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Degradation of various fruit juice anthocyanins by hydrogen peroxide

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Abstract

Degradation kinetics of anthocyanins was studied in sour cherry nectar, pomegranate and strawberry juices at high hydrogen peroxide (H_2O_2) concentrations $(9.31-27.92 \text{ mmol } 1^{-1})$ at $10-30 \,^{\circ}\text{C}$ and in only sour cherry nectar at low H_2O_2 concentrations $(0.23-2.33 \text{ mmol } 1^{-1})$ at $20 \,^{\circ}\text{C}$. Degradation of anthocyanins followed the first-order reaction kinetics. Sour cherry anthocyanins were the most resistant to H_2O_2 , followed by pomegranate and strawberry anthocyanins. Degradation of anthocyanins was also studied in sour cherry nectar and pomegranate juice in the presence of ascorbic acid at $60 \,^{\circ}$ and $80 \,^{\circ}$ mg 1^{-1} concentrations at $20 \,^{\circ}$ C. At $80 \,^{\circ}$ mg level, ascorbic acid significantly accelerated the degradation of anthocyanins in sour cherry nectar at 4.65, $6.98 \,^{\circ}$ md $9.31 \,^{\circ}$ mmol $1^{-1} \,^{\circ}$ H $_2O_2$ concentrations. In contrast, ascorbic acid at both $60 \,^{\circ}$ mg levels protected the anthocyanins from degradation by H_2O_2 in pomegranate juice.

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

1. Introduction

With FDA approval (1981), hydrogen peroxide (H₂O₂) has found widespread use as a packaging sterilant in aseptic processing systems (Nelson, 1993). The sterilization of the packaging material used in aseptic systems is carried out by either dipping the packaging material into H₂O₂ bath or spraying H₂O₂ onto the polyethylene food contact surfaces. Although the excessive H₂O₂ is removed form the food contact surfaces by pressure roller in combination with scrapers and subsequent drying with sterile hot air at 180–205 °C (Von Bockelmann & Von Bockelmann, 1986), residues left on the packaging material or vapors generated during drying may sometimes get trapped inside the package

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upon sealing (Toledo, 1986). H_2O_2 may also derive from the aerobic degradation of ascorbic acid (Davídek, Velísek, & Pokorny, 1990). Therefore, residues left inside packages, together with H_2O_2 formed from the degradation of ascorbic acid, may occasionally cause a quality loss in foods, especially fruit juices. The current FDA regulation limits residual H_2O_2 to 0.5 ppm in finished food packages (Code of Federal Regulations, 2000).

Anthocyanins and ascorbic acid are the two important components adversely affected by H_2O_2 in fruit juices. The deleterious effect of H_2O_2 on anthocyanins was demonstrated in strawberry juice (Sondheimer & Kertesz, 1952), and ascorbic acid in orange juice (Johnson & Toledo, 1975) and in orange, grape and pomegranate juices as well as sour cherry nectar (Özkan, Kırca, & Cemeroğlu, 2004). Sondheimer and Kertesz (1952) showed the extreme susceptibility of strawberry anthocyanins to H_2O_2 . Johnson and Toledo (1975) reported a 2-fold increase in the degradation rate of

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ascorbic acid when the aseptic chamber was pre-sterilized with H₂O₂ instead of steam.

