Changes in Amylose and Total Starch Content in 'Fuji' Apples during Maturation

X. Fan¹

Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414

J.P. Mattheis²

U.S. Department of Agriculture, Agricultural Research Service, Tree Fruit Research Laboratory, 1104 North Western Avenue, Wenatchee, WA 98801

M.E. Patterson³

Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164-6414

J.K. Fellman⁴

Department of Plant, Soil, and Entomological Sciences; University of Idaho; Moscow; ID 83843

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Abstract. Total starch and amylose (AM) concentration and a starch index (SI) were determined in 'Fuji' apple (*Malus domestica* Borkh.) fruit from weekly harvests in 1990 and 1991. As apples matured, SI scores increased and total starch and amylose content decreased. The percentage of AM in the total starch decreased as the apples matured. Because KI solutions interact efficiently only with AM, the SI is less reliable in representing total starch during later stages of 'Fuji' apple maturation.

The starch iodine test, widely used as a simple maturity index for apples, provides an estimate of relative fruit starch content (Bigelow et al., 1905; Ingle and D'Souza, 1989; Onmiya and Kakiuchi, 1990; Reid et al., 1982; Smith et al., 1979). Starch reacts with a I_2 -KI solution to form a polyiodine complex that is blue-black. The progression of starch degradation visible after staining with an I_2 -KI solution is cultivar specific, a characteristic that requires the development of visual starch charts for each cultivar (Smith et al., 1979). Apple starch consists of two components: amylose (AM) and amylopectin (AP). Only AM can efficiently react with I_2 -KI to produce the blue-black pigment (McCready and Hassid, 1943). Morrison and Karkalas (1990) found I_2 binding capacity for AM to be 20 to 21 g per 100 g compared with 0.3 to 2.6 g per 100 g for AP. The absorbance maximum (λ_m) is between 630 to 680 nm for the AM-polyiodine

complex and 530 to 585 nm for the AP-polyiodine complex, depending on analytical conditions. The AP-polyiodine complex develops a purple-red pigment only at high AP concentrations.

Using the starch index (SI) as an indicator of total starch content depends on the proportions of AM and AP remaining constant during apple maturation. Cultivar differences in starch AM and AP composition and degradation rates may account for the different staining patterns observed during apple maturation. Potter et al. (1949) reported that AM comprises 24.8% of the total starch in 'Newtown Pippin' apples; however, we found no literature that reported changes in AM and total starch during apple maturation. 'Fuji' is a late-maturing apple that stores well when harvested with nearly all starch hydrolyzed (Fan, 1992). Our objectives were to evaluate SI as an indicator of total starch content in 'Fuji' apples and to determine if the AM and AP proportions remain constant during 'Fuji' maturation.

Materials and Methods

'Fuji' apples were harvested weekly from 17 Sept. to 22 Oct. in 1990 and from 4 Sept. to 24 Oct. in 1991 in a commercial orchard near Orondo, Wash. SI was rated visually using a 1 to 6 scale (1 = 100% stained, full starch; 2 = clear to core line; 3 = 50% cortex and core area clear; 4 = 75% cortex and core area clear; 5 = 90% cortex and core area clear; 6 = 0% stained,

no apparent starch) after immersing a radial section in I_2 -KI solution. Apples were peeled, cored, and cut into wedges. The sample was randomized into five replications, each consisting of 5 g from four apples. The tissues were frozen in liquid N and then stored at -20°C before freeze-drying. Samples were reweighed after freeze-drying, then ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia) using a screen with holes 354 μm in diameter.

