dowex-50. The column was eluted with 200 ml water followed by 200 ml each of 1.5 N and 5.0 N NH_4OH (6). The combined ammonia eluates were evaporated to near dryness. An aliquot was taken up in alcohol and streaked on Whatman 3M paper. The chromatogram was developed with water-saturated n-butanol as solvent, which, on drying, cut into 10 strips of equal width and assayed with the soybean callus technique (4). Aliquots of samples were treated with alkaline phosphatase or B-glucosidase according to Miller (4). After a 24-hr incubation period, cytokinins were isolated, chromatographed, and the chromatograms were assayed as above.

Results and Discussion

Total carbohydrate increased rapidly initially, keeping pace with fruit growth (Fig. 1, 2). The fruit, which contains chlorophyll except in the epidermis and the central pithy zone, accumulated starch centripetally from the sub-epidermal zone. By mid-September, even the cells in the white central core were filled with starch grains as tested with KI-I_2 solution. Thereafter, starch hydrolysis began which coincided with the small but noticeable final increase in size (Fig. 2). Sucrose increased from less than 1% to 2% by October on a dry weight basis, only to decrease as the fruit matured. Inositol, a growth-promoting substance, decreased steadily from 2% in the earliest sample to less than 1% by harvest. Green immature fruit was rich in glucose but the level decreased while starch was accumulating rapidly during late July and August. As starch hydrolysis began, glucose level increased rapidly, attaining nearly 10% by harvest. Fructose increased gradually from the youngest stage of fruit development until harvest.

Continued accumulation of dry matter by the fruit while starch was hydrolyzing is reflected by a noticeable increase in total soluble solids. This accumulation indicates that photosynthates were still being imported until the fruits were harvested.

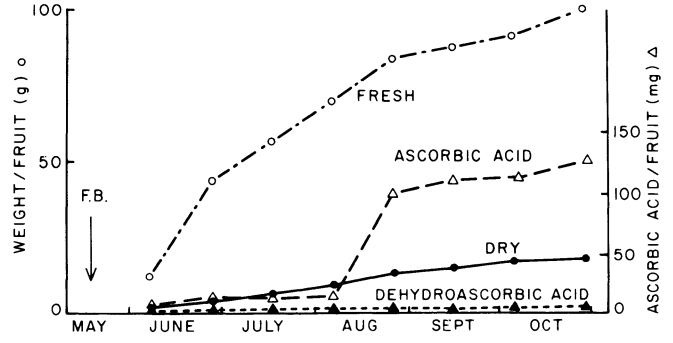

Fig. 1. Seasonal changes in weight of 'Hayward' kiwi fruit and ascorbic and dehydroascorbic acid content.

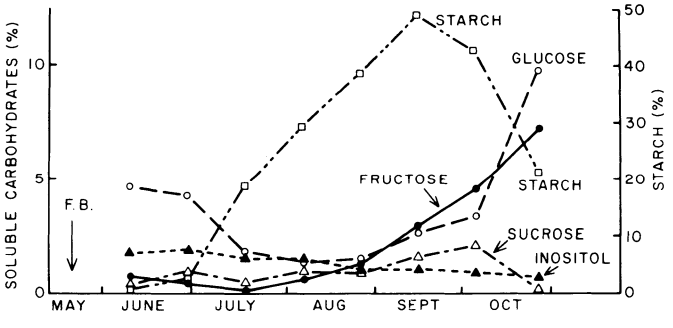

Fig. 2. Seasonal changes in carbohydrate composition of 'Hayward' kiwi fruit (dry weight basis).

Protein concentration was high in the initial sample but subsequently decreased, only to increase again to a mid-summer maximum (Table 1). This maximum coincided with the period of seed development when ascorbic acid content also increased rapidly. The protein value then decreased until harvest when it was 112 mg/100 g fresh weight. On a per fruit basis, protein content increased proportionately with fresh weight and starch content between 40 and 100 days after full bloom; thereafter, protein content decreased while fruit size continued to increase (Table 1, Fig. 1).