Pomegranate and strawberry juices, and sour cherry nectar were selected in this study because of their high anthocyanin contents. In fact, anthocyanin contents were reported between 267 and 688 mg l⁻¹ for sour cherry juice (Erbaş & Cemeroğlu, 1992), $271-316 \text{ mg l}^{-1}$ for pomegranate juice (Bodur & Yurdagel, 1986; Cemeroğlu & Artık, 1990) and 176–445 mg l^{-1} for strawberry juice (Pilando, Wrolstad, & Heatherbell, 1985). In addition to its high anthocyanin content, sour cherry nectar is the most widely consumed anthocyanin containing fruit juice in Turkey and marketed as a clear nectar in carton-based laminated packages. The color loss in aseptically packaged sour cherry nectar has been brought to our attention by one of the major juice producers. Therefore, we conducted extensive research to show the adverse effects of H_2O_2 on the anthocyanins from sour cherry nectar, and pomegranate and strawberry juices (Ozkan, Yemenicioğlu, Çıtak, & Cemeroğlu, 2000; Özkan, Yemenicioğlu, Asefi, & Cemeroğlu, 2002). Anthocyanin degradation was also studied in sour cherry nectar and pomegranate juice in the presence of added ascorbic acid (Ozkan, 2002). These two juices were chosen because sour cherry (Herrmann, 1978) and pomegranate juices (Cemeroğlu, Artık, & Erbaş, 1992) contain insignificant amounts of ascorbic acid. This article summarizes the results from the studies conducted in our laboratory.

2. Materials and methods

2.1. Materials

Pomegranates (*Punica granatum* L.) and fully ripened strawberries (*Fragaria vesca* L.) were purchased from a local market in Ankara. Fruits were brought to the fruit juice plant at Food Engineering Department and washed in cold tap water. The outer skins of pomegranates were hand-peeled. The juicy sacs from the fruit pericarp were separated by hand and pressed on a rack and cloth press (Bucher–Guyer, Niederweningen, Switzerland). The extracted juice was kept frozen at -30 °C in glass bottles. Before use, the juice was clarified at 4 °C overnight only with gelatin (0.5%) and then filtered.

Strawberries were homogenized in a high speed blender. The homogenate was depectinized with the enzyme Pectinex Ultra SP-L (Novo Nordisk, Dittingen, Switzerland) at 50 °C for 2 h and filtered through seven layers of muslin cloth and then filter paper. The filtered juice was kept frozen at -30 °C in glass bottles until used for analysis. Sour cherry (*Prunus cerasus* L.) nectar was obtained in 200-ml bottles directly from the commercial production of fruit juice plant at Food Engineering Department.

2.2. 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 and filtered again prior to degradation studies. The absorption spectra were scanned from 350 to 700 nm with a Unicam UV2-100 spectrophotometer (Unicam, Cambridge, England), using 1-cm quartz cuvettes. The wavelengths of maximum absorptions were 499, 512 and 515 nm for strawberry, sour cherry and pomegranate anthocyanins, respectively. All absorbance readings were made against distilled water as a blank. The pH of diluted pomegranate, strawberry and sour cherry juices at 20 °C was 3.32, 3.40 and 3.50, respectively.

2.3. Degradation studies

The effects of various H₂O₂ concentrations on the anthocyanins of sour cherry, pomegranate and strawberry juices were studied at 10, 20 and 30 °C. The diluted juice samples were allowed to reach the required temperature in a Sanyo MIR 153 model refrigerated incubator (Sanyo, Gunma, Japan). Then, the predetermined volumes of diluted H₂O₂ solutions (prepared from 35% stock H₂O₂ solution) were added rapidly to the juice samples to obtain final H₂O₂ concentrations of 9.31, 13.96, 18.60, 23.27 and 27.92 mmol 1^{-1} . 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 H₂O₂. At 10-30 °C, the change in absorbance of the sample solution containing no H₂O₂ was insignificant over time. The anthocyanin retention for each time period was calculated as percentage of zero-time absorbance readings, taken as 100% retention. Hydrogen peroxide was purchased from Merck Co. (Darmstad, Germany).

The degradation of anthocyanins was also studied in only sour cherry nectar at low H_2O_2 concentrations (0.23–2.33 mmol l^{-1}) at 20 °C. Finally, degradation of anthocyanins was studied in the presence of 60 and 80 mg l^{-1} ascorbic acid at 4.65, 6.98 and 9.31 mmol l^{-1} H_2O_2 concentrations in sour cherry nectar and pomegranate juice.

