Effect of Ethephon and Seniphos Treatments on the Anthocyanin Composition of Starking Apples

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Anthocyanins were extracted from the skin of apples (*Malus domestica* Borkh L. cv. Starking Delicious) with acidified methanol and were identified by HPLC. Cyanidin 3-galactoside was the most abundant anthocyanin. Other anthocyanins present in smaller amounts were cyanidin 3,5-diglucoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-rutinoside, cyanidin 3-xyloside, cyanidin, and cyanidin 3-acetylglucoside. Treatment with ethephon and seniphos increased anthocyanin concentration and altered the percentage composition during ripening.

Keywords: *Apple; ripening; anthocyanin; ethephon; seniphos*

INTRODUCTION

The phenolic compounds present in agricultural foodstuffs affect the nutritional, organoleptic, and commercial properties of those products. Anthocyanins are a class of phenolic compounds that is mainly responsible for red, blue, purple, etc. pigments in such foodstuffs. Red-skinned apple varieties are rich in anthocyanins. Because of the important influence of color on fruit quality, the anthocyanins have been studied by a number of workers (Ferreira and Tomás, 1984; Lin *et al*., 1989; Lister *et al*., 1994). To date, all the anthocyanins present have been found to be cyanidin derivatives, with cyanidin 3-galactoside present in the highest concentration (Duncan and Dustman, 1936). Other anthocyanins reported include cyanidin 3-arabinoside and cyanidin 7-arabinoside (Sun and Francis, 1967), cyanidin 3-xyloside (Timberlake and Bridle, 1971), cyanidin 3-glucoside (Pais and Gombkötö, 1967), together with the acylated derivatives of all the foregoing except cyanidin 7-arabinoside (Timberlake and Bridle, 1971).

Various treatments are employed to increase the commercial value of apples either to enhance quality or to yield produce out of season. Application of ethephon [(2-chloroethyl)phosphonic acid] is one treatment that yields fruit before the start of the season. This compound produces ethylene by solvolytic hydrolysis inside the fruit (Swindeman *et al*., 1988); ethylene is a hormone involved in maturation processes that can accelerate ripening of the fruit by ~2 weeks (Pollard, 1974), and it is related to PAL activity and anthocyanin accumulation in unripe apples (Faragher and Brohier, 1984). Seniphos can be used to achieve the opposite effect; that is, extending the mean life of fruit to delay ripening and obtain produce after the end of the season. The main components of this chemical are mineral ions, and treatment extends the storage life of apples at low temperature over longer periods, postponing such storage-related forms of deterioration as storage-induced senescence, cold-induced senescence, and bitter pit (small brownish depressions on the surface of the fruit; Harmerhill International, 1980).

In the present paper, we examine the effect of treatments with ethephon and seniphos on the anthocyanin composition of Starking apples during the 20 days subsequent to application during the ripening period.

MATERIALS AND METHODS

Plant Material and Treatments. Apples (*Malus domestica* Borkh L. cv. Starking Delicious) were obtained from Lleida, Spain, during the summer of 1992. A block of trees planted in 1975 was used for these tests. Treatments were assigned to two-tree plots in a completely randomized design. The rootstock was "Franco".

Ethephon was supplied by Compagnie Française de Produits Industriels (CFPI) in the Union Carbide Formulation (0.48 kg L^{-1}) and was applied at a rate of 0.1% (v/v) in water with a manual sprayer on September 9, 2 weeks before the commercial harvest. Seniphos is a mineral mixture (310 g/L P_2O_5, 56 g/L CaO, and 30 g/L total nitrogen; 1% NO_3 and 2% NH_3) subministered by the Phosyn Ltd. Company (Phosyn PLC, York, U.K.). Seniphos was applied with a manual sprayer on September 2, 3 weeks before the commercial harvest and according to the company recommendation of 1%. Controls were sprayed with water.

Samples. Samples were collected 4, 12, and 20 days after treatment. The apple skins were separated from the pulp and freeze-dried.

Sample Preparation. A 0.5-g sample (lyophilized skin) was steeped in 100 mL of a solution of MeOH/HCl (1000/1) at room temperature for 0.5 h. The resulting solution was filtered through no. 238 Albert paper and concentrated to dryness at 30 °C and a pressure of 100 mmHg in a rotary evaporator. The residue was redissolved in 25 mL of ethanol/water (20/80). This solution was extracted four times by shaking with 15 mL of ethyl acetate each time in a decantation flask for 1 min. The anthocyanins transferred to the aqueous phase. The aqueous solution was concentrated to one-third of its initial volume and was filtered through a 0.45-μm filter for injection into an HPLC column.

Standards. Anthocyanin standards from Extrasynthèse (Genay, France) in methanol/formic acid/water (48/2/48) at a concentration of 100 mg/L were used.