Young fruit are especially rich in quinic acid which disappeared as size increased (Fig. 1, 3). Malic acid attained a low mid-summer maximum and decreased towards harvest; the trend of citric acid followed that of starch reaching a peak in mid-September (Fig. 3). The 3 acids constituted about 3% of the dry matter at harvest so the fruit was quite tart. Some of the tartness is due to the high ascorbic acid content (Fig. 1). Dehydroascorbic acid was detectable but always in small quantity. Ascorbic acid increased rapidly during mid-season when the seeds enlarged and fruit growth slowed.

Table 1. Seasonal changes in protein[z] and Folin-Denis sensitive substances[y] in 'Hayward' kiwi fruit, (*Actinidia chinensis*, Planch.).

Days after full bloom	Protein (mg/100 g)	Protein (mg/fruit)	Folin-Denis-sensitive substances[x] (mg/100 g)	Folin-Denis-sensitive substances[x] (mg/fruit)
20	195	23.4	490	58.9
40	121	52.8	237	103.0
60	139	78.0	184	103.5
80	152	105.9	177	123.3
100	162	135.5	219	182.2
120	137	120.2	122	107.1
140	116	106.3	105	96.1
163	112	111.8	84	84.3

[z]Determined by the method of Lowry et al (3) on alcohol-insoluble residue.
[y]Determined by the method of Smith et al. (12) on ethanol-soluble fraction which includes tannins, polyphenolic substances and ascorbic acid.
[x]Computed on fresh weight basis.

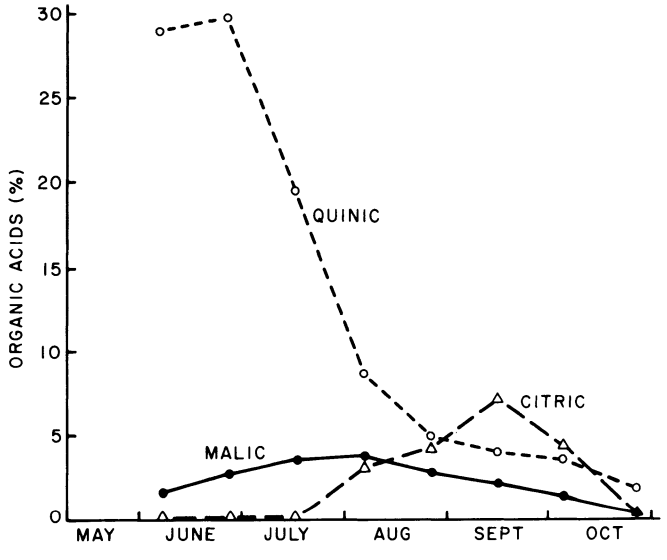

Fig. 3. Seasonal changes in 3 major organic acids in 'Hayward' kiwi fruit (dry weight basis).

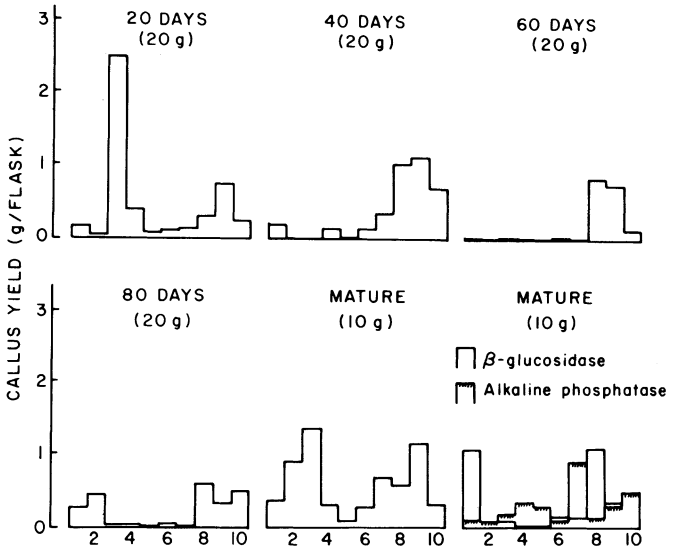

Fig. 4. Seasonal changes in composition of cytokinin-like substances in 'Hayward' kiwi fruit.

