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Degradation of anthocyanins in sour cherry and pomegranate juices by hydrogen peroxide in the presence of added ascorbic acid

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Abstract

Effects of hydrogen peroxide in the presence of ascorbic acid on the degradation of sour cherry and pomegranate juice anthocyanins were studied at three H_2O_2 concentrations (4.65, 6.98 and 9.31 mmol l^{-1}) and two ascorbic acid concentrations (60 and 80 mg l^{-1}) at 20 °C. Degradation of anthocyanins by H_2O_2 was fitted to first-order reaction kinetics in sour cherry and pomegranate juices. A similar degradation pattern was found at the 60 and 80 mg ascorbic acid levels for pomegranate juice. Degradation of sour cherry anthocyanins at the 60 mg ascorbic acid level was fitted to a second-order reaction kinetic, whereas it was a first-order reaction kinetic after a lag period occurred at the 80 mg level. Ascorbic acid, at 80 mg l^{-1}, markedly accelerated the degradation of anthocyanins in sour cherry juice at all the H_2O_2 concentrations studied. In contrast, ascorbic acid, at both 60 and 80 mg l^{-1}, protected the anthocyanins from the degradation by H_2O_2 in pomegranate juice.

Keywords: Anthocyanins; Hydrogen peroxide; Ascorbic acid; Degradation kinetics; Fruit juices

1. Introduction

Hydrogen peroxide is the most commonly used packaging sterilant in aseptic processing systems (Kunz & Binning, 1987; Von Bockelmann & Von Bockelmann, 1986). FDA classifies H_2O_2 as generally recognized as safe (GRAS) and limits the residual H_2O_2 to 0.5 ppm in the finished food packages (Code of Federal Regulations, 2000). Although aseptic processing systems have been designed to remove the residual H_2O_2 during the sterilization of aseptic chambers or packaging material with H_2O_2, residues left on the packaging material or vapours generated during drying may get trapped inside the package upon sealing (Toledo, 1986). Therefore, the residues left inside the packages may occasionally be over the FDA limit and particularly cause the degradation of anthocyanins and ascorbic acid in fruit juices.

The deleterious effects of H_2O_2 on anthocyanins and ascorbic acid have been reported in fruit juices and minimally processed fruits. Sondheimer and Kertesz (1952) found the rapid degradation of anthocyanins in both model systems of strawberry anthocyanins and strawberry juice. Özkan, Yemenicioğlu, Çıtak, and Cemeroğlu (2000) also showed the degradation of anthocyanins in sour cherry juice at various H_2O_2 concentrations and temperatures, and recommended low temperature storage. Sapers and Simmons (1998) used H_2O_2 as a surface sterilant in sweet cherries, raspberries and strawberries, and found rapid bleaching of anthocyanins in the last two fruits. Johnson and Toledo (1975) found that the half-life of ascorbic acid in orange juice concentrate was only 21 days at 24 °C when the aseptic chamber was pre-sterilized with H_2O_2 and 42 days when pre-sterilized with steam.

Ascorbic acid and its derivatives are used in many foods as antioxidants and act primarily as singlet oxygen quenchers (Elliott, 1999). They are also added to some foods, including fruit juices, to prevent browning and improve the nutritional value (Freedman & Francis, 1984; Starr & Francis, 1968). Aside from their use as antioxidants, Sapers and Simmons (1998), and Sapers, Miller, Choi, and Cooke (1999) recommended the use of Na-erythorbate, an ascorbic acid derivative, for the removal of H_2O_2 residues from H_2O_2-treated foods.

In this study, pomegranate and sour cherry juices were chosen because both juices contain copious

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amounts of anthocyanins and an insignificant amount of ascorbic acid. In fact, sour cherry and pomegranate juices contain 267–688 mg l^{-1} (Erbaş & Cemeroğlu, 1992) and 271–316 mg l^{-1} (Bodur & Yurdagel, 1986; Cemeroğlu & Artık, 1990) anthocyanins, respectively. On the other hand, both sour cherry (Herrmann, 1978) and pomegranate juices (Cemeroğlu, Artık, & Erbaş, 1992) contain insignificant amounts of ascorbic acid. No published data have been found to show the effects of either H_2O_2 or ascorbic acid on the anthocyanins of fruit juices. Therefore, this study was undertaken to determine the effects of H_2O_2 on the degradation of anthocyanins in sour cherry and pomegranate juices in the presence of added ascorbic acid.