Chemical Analysis. Individual anthocyanins were analyzed by reversed-phase HPLC (Gónzalez-San José *et al*., 1988) with a Waters liquid chromatograph equipped with two model 510 pumps; a U6K injector; a dual-chanel, UV−visible light detector; 313- and 546-nm fixed wavelengths; and a Novapak C_{18} column (15 cm long, 0.45 cm i.d.).

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Table 1. Values for Anthocyanins Analyzed, Expressed as Micrograms per Gram Dry Weight in Cyanidin 3-Glucoside

anthocyanin	control	control+4	control+12	control+20	ethep+4	ethep+12	ethep+20	senip+4	senip+12	senip+20
Cy-3,5-digl		0.258	0.231	0.334	0.086	0.358	0.487	0.521	0.631	0.392
peak 2 (Uk)			0.626	0.636	1.606	2.236	2.568	1.166	0.821	1.247
Cy-3-gal	49.321	85.72	150.221	246.779	262.551	463.595	547.314	144.646	246.981	465.259
Cy-3-gluc	0.462	1.105	1.875	3.377	3.719	7.036	9.073	2.728	3.357	5.568
Cy-3-arab	2.716	7.199	17.094	27.306	31.316	56.726	72.453	18.165	25.834	46.099
Cy-3-rut		2.688	1.548	0.41	0.075	0.272	2.46	0.106	0.113	0.213
peak 7 (Uk)	2.023	4.16	9.232	17.641	21.415	39.645	45.2	10.354	16.845	26.719
peak 8 (Uk)	2.369	4.78	9.144	13.9	15.169	28.02	33.687	6.421	13.557	26.874
Cy-3-xil	1.494	2.517	3.731	5.282	1.373	6.751	13.725	1.599	4.359	8.629
cyanidin		0.427	2.203	0.321	1.057	0.581	3.249	0.564	1.599	4.834
Cy3(6ac)gl.			1.403	0.614		0.953	1.19	0.325	0.346	0.716
total	58.385	108.854	197.308	316.6	338.367	606.173	731.406	186.595	314.443	586.55

Sugars were analyzed as trimethylsilyl (TMS) derivates (Ausin and Gómez-Cordovés, 1996) with a Perkin-Elmer 8310 gas chromatograph equipped with flame ionization detector (FID) and an OV 101 fused silica gel column (25 m × 0.2 mm). The initial column temperature was 185 °C for 6 min, and the final column temperature was 270 °C for 4 min, which was attained in increments of 30 °C/min. Injector and detector temperatures were both 320 °C. Nitrogen was used as the carrier gas.

RESULTS AND DISCUSSION

The first step was to identify the anthocyanins present in the apple skins. The chromatogram for a sample treated with ethephon 20 days after treatment is shown in Figure 1. Identification of the anthocyanidins that corresponded to the glycosides present was accomplished by acid hydrolysis of one of the samples (Hebrero *et al.*, 1988). The hydrolysate was analyzed by HPLC, which yielded a single anthocyanidin peak that matched the standard for cyanidin under the same chromatographic conditions.

Identification of anthocyanins **1**, **4**, **6**, and **11** was achieved by matching them to the standards analyzed under the same chromatographic conditions. These compounds were identified as follows: **(1)** cyanidin 3,5-diglucoside (Cy-3,5-digl); **(4)** cyanidin 3-glucoside (Cy-3-gluc); **(6)** cyanidin 3-rutinoside (Cy-3-rut); and **(10)** cyanidin. Anthocyanin **3** was isolated by HPLC and hydrolyzed enzymatically. An aliquot of the hydrolyzed solution was used to identify the glycosidic portion by GC of the TMS derivative. That portion proved to be galactose, and peak 3 was thus cyanidin 3-galactoside (Cy-3-gal). Anthocyanins **5** and **9** were identified by comparison with the elution order and retention times reported by Mazza and Veliglou (1992) as cyanidin 3-arabinoside (Cy-3-arab) and cyanidin 3-xyloside (Cy-3-xil), respectively. Anthocyanin **11** was identified by comparing the retention times for the components of an anthocyanin extract of grape skins of *Vitis vinifera* var. Graciano of known composition that was analyzed by HPLC under the same chromatographic conditions. This anthocyanin turned out to be cyanidin 3-acetylglucoside (Cy-3-(6ac)gl). The quantitative values for the anthocyanins analyzed, expressed as μg/g dry weight, are presented in Table 1.

The changes in all the individual anthocyanins over the study period for the treated and control samples are shown in Figure 2. The anthocyanin content increased during ripening in all three batches. A comparison of the treatments showed that fruit treated with ethephon had the highest anthocyanin concentrations and that the fruit treated with seniphos had a higher anthocyanin content than the fruit in the control batch.