3. Results and discussion

3.1. Degradation mechanism

The decomposition of H₂O₂ in an aqueous solution occurs in two ways: (1) dissociation and (2, 3) homolytic cleavage of O–H or O–O bonds. These reactions leads to the formation of highly reactive products: perhydroxyl anion (HOO⁻), and perhydroxyl ('OOH) and hydroxyl ('OH) radicals (De, Chaudhuri, & Bhattacharjee, 1999).

$$H_2O_2 \to H^+ + HOO^- \quad K = 1.55 \times 10^{-12}$$
 (1)

$$HOOH \rightarrow "OOH + "H$$
 (2)

$$HOOH \rightarrow 2^{\bullet}OH$$
 (3)

The decomposition and dissociation products of H₂O₂ have been shown to be responsible for the oxidation and subsequent degradation of phenolic compounds, including anthocyanins (Sapers & Simmons, 1998; Sapers, Miller, Choi, & Cooke, 1999). In fact, De et al. (1999) found that 'OH radical is the main reactive species to cleave the benzene ring in phenolic compounds and degrade the substrate eventually into CO₂ and H₂O. Von Elbe and Schwartz (1996) reported that quinones, formed by the oxidation of phenols, also have deleterious effects on anthocyanins. 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 the decomposition and dissociation of H_2O_2 , respectively; (2) the amount of quinones formed by the H₂O₂-catalyzed oxidation of phenolic compounds.

Sondheimer and Kertesz (1952) were among the first to investigate the kinetics of anthocyanin degradation by H_2O_2 in both strawberry juice and pure solutions of the major strawberry anthocyanin (pelargonidine-3-glucoside). According to these workers, the oxidative degradation of anthocyanins occurs in two steps: an initial reversible reaction with the formation of anthocyanin- H_2O_2 adduct, followed by a slower irreversible one. H_2O_2 or its decomposition products cleaves the neighboring carbon–carbon bond at the C-2 and C-3 positions of anthocyanins to form colorless malvones (Coultate, 1989).

3.2. Degradation kinetics

The degradation of anthocyanins by H_2O_2 was fitted to a first-order kinetic model (Fig. 1). The reaction rate constant (k) and half-life $(t_{1/2})$, the time needed for 50% degradation of anthocyanins at a given H_2O_2 concentration and temperature, were calculated by the following equations:

$$\ln(A_t/A_0) = -k \times t,\tag{4}$$

$$t_{1/2} = -\ln 0.5/k,\tag{5}$$

where A_0 is the initial absorbance of diluted fruit juice and A_t is the absorbance value after t min incubation at a given temperature.

The effect of H_2O_2 on anthocyanins was studied at both high H_2O_2 concentrations (9.31–27.92 mmol l⁻¹) at 10–30 °C and low H_2O_2 concentrations (0.23–2.33 mmol l⁻¹) at 20 °C. At high H_2O_2 concentrations, strawberry anthocyanins showed the highest susceptibility to H_2O_2 , followed by pomegranate and sour cherry

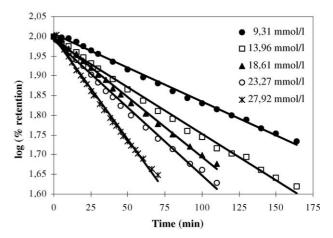


Fig. 1. Degradation of pomegranate juice anthocyanins at various H_2O_2 concentrations and 20 °C.

anthocyanins, as indicated by the highest k and the lowest $t_{1/2}$ values (Table 1). Sondheimer and Kertesz (1952) reported the $t_{1/2}$ values for anthocyanins in strawberry juice as 6, 9 and 13 min for 77.4, 10.7 and 2.4 mmol l⁻¹ H_2O_2 concentrations at 20 °C, respectively. Compared to our $t_{1/2}$ values for strawberry anthocyanins, their $t_{1/2}$ values were much lower. These results clearly indicate that, in the production of anthocyanin-rich fruit juices which have higher susceptibility to H_2O_2 , the removal of H_2O_2 from juice contact surfaces of aseptic packages should be controlled very carefully to minimize anthocyanin losses.