2. Materials and methods

2.1. Materials

Sour cherries (*Prunus cerasus* L.) were obtained from the Çubuk region of Ankara and brought to the fruit juice pilot plant in the Department of Food Engineering. Fruits were washed in cold tap water and crushed. The mash was heated in a tubular heat exchanger at 88 °C for 2 min and pressed on a Bucher Model Packaged Press. The juice was depectinized (Pectinex 3XL; Novo Nordisk, Dittingen, Switzerland), clarified and filtered. The filtered juice was then pasteurized in a plate heat exchanger at 90 °C, bottled and stored at room temperature.

Pomegranates (*Punica granatum* L.) were purchased from a local market in Ankara. Fruits were washed in cold tap water and the outer skins were hand-peeled. The juicy sacs from the fruit pericarp were separated by hand. The separated sacs were pressed on the same packaged press. Before use, pomegranate juice was clarified with gelatin at 4 °C overnight. For 1 l of pomegranate juice, 50 ml of per cent 0.5 gelatin solution were added and the resulting clarified juice was then filtered. Pomegranate juice was filled into glass bottles and kept frozen at −30 °C until used for analysis.

2.2. Methods

2.2.1. Sample preparation and absorption spectra

The juice samples were diluted with distilled water to give an absorbance reading between 0.6 and 0.8 units. The pH values of the diluted pomegranate and sour cherry juices were 3.32 and 3.50, respectively. The diluted juice was filtered before using for degradation studies. The absorption spectra were scanned from 350 to 700 nm. The wavelength of maximum absorption were 512 and 515 nm for sour cherry and pomegranate anthocyanins, respectively. All absorbance readings were made against distilled water as a blank. Spectrophotometric measurements were carried out using a Unicam UV2–100 spectrophotometer (Unicam, Cambridge, England).

2.2.2. Effect of H_2O_2 concentration

The effects of three H_2O_2 concentrations (4.65, 6.98 and 9.31 mmol l^{-1}) on the anthocyanins of sour cheery and pomegranate juices were studied at 20 °C. The diluted juice samples were allowed to reach 20 °C in a Sanyo MIR 153 Model Refrigerated Incubator (Sanyo, Gunma, Japan). Then, the diluted H_2O_2 solution was rapidly added to the juice sample which was made up to volume.

The absorbance of the sample solutions was measured periodically. The zero-time absorbance values were determined by preparing the samples with the same amount of distilled water instead of H_2O_2. At 20 °C, the change in absorbance of the sample solution containing no H_2O_2 is insignificant over the time. The anthocyanin retention for each time period was calculated as a percentage of zero-time absorbance readings, taken as 100% retention.

2.2.3. Effect of ascorbic acid concentration

The effects of two ascorbic acid concentrations (60 and 80 mg l^{-1} fruit juice) on the anthocyanins of sour cherry and pomegranate juices were determined at 20 °C. The same diluted H_2O_2 solutions were added to the juice samples containing 60 and 80 mg ascorbic acids l^{-1} fruit juice. The anthocyanin retention for each time period was calculated as described earlier.

3. Results and discussion

3.1. Degradation kinetics

The decomposition of H_2O_2 in an aqueous solution occurs by dissociation (1) and homolytic cleavage of O–H or O–O bonds (2, 3) with the formation of highly reactive products: perhydroxyl anion (HOO^-), and perhydroxyl (*OOH) and hydroxyl (*OH) radicals (De, Chaudhuri, & Bhattacharjee, 1999).

$$H_2O_2 \rightarrow H^+ + HOO^- \quad K = 1.55 \times 10^{-12} \tag{1}$$

$$HOOH \rightarrow {}^*OOH + {}^*H \tag{2}$$

$$HOOH \rightarrow 2{}^*OH \tag{3}$$

De et al. (1999) reported that *OH radical is the main reactive species to cleave the benzene ring and degrade the substrate into CO_2 and H_2O. Sondheimer and Kertesz (1952) also suggested that the *OH radical is responsible for the oxidation and subsequent degradation of anthocyanins. Von Elbe and Schwartz (1996) reported that quinones also have deleterious effects on anthocyanins. The quinones are formed by the oxidation

of phenolic substances and this reaction can also be catalyzed by the decomposition products of H_2O_2 (De et al., 1999; Sapers & Simmons, 1998).