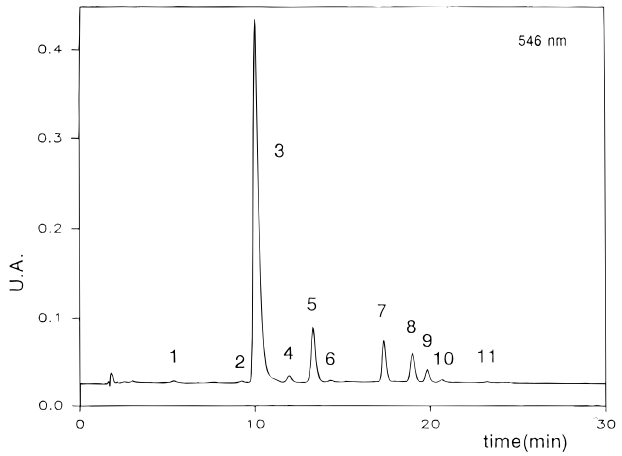

Figure 1. HPLC chromatogram of apple skin anthocyanins for a sample treated with ethephon, 20 days after treatment (for peak numbers, see text).

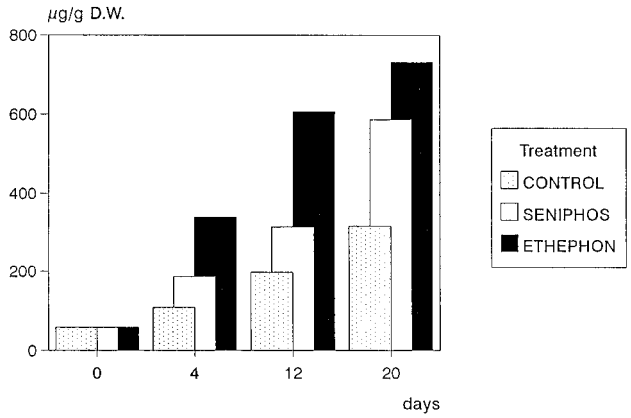

Figure 2. Evolution of total anthocyanins 20 days after treatment.

The changes in anthocyanin content produced by each of the treatments with respect to the control batch are shown in Figure 3. In the samples treated with ethephon, the sharpest increase took place in the first 12 days, and in the samples treated with seniphos, values during that same period were slightly higher than those for the control batch. From day 12 to day 20, anthocyanin accumulation decreased in the batch treated with ethephon but increased in the batch treated with seniphos as compared with the first 12 days. The curves illustrate the differing effects of the two treatments, with a faster accumulation rate in the initial period in the fruit treated with ethephon. In any event, the period considered was still the early stages of ripening because the slopes of the accumulation curves for the batch treated with seniphos and the control batch were still rising at the time of the final sample. Conversely,

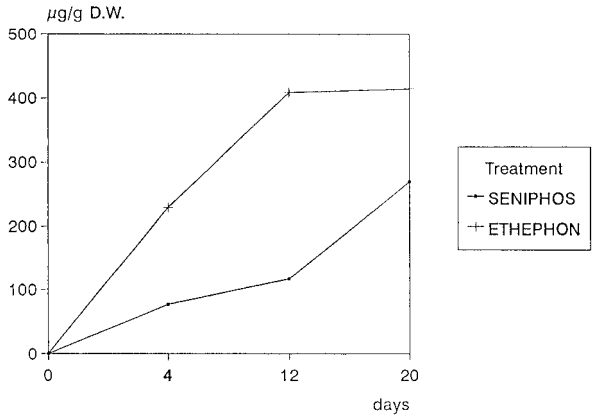

Figure 3. Changes of total anthocyanins following treatment with respect to control.

treatment with ethephon speeded up ripening and, by the end of the period, the samples treated with ethephon were nearing maturity as shown by the decline in anthocyanin synthesis.

Taking peak anthocyanin accumulation as the marker index for ripening, it was only to be expected that the different treatments would yield peaks at different times, as was the case just described. Accordingly, to obtain the same degree of ripening the fruit should be harvested at the respective peak for each treatment.