The different susceptibilities of anthocyanins to H_2O_2 were also reported by Sapers and Simmons (1998). These workers observed much faster bleaching of raspberry and strawberry anthocyanins by H_2O_2 , as compared to sweet cherry anthocyanins. Although our results correlate well with those of Sapers and Simmons (1998) who applied H_2O_2 for surface sterilization of these fruits, while we added H_2O_2 directly to the fruit juices where H_2O_2 and anthocyanins reacted easily. The protective skins of cherries probably prevented H_2O_2 to diffuse freely to the interior of fruits.

The high reactivity to H₂O₂ suggests the greater antioxidant potential of strawberry juice anthocyanins against reactive oxygen species (ROS). The different susceptibilities of fruit juice anthocyanins to H₂O₂ may be due to their varying anthocyanidin composition. The cyanidins were reported as the main anthocyanidins in pomegranate seed coats (Du, Wang, & Francis, 1975) and sour cherries (Dekazos, 1970), whereas the pelargonidins were reported as the major ones in strawberries (Fuleki, 1969). Thus, higher resistance of sour cherry and pomegranate juice anthocyanins to H₂O₂ may be attributed to the presence of cyanidins in these juices. Moreover, the quinones, formed by the H₂O₂-catalyzed oxidation of phenolic compounds, can also contribute to the degradation of anthocyanins. Therefore, the

Table 1 Effect of temperature on the degradation of sour cherry, pomegranate and strawberry anthocyanins at various H_2O_2 concentrations

H ₂ O ₂ concentration (mmol l ⁻¹)	Temperature (°C)	Sour cherry		Pomegranate		Strawberry	
		$k^{\rm a} (10^3 \times \rm min^{-1})$	t _{1/2} (h)	$k^{\rm a} (10^3 \times \rm min^{-1})$	t _{1/2} (h)	$k^{\rm a} (10^3 \times \rm min^{-1})$	t _{1/2} (h)
9.31	10	1.54	7.5	1.80	6.4	2.37	4.9
	20	2.86	4.0	3.92	2.9	4.08	2.8
	30	5.02	2.3	7.14	1.6	7.19	1.6
13.96	10	2.49	4.6	2.42	4.8	3.11	3.7
	20	4.49	2.6	5.46	2.1	5.71	2.0
	30	7.97	1.4	9.72	1.2	9.90	1.2
8.61	10	2.72	4.2	3.62	3.2	3.94	2.9
	20	5.57	2.1	7.00	1.7	7.85	1.5
	30	10.36	1.1	14.46	0.8	14.07	0.8
23.27	10	3.96	2.9	4.38	2.6	4.97	2.3
	20	6.96	1.7	7.88	1.5	9.70	1.2
	30	13.50	0.9	16.72	0.7	18.40	0.6
27.92	10	5.00	2.3	5.09	2.3	5.50	2.1
	20	9.40	1.2	12.02	1.0	12.23	0.9
	30	15.25	0.8	21.19	0.5	19.46	0.6

^a Determination coefficients for k values are over 0.99.

differences in the amount and composition of phenolics in sour cherry, pomegranate and strawberry juices could also have affected the degradation rate of anthocyanins. Furthermore, the high amount of ascorbic acid in strawberry juice may have also contributed to the degradation of anthocyanins. In fact, the adverse effects of ascorbic acid on anthocyanins have been shown in strawberry juice (Sondheimer & Kertesz, 1953) and model systems containing strawberry anthocyanins (Markakis, Livingston, & Fellers, 1957). Contrary to strawberry juice, both sour cherry (Herrmann, 1978) and pomegranate juices (Cemeroğlu et al., 1992) contain insignificant amounts of ascorbic acid.