Thus, two factors can primarily affect the degradation of anthocyanins by H_2O_2 in fruit juices which generally contain copious amounts of phenolic compounds: (1) the amount of free radicals and HOO^- anion formed by decomposition and dissociation, respectively; (2) the amount of quinones formed by the oxidation of phenolic compounds.

The degradation of anthocyanins by H_2O_2 was fitted to first-order reaction kinetics in sour cherry (Fig. 1) and pomegranate juices (Fig. 2). As a similar degradation pattern was found at 60 mg (Fig. 3) and 80 mg (Fig. 4) ascorbic acid levels for pomegranate juice. The degradation of sour cherry anthocyanins at the 60 mg (Fig. 5) ascorbic acid level was fitted to a second-order reaction

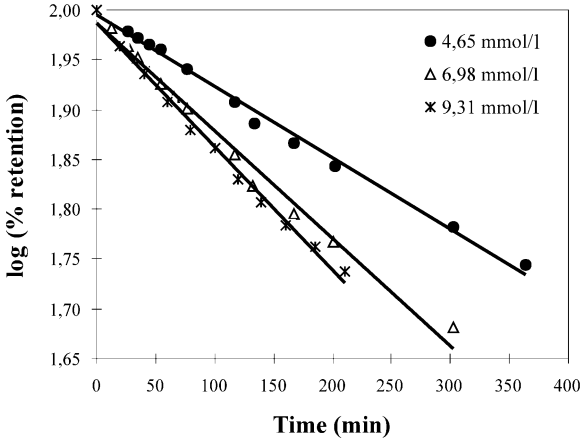

Fig. 1. Degradation of sour cherry anthocyanins at various H_2O_2 concentrations at 20 °C.

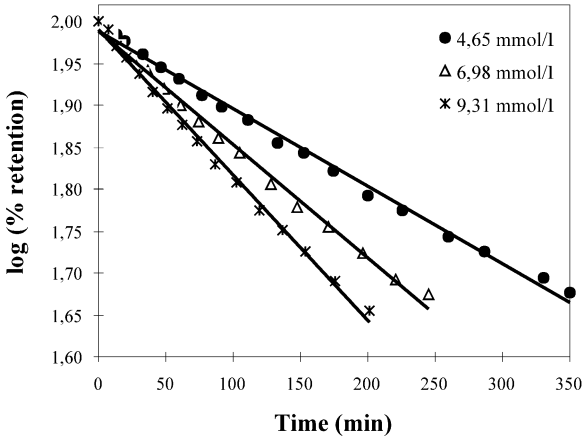

Fig. 2. Degradation of pomegranate anthocyanins at various H_2O_2 concentrations at 20 °C.

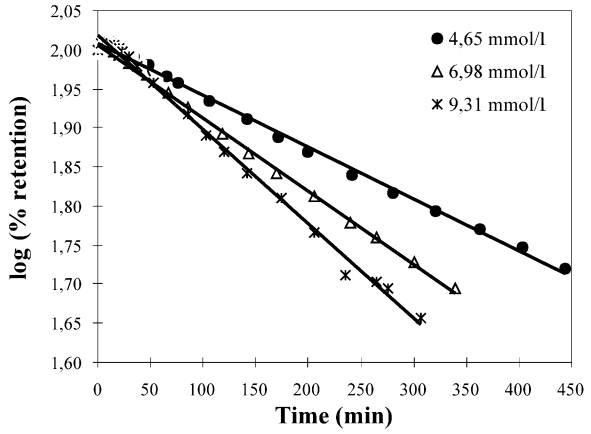

Fig. 3. Degradation of pomegranate anthocyanins at various H_2O_2 concentrations in the presence of added ascorbic acid (60 mg l^{-1}) at 20 °C.