The question of whether either of the treatments affected the percentage composition of the anthocyanins in the fruit is an important one and requires comparison of the fruit at similar maturity stages. The percentages of anthocyanins in samples from the batch treated with ethephon at 4 days, the batch treated with seniphos at 12 days, and the control batch at 20 days are shown in Figure 4. The values for the main component present, cyanidin 3-galactoside, which were 77.4, 78.3, and 77.9% in each of the three batches, respectively, have not been included in Figure 4. The remaining share, ~23%, was distributed among the other anthocyanins. The three main secondary anthocyanins, cyanidin 3-arabinoside, cyanidin 3-rhamnoside, and cyanidin 7-arabinoside, are depicted in Figure 4A. The profiles were similar in all three batches, except that the batch treated with ethephon had a higher percentage of cyanidin 3-arabinoside. The percentage values for the anthocyanins present in smaller amounts are shown in Figure 4B. The proportion of cyanidin was higher and the proportions of cyanidin 3-xyloside and cyanidin 3-acetylglucoside were lower in the treated fruit batches than in the control batch and this effect was more pronounced in the batch treated with ethephon.

CONCLUSIONS

In addition to the anthocyanins described in the literature, small quantities of cyanidin and the anthocyanidins cyanidin 3,5-diglucoside, cyanidin 3-rutinoside, and cyanidin 3-acetylglucoside were identified. Treatment with ethephon and with seniphos increased the concentration of anthocyanins in the skin over the ripening period considered. The increase was more pronounced in the batch treated with ethephon. The treatments also altered the percentage composition of the anthocyanins, the most salient differences being the decrease in cyanidin 3-xyloside formation and the increase in cyanidin production. The results for the sampling period considered demonstrated that ripening

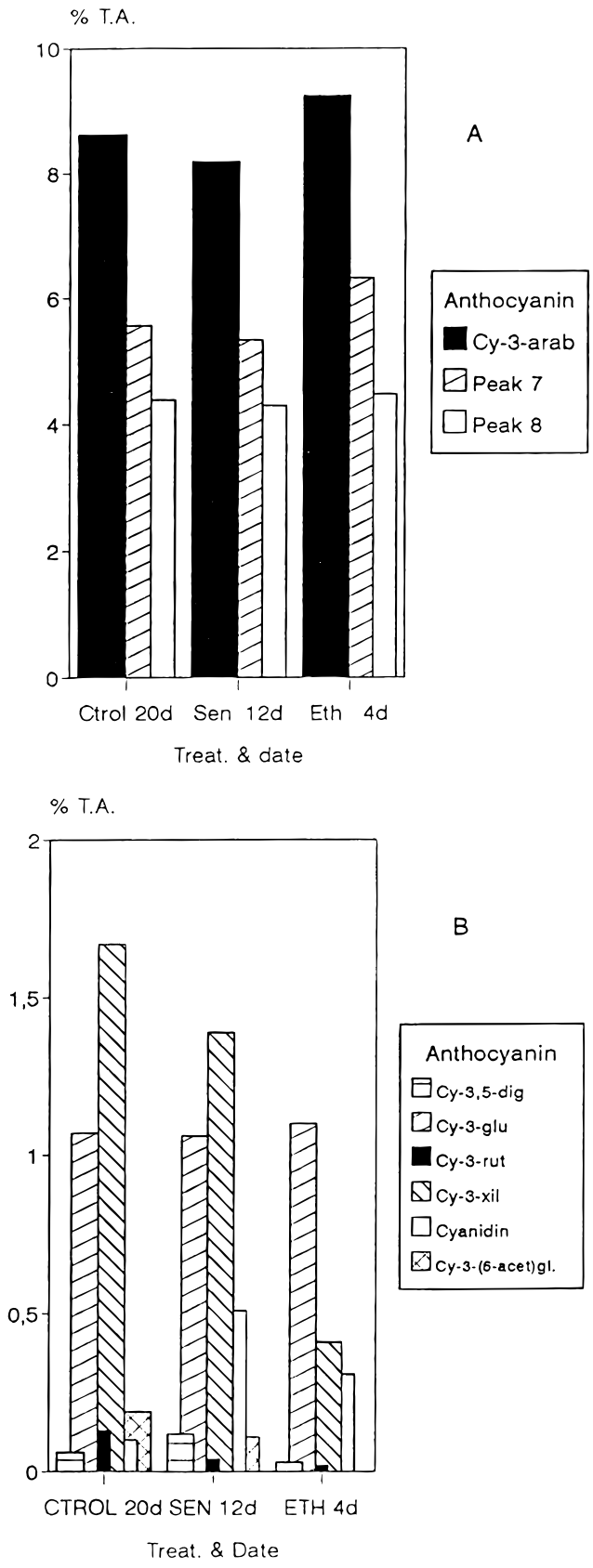

Figure 4. Percentage composition of the samples: control, 20 days; seniphos, 12 days; ethephon, 4 days; (A) main secondary anthocyanins; (B) minor secondary anthocyanins.

was accelerated in the treated fruit batches, but the accumulation curves presented in Figure 2 indicate that, compared with ethephon, treatment with seniphos both accelerates ripening and at the same time extends the mean storage life of the fruit.

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