The effect of low H_2O_2 concentrations (0.23–2.33 mmol l⁻¹) on anthocyanin degradation was studied only in sour cherry nectar at 20 °C. At these H_2O_2 concentrations, the $t_{1/2}$ values varied between 111 and 20 h, respectively (Table 2). A quadratic relationship was obtained between k and H_2O_2 concentrations and the equation for this relationship is expressed as

Table 2 Effect of different H_2O_2 concentrations on the degradation of sour cherry anthocyanins at 20 °C

H_2O_2 concentration (mmol l^{-1})	$k\ (10^3 \times \mathrm{h}^{-1})$	t _{1/2} (h)
0.23	6.22 (0.982) ^a	111
0.47	10.59 (0.975)	65
0.70	15.56 (0.969)	45
0.93	18.09 (0.981)	38
1.16	20.96 (0.990)	33
2.33	34.81 (0.982)	20

^a Number in brackets is the determination coefficient.

$$k = -0.0031[H_2O_2]^2 + 0.0218[H_2O_2] + 0.0008$$

($R^2 = 0.996$).

If one places $0.0147 \text{ mmol } 1^{-1}$ (0.5 ppm) in the above equation, which is the maximum allowed level of H_2O_2 in the finished food packages by FDA, a k of $1.12 \times 10^{-3} \text{ h}^{-1}$ and $t_{1/2}$ of 26 days at 20 °C are calculated. Such values indicate that fruit juice anthocyanins may be degraded even at very low H_2O_2 concentrations.

3.3. Effect of ascorbic acid concentration

In sour cherry nectar and pomegranate juice, the stability of anthocyanins to H₂O₂ (4.65, 6.98 and 9.31 mmol 1^{-1}) was also studied at 60 and 80 mg 1^{-1} ascorbic acid concentrations at 20 °C. The stability of anthocyanins was higher in samples containing both ascorbic acid (60 mg l⁻¹) and H₂O₂ than in samples containing only H₂O₂. However, ascorbic acid at 80 mg level markedly increased the degradation of anthocyanins in sour cherry nectar samples in the presence of H₂O₂ (Fig. 2). For example, the $t_{1/2}$ values at 60 mg ascorbic acid level for sour cherry anthocyanins were over 450 min for all three H₂O₂ concentrations studied, whereas the $t_{1/2}$ values decreased sharply, down to 8 min, at 80 mg level (Table 3). Similarly, Freedman and Francis (1984) found that the anthocyanins in blackberry jelly showed little change during 32 weeks of storage at both 0 and 35 mg ascorbic acid in 100 ml jelly. On the contrary, raising ascorbic acid level to 70 mg resulted in a lighter colored product, as indicated by the shift in the hue values from "red" region to "yellow" region.

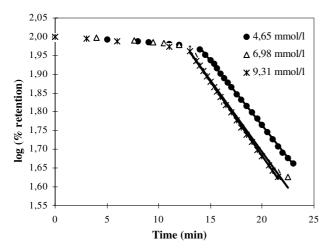


Fig. 2. Degradation of sour cherry anthocyanins at various H_2O_2 concentrations in the presence of added ascorbic acid (80 mg l⁻¹) at 20 °C.

The accelerated degradation of anthocyanins in the presence of both H₂O₂ and ascorbic acid can be attributed to the degradation products of ascorbic acid. In fact, Sondheimer and Kertesz (1953) showed the maximum loss of strawberry anthocyanins under conditions most favorable to ascorbic acid degradation. This indicates that the degradation products of ascorbic acid, not ascorbic acid itself, are responsible for the anthocyanin degradation (Jackman, Yada, Tung, & Speers, 1987). Of the degradation products, dehydroascorbic acid, furfurals and H₂O₂ were thought to be responsible for the degradation of anthocyanins (Meschter, 1953). The combined presence of O₂ and ascorbic acid has also been demonstrated to have a synergistic effect on anthocyanin degradation (Markakis et al., 1957). 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 in dark or daylight, respectively. The authors concluded that the loss of anthocyanins is favored 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 anthocyanins to its stabilized radical form.