Table 2. Variation in sensitivity of various polyphenolic substances and ascorbic acid to Folin-Denis reagent.

Compound	Molecular wt	Variation in sensitivity mg/100 ml[z]	Variation in sensitivity Molarity ($\times 10^{-3}$)
Catechol	110.1	15	1.36
Chlorogenic acid	354.3	95	2.68
Caffeic acid	180.2	46	2.55
Ascorbic acid	176.1	57	3.24

[z]Values taken at optical density of 0.50 at 760 mμ

Analysis of mature fruit imported from New Zealand revealed that the green carpellary tissue contained about 80 mg ascorbic acid/100 g of fruit while the white pithy part contained 30 mg per 100 g. This vitamin is very stable (unpublished data) probably because the fruit is an oxalate-accumulating organ. Numerous raphides, presumably of calcium oxalate, can be observed in certain cells. The accumulation of oxalate may serve as a base of feedback inhibition of ascorbic acid metabolism. In grapes and *Pelargonium* (10, 15), ascorbic acid is metabolized to tartaric acid and a 2-carbon fragment. This 2-carbon fragment has been identified in *Pelargonium* as oxalic acid (16). In 'Bruno' fruit, oxalic acid was detected in low amounts (2); a trace of oxalic acid was found in our 'Hayward' samples but tartaric acid was not.

Folin-Denis reagent is commonly used for estimating tannins and polyphenolic substances in food and beverages (5, 12). In mature kiwi fruit which is rich in ascorbic acid, its value needs to be taken into consideration when calculating total tannins plus other polyphenols because the vitamin also reacts with this chromogenic reagent. The reasons why the level of ascorbic acid appears greater on dry wt basis than the total Folin-Denis-sensitive substances (Fig. 1, Table 1) are due to the vagaries of the colorimetric methods and the means by which the concentrations are expressed. That is, the reactivity of catechol, ascorbic acid and 2 other polyphenols, chlorogenic and caffeic acids, with Folin-Denis reagent vary as do their molecular weights (Table 2). Since the crude extract contains an unknown number of these polyphenols and in equally unknown proportions, we can only surmise that

these compounds made up most of the Folin-Denis-sensitive substances early in the development of the fruit. As ascorbic acid increased towards harvest maturity, it probably was the predominant Folin-Denis-sensitive compound. Smith et al. (12) reported similar variations when this colorimetric method was compared with a volumetric one.

Incidentally, acetone powder prepared from mature kiwi fruit according to Conn and Stumpf (1) possessed polyphenoloxidase activity which oxidized catechol at 0.039 absorbance unit at 420 mμ per min per mg protein. The mean rate of oxidation for 7 apple cultivars was 0.174 units (17). The relatively low tannin content coupled with a low polyphenoloxidase activity and high ascorbic acid content could well explain why bruised kiwi fruits do not exhibit the browning reaction so common with other fruits (7).

Bioassays of carpellary tissue for cytokinin-like compounds revealed that the young fruit was relatively rich in zeatin ribotide and zeatin (Fig. 4). The ribotide form disappeared as the fruit developed but reappeared in extracts from mature fruit. Treatment of these extracts with a β-glucosidase or alkaline phosphatase in a medium, with an appropriately adjusted pH, according to Miller (4), yielded zones of activities comparable to zeatin and zeatin riboside, respectively, on chromatograms derived from hydrolyzates. The compound which promoted callus growth at strip 1 is unidentified. A concurrent disappearance of growth activity observed on chromatograms derived from untreated extracts was noted.

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