Measurement of starch, AM, and AP. Ground samples (60 to 80 mg) were placed into 16 $\times$ 125-mm test tubes, and 1 ml of 18% HCl was added to each tube. The ground cortex tissue and 18% HCl were well-mixed, then incubated at 20°C for 30 min. Then, 10 ml distilled H_2O was added to each tube; the supernatant was mixed and centrifuged at $3800\times g$ using a clinical centrifuge (model CL; International Clinical Centrifuge, Boston). It (0.2 to 1.0 ml) was transferred to 10 $\times$ 100-mm test tubes, and 5.0 ml of 1.8% HCl was added to each tube. After mixing, 200 μl of I_2 -KI (2 mg I_2 and 20 mg KI per ml H_2O) solution was added. Each sample was mixed, and after 10 min, absorbance was measured at 530 (for AP) and 606 nm (AM) using a diode array spectrophotometer (model HP 8451A; Hewlett-Packard Co., Avondale, Pa.). AM and AP standards (Sigma Chemical Co., St. Louis) were dissolved in 18% HCl for 30 min and then diluted 10-fold. The two standards were mixed in various ratios, and absorbance was measured at 530 and 606 nm to generate a standard curve. Absorption coefficients (A), determined by Beer's law for AM and AP standards, were $A_{AM606} = 12.34$, $A_{AM530} = 8.31$, $A_{AP606} = 4.53$, and $A_{AP530} = 6.97$, and a standard curve typically had an r^2 value of 0.99. These A values were lower than those reported by Magel (1991), perhaps due to using lower concentrations of I_2 and KI in the staining solution. However, the $A_{AM530} : A_{AP530}$ and $A_{AM606} : A_{AP606}$ ratios were similar to those calculated using A values reported by Magel. The amounts of AM and starch concentration (SC) in the samples were calculated using equations derived by Magel (1991); AP concentration then can be obtained by subtracting AM from SC.

Results and Discussion

SC of 'Fuji' apples harvested in 1990 and 1991 decreased as fruit matured (Figs. 1 and 2). Total SC was correlated with SI in 1991 but not in 1990 (Table 1). Differences in the SC and SI relationship between the two years may have been due to earlier harvest initiation in 1991 compared to 1990. During 1991, SC decreased by 70% between 15 Aug. and 4 Sept., and the loss rate slowed over the next 7 weeks. The AM amount in the total starch decreased as fruit maturation progressed. AM concentration was negatively correlated with SI in both years. Therefore, SI is a better indicator of 'Fuji' AM concentration than SC.

The percentage of AM in the starch decreased from 52% on 16 Sept. to 39% on 22 Oct. in 1990 and from 60% on 4 Sept. to 37% on 24 Oct. in 1991. The decrease in the per-

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¹Graduate Research Assistant.

²Research Plant Physiologist.

³Professor Emeritus.

⁴Associate Professor.

centage of AM was greater during the later part of the season, indicating that SI is less reliable in representing total SC during this period of 'Fuji' apple development. SI continued to increase during the mid- and late stages of maturation, primarily due to the decrease in AM rather than a decrease in SC. Therefore, during the late maturation period, most of the starch was AP, which as previously stated is not efficient at forming an iodine complex.

Potter et al. (1949) reported that the percentage of AM in total starch of 'Newtown

Pippin' apples was 24.8. In that report, AM was measured only once in apples harvested in August. Our results showed the percentage of AM in total SC of 'Fuji' apples was >25%, and that this percentage changed during fruit maturation.

The SI is a useful commercial tool for determining apple harvest maturity. Our objective was to determine if the percentage of AM in total starch changes during 'Fuji' apple maturation and, therefore, if SI is a good measure of total SC. Although our results

Table 1. Correlation coefficients (r^2) for total starch concentration, amylose, and starch index from 'Fuji' apples harvested in two consecutive years (1990 to 1991).

Variable	Starch index	
	1990	1991
Starch concentration	-0.63 ^{ns}	-0.94 ^{***}
% Amylose	-0.83 [*]	-0.92 ^{***}

^{ns}, ^{*}, ^{***} Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

indicate that SI is not a reliable SC measure in 'Fuji' apples, using the SI as a maturity index is not questioned because storage studies and practical experience indicate that SI is a useful tool for relative determination of 'Fuji' apple development (Fan, 1992). Our results provide insight into a possible reason why the characteristic patterns of starch degradation differ between apple cultivars. Further studies using other apple cultivars are necessary to determine if the different patterns characteristic of the visual SI for various apple cultivars is related to cultivar differences in the AM : AP ratio, degradation rates for each polymer during maturation, or both.

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Fig. 1. Changes in total starch concentration (SC), percentage of amylose (AM), and starch index (SI) during 'Fuji' apple maturation in 1990. Vertical bars represent SE.

Fig. 2. Changes in total starch concentration (SC), percentage of amylose (AM), and starch index (SI) during 'Fuji' apple maturation in 1991. Vertical bars represent SE; where no bars appear, SE was smaller than the symbol size.