3.4. Temperature dependence

The temperature dependence of anthocyanin degradation by H_2O_2 was determined by calculating activation energies (E_a) and temperature quotients (Q_{10}) from the following equations:

$$k = k_0 \times e^{-E_a/RT},\tag{6}$$

$$Q_{10} = k_{(T+10)}/k_{(T)}. (7)$$

At 9.31–27.92 mmol 1^{-1} H₂O₂ concentrations and 10–30 °C, the E_a values varied between 39.3 and 46.4 kJ mol⁻¹ for strawberry, 39.7–47.7 kJ mol⁻¹ for sour cherry and 47.7–51.0 kJ mol⁻¹ for pomegranate anthocyanins (Özkan et al., 2002). At the same concentration and temperature ranges, the respective Q_{10} values were between 1.59–2.22, 1.62–2.05 and 1.76–2.36 for strawberry, sour cherry and pomegranate anthocyanins, respectively. Higher E_a and Q_{10} values indicated the higher temperature dependence for the degradation of pomegranate juice anthocyanins by H₂O₂.

In long-term storage, the cold storage temperatures prevent the degradation of anthocyanins. In fact, Cemeroğlu, Velioğlu, and Işık (1994) showed that storing sour cherry concentrate at 5 °C rather than 20 °C resulted in an almost 10-fold increase in the $t_{1/2}$ values of sour cherry anthocyanins, 38–356 days. Similarly, $t_{1/2}$ values of anthocyanins for aseptically packed cranberry juice cocktail were reported to be 210 days at -18 °C, 112 days at 21 °C and 86 days at 36 °C (Toledo, 1986). The effect of storage temperature was found to be more pronounced for the anthocyanins of aseptically packaged blueberry juice, which has a Q_{10} value of 2.4 at 25–38 °C as compared to cranberry juice anthocyanins with a Q_{10} value of 1.2 at 21–36 °C (Toledo, 1986).

The plots of k vs H_2O_2 concentrations at different temperatures showed that anthocyanin degradation increased as the temperature and H_2O_2 concentration increased in all three samples (Özkan et al., 2002). Thus,

Table 3
Effect of H₂O₂ and ascorbic acid concentrations on anthocyanins from sour cherry nectar and pomegranate juice at 20 °C

H ₂ O ₂ concentration (mmol l ⁻¹)	Ascorbic acid concentration	$k^{\rm a} (10^3 \times {\rm min}^{-1})$		t _{1/2} (min)	
	(mg l^{-1})	Sour cherry	Pomegranate	Sour cherry	Pomegranate
4.65	0	1.66	2.14	418	324
6.98	0	2.49	3.13	279	221
9.31	0	2.86	4.01	243	173
4.65	60	0.019	1.52	450	456
6.98	60	0.026	2.14	450	324
9.31	60	0.038	2.79	450	249
4.65	80	79.5	1.66	9	418
6.98	80	87.5	2.40	8	289
9.31	80	91.5	3.11	8	223

^a Determination coefficients for k values are over 0.99.

greater anthocyanin losses should be expected as residual H₂O₂ concentration and storage temperature increase in aseptically packaged fruit juices.

4. Conclusions

Anthocyanins from various fruit juices were found to be very susceptible to H₂O₂. A rapid degradation may occur even at H₂O₂ concentrations as low as FDA limit. Therefore, aseptic systems should be frequently controlled to ensure the effective removal of residual H₂O₂ from the food contact surfaces. Since the rate of anthocyanin degradation by H₂O₂ is highly dependent on temperature, cold storage of aseptically packed anthocyanin-rich fruit juices is strongly recommended to minimize anthocyanin degradation by residual H₂O₂ and temperature. Compared to pomegranate and sour cherry anthocyanins, strawberry anthocyanins were much more susceptible to H_2O_2 . At 60 mg l^{-1} ascorbic acid, the susceptibility of pomegranate and sour cherry anthocyanins to H₂O₂ reduced. However, the degradation of anthocyanins in sour cherry nectar markedly increased at 80 mg ascorbic acid level. Therefore, the fortification of aseptically packed anthocyanin-rich fruit juices with ascorbic acid should be avoided or carried out very carefully.

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