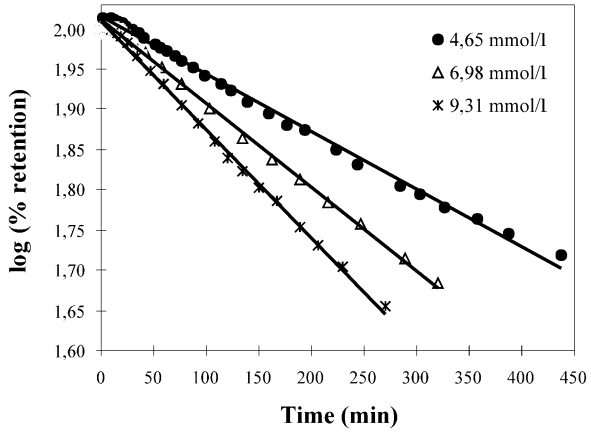

Fig. 4. Degradation of pomegranate anthocyanins at various H_2O_2 concentrations in the presence of added ascorbic acid (80 mg l^{-1}) at 20 °C.

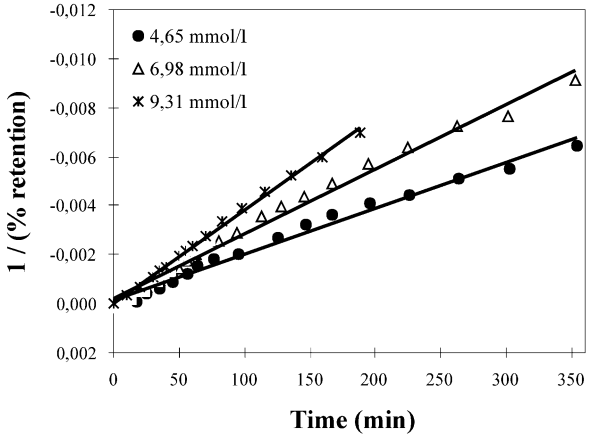

Fig. 5. Degradation of sour cherry anthocyanins at various H_2O_2 concentrations in the presence of added ascorbic acid (60 mg l^{-1}) at 20 °C.

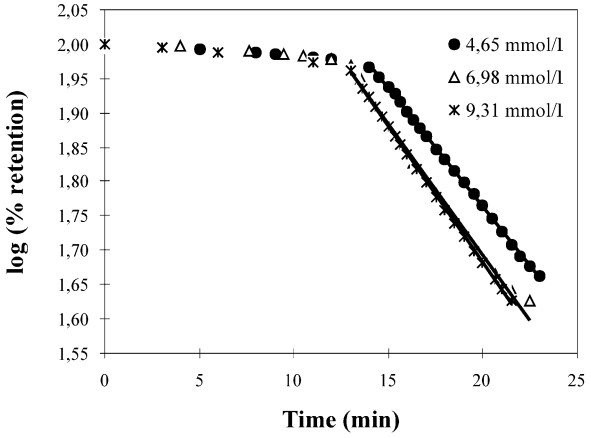

Fig. 6. Degradation of sour cherry anthocyanins at various H_2O_2 concentrations in the presence of added ascorbic acid (80 mg l^{-1}) at 20 °C.

kinetic, whereas first-order reaction kinetic was fitted after a lag period occurred at the 80 mg (Fig. 6) level.

The first-order reaction rates (k) and half-lives ($t_{1/2}$), i.e. the time needed for 50% degradation of anthocyanins at a given H_2O_2 concentration and temperature, were calculated by the following equations:

$$\ln (A/A_o) = -kt \quad (4)$$

$$t_{1/2} = \ln 0.5/k \quad (5)$$

where A_o is the initial absorbance of diluted fruit juice and A is the absorbance value after t min incubation at 20 °C.

3.2. Effect of H_2O_2 concentration

The anthocyanin loss occurred faster with increasing H_2O_2 concentrations from 4.65 to 9.31 mmol l^{-1} in both sour cherry (Fig. 1) and pomegranate (Fig. 2) juices at 20 °C. The k values (Table 1) varied between (1.7–2.9) × 10^{-3} min^{-1} and (2.1–4.0) × 10^{-3} min^{-1} at 20 °C in sour cherry and pomegranate juices, respectively. The $t_{1/2}$ values (Table 1) at the same H_2O_2 concentrations ranged from 418 to 243 and 324 to 173 min in sour cherry and pomegranate juices, respectively. The comparison of k and $t_{1/2}$ values indicated that the anthocyanins in sour cherry juice were more stable to H_2O_2 than those in pomegranate juice.

There are only a few studies on the degradation of anthocyanins by H_2O_2. Sondheimer and Kertesz (1952) reported that $t_{1/2}$ values for strawberry anthocyanins were 6, 9 and 13 min for 77.4, 10.7 and 2.42 mmol l^{-1} H_2O_2 concentrations at 20 °C, respectively. On the other hand, Özkan et al. (2000) found that the $t_{1/2}$ values for sour cherry anthocyanins were 111–20 h at 0.233–2.327 mmol l^{-1} H_2O_2 concentrations at 20 °C. Similarly, Özkan, Yemenicioğlu, Asefi, and Cemeroğlu (in press) compared the stabilities of anthocyanins from various fruit juices and found that sour cherry anthocyanins were the most resistant to H_2O_2, followed by pomegranate and strawberry anthocyanins. The different susceptibilities of fruit juice anthocyanins to H_2O_2 may be due to their varying anthocyanidin composition. The reported anthocyaninidins in pomegranate seed coats, in decreasing order, are cyanidin 3-glucoside, delphinidin 3-glucoside, cyanidin 3,5-diglucoside, delphinidin 3,5-diglucoside, pelargonidin 3-glucoside and pelargonidin 3,5-diglucoside (Du, Wang, & Francis, 1975; Hernández, Melgarejo, Tomás-Barberán, & Artés, 1999). The major anthocyanidins in sour cherries are cyanidin 3-glucosylrhamnosylglucoside, followed by cyanidin 3-rutinoside, cyanidin 3-glucoside and peonidin 3-rutinoside (Dekazos, 1970).

3.3. Effect of ascorbic acid concentration

Ascorbic acid, at 80 mg l^{-1}, markedly accelerated the degradation of anthocyanins in sour cherry juice samples

Table 1
k and $t_{1/2}$ values of anthocyanins from sour cherry and pomegranate juices at various H_2O_2 and ascorbic acid concentrations at 20 °C

H_2O_2 conc. (mmol l^{-1})	Ascorbic acid conc. (mg l^{-1})	$k \times 10^3$ (min^{-1})		$t_{1/2}$ (min^{-1})	
		Sour cherry	Pomegranate	Sour cherry	Pomegranate
4.65	0	1.66 (0.9934[a])	2.14 (0.9952)	418	324
6.98	0	2.49 (0.9873)	3.13 (0.9948)	279	221
9.31	0	2.86 (0.9924)	4.01 (0.9953)	243	173
4.65	60	0.019 (0.9858)	1.52 (0.9975)	>450	456
6.98	60	0.026 (0.9902)	2.14 (0.9986)	>450	324
9.31	60	0.038 (0.9977)	2.79 (0.9953)	>450	249
4.65	80	79.5 (0.9995)	1.66 (0.9949)	9	418
6.98	80	87.5 (0.9933)	2.40 (0.9977)	8	289
9.31	80	91.5 (0.9994)	3.11 (0.9977)	8	223

[a] Numbers in parentheses are the determination coefficients.

containing 4.65, 6.98 and 9.31 mmol l^{-1} H_2O_2 at 20 °C (Table 1). The degradation pattern was changed dramatically by raising ascorbic acid concentration from 60 (Fig. 5) to 80 mg l^{-1} in sour cherry juice (Fig. 6). The $t_{1/2}$ values (Table 1) at 60 mg ascorbic acid level for sour cherry anthocyanins were over 450 min for all the H_2O_2 concentrations studied, whereas the $t_{1/2}$ values decreased sharply, down to 8 min, at the 80 mg ascorbic acid level. Similarly, Freedman and Francis (1984) found that the anthocyanins in blackberry jelly showed little change during 32 weeks of storage at both zero and 35 mg ascorbic acid in 100 ml jelly. On the other hand, raising the ascorbic acid level to 70 mg resulted in a lighter coloured product, as indicated by the shift in the hue values from the "red" region to "yellow" region.

The accelerated degradation of anthocyanins in the presence of both H_2O_2 and ascorbic acid can be attributed to the degradation products of ascorbic acid. In fact, Sondheimer and Kertesz (1952) showed a maximum loss of strawberry anthocyanins under conditions most favourable to ascorbic acid degradation. This indicates that the degradation products of ascorbic acid, not ascorbic acid itself, are responsible for the anthocyanin degradation (Adams, 1973; Jackman, Yada, Tung, & Speers, 1987). Of the degradation products, dehydroascorbic acid, furfurals and H_2O_2 were thought to be responsible for the degradation of anthocyanins (Meschter, 1953). The combined presence of O_2 and ascorbic acid has also been demonstrated to have a synergistic effect on anthocyanin degradation (Markakis, Livingston & Fellers, 1957; Starr & Francis, 1968). Iversen (1999) found that the degradation rate of anthocyanins in black currant nectar was 3–4 times faster than that of ascorbic acid, depending on the storage conditions (dark or daylight). The authors concluded that the loss of anthocyanins is favoured for the protection of ascorbic acid and they attributed the ascorbic acid protecting effect of anthocyanins to the conversion of ascorbic acid radicals into ascorbic acid by oxidizing one molecule of anthocyanin to its stabilized radical form.

In pomegranate juice, the addition of ascorbic acid slowed down the rate of anthocyanin degradation at both 60 mg (Fig. 5) and 80 mg (Fig. 6) levels for all three H_2O_2 concentrations studied, as compared with pomegranate juice treated only with H_2O_2. However, increasing ascorbic acid level from 60 to 80 mg increased the anthocyanin loss. The $t_{1/2}$ values were between 456–249 and 418–223 min at 60 and 80 mg ascorbic acid levels, respectively. The comparison of $t_{1/2}$ values (Table 1) revealed that ascorbic acid added at the 60 mg level has a protective effect on anthocyanins, in both sour cherry and pomegranate juices.

The faster anthocyanin degradation, at 80 mg ascorbic acid per litre in sour cherry juice, may be attributed to the degradation of ascorbic acid by H_2O_2. At this ascorbic acid level, the ascorbic acid degradation products may have reached the critical concentration at which they degrade the anthocyanins at a rapid rate. At the 60 mg ascorbic acid level, not enough degradation products may have formed from the degradation of ascorbic acid by H_2O_2 and the ascorbic acid may have been used in the removal of H_2O_2, thereby protecting the anthocyanins from the degradation.

Moreover, the flavonols in sour cherry and pomegranate juices may have prevented the reaction between ascorbic acid and anthocyanins. The flavonols always accompany anthocyanins in fruit juices (Shrikhande & Francis, 1974) because reductive acylation of flavonols give rise to their corresponding anthocyanin pigments (Buckmire & Francis, 1976). Shrikhande and Francis (1974) showed the protective effects of flavonols on ascorbic acid and anthocyanins of cranberries in citrate buffer. The protective effect of flavonols has been attributed to their actions as free radical acceptors and complexors of metals (Shrikhande & Francis, 1974). Flavonols interfere in the free radical chain reaction in the autoxidation of ascorbic acid, thereby protecting the anthocyanins from the action of ascorbic acid degradation products. A similar protection of anthocyanins and ascorbic acid has been observed for the strawberry juice anthocyanins (pg-3-glucoside) in the presence of citrate and fluoride ions (Sondheimer & Kertesz, 1952), and thiourea (Sondheimer & Kertesz, 1953). The authors attributed this protection to the metal-complexing properties of these compounds. Jackman et al. (1987) also reported that the antioxidative activity of flavonols may be attributed to their action as copigments, by weakly binding to anthocyanins. Therefore the flavonols prevent complex formation between anthocyanins and ascorbic acid, thereby stabilizing both compounds.

The marked shift in the degradation pattern of sour cherry anthocyanins by raising ascorbic acid level from 60 to 80 mg l^{-1} may be due to the exhausting of antioxidative substances such as flavonols in sour cherry juice, thereby resulting in the oxidation of ascorbic acid by H_2O_2 with the formation of its breakdown products which, in turn, cause the degration of anthocyanins.

4. Conclusions

The results show that the anthocyanins from sour cherry and pomegranate juices were sensitive to hydrogen peroxide. Compared with pomegranate juice anthocyanins, sour cherry anthocyanins were more resistant to hydrogen peroxide. In the presence of added ascorbic acid, the degradation pattern of anthocyanins changed markedly in sour cherry anthocyanins containing 80 mg l^{-1} ascorbic acid. Ascorbic acid, at both 60 and 80 mg l^{-1}, seemed to increase the stability of anthocyanins in pomegranate juice. The fortification of sour cherry juice with ascorbic acid should be avoided.

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