

Degradation Kinetics of Anthocyanins from Sour Cherry, Pomegranate, and Strawberry Juices by Hydrogen Peroxide

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ABSTRACT: Degradations were studied at different hydrogen peroxide (H_2O_2) concentrations (9.31 to 27.92 mmol. L^{-1}) over a range of 10 ° to 30 °C. Degradation of anthocyanins by H_2O_2 was described by first-order function. Comparison of $t_{1/2}$ values revealed that sour cherry anthocyanins were the most resistant to H_2O_2 , followed by pomegranate and strawberry anthocyanins. Thus, the removal of residual H_2O_2 from the juice contact surfaces of aseptically packaged strawberry juices should be controlled more carefully to prevent anthocyanin degradation. Respective E_a values were between 9.4 to 11.1, 9.5 to 11.4, and 11.4 to 12.2 kcal.mol⁻¹; and Q_{10} values between 1.59 to 2.22, 1.62 to 2.05, and 1.76 to 2.36 for strawberry, sour cherry, and pomegranate anthocyanins.

Keywords: anthocyanins, hydrogen peroxide, degradation, kinetics, fruit juices

Introduction

HYDROGEN PEROXIDE (H_2O_2) HAS BEEN USED IN FOODS AND food-packaging materials for various purposes in many European countries for over 30 years (Andres 1981; Toledo 1986). In the United States, the FDA has approved H_2O_2 for the sterilization of polyethylene food-contact surfaces only after February 1981 (Nelson 1993). From this date on, H_2O_2 has been the choice of chemical sterilant for treating plastic packaging materials used in aseptic processing systems (Tillotson 1984; Wang and Toledo 1986; Kunz and Binnig 1987; Mitchell 1988).

Aside from its common use as a packaging sterilant, H_2O_2 has been recommended for the surface disinfection of fruits and vegetables as an alternative to chlorine (Fallik and others 1994; Sapers and Simmons 1998). Hydrogen peroxide has also been used to eliminate certain chemical residues from various foods. Altug and others (1990) used H_2O_2 to reduce aflatoxins in figs, and Sreedhara and Subramanian (1991) and Clavero and others (1993) in groundnuts (peanuts). McFeeters (1998) successfully applied H_2O_2 to remove sulfites from fresh cucumbers. FDA classifies H_2O_2 as a food additive generally recognized as safe (GRAS) and requires that residual H_2O_2 be removed by appropriate physical and chemical means during processing (Code of Federal Regulations 2000a).

When H_2O_2 is used as a packaging sterilant in aseptic processing, the excessive H_2O_2 is removed from the food contact surfaces by pressure roller in combination with scrapers and subsequent drying with sterile hot air at 180 ° to 205 °C (von Bockelmann and von Bockelmann 1986). FDA regulations currently limit residual H_2O_2 to 0.5 ppm, leached into distilled water, in finished food packages (Code of Federal Regulations 2000b). However, during the sterilization of aseptic chambers or packaging materials with H_2O_2 , residues left on the packaging material or vapors generated during drying may get trapped inside the package upon sealing (Stannard and Wood 1983; Toledo 1986). Residues left inside packages may occasionally be over the legal limit. H_2O_2 may also derive from the aerobic degradation of ascorbic acid

(Sondheimer and Kertesz 1952; Adams 1973; Davidek and others 1990).

The deleterious effect of H_2O_2 on anthocyanins and ascorbic acid in fruit juices is well-known. The degradation of anthocyanins by H_2O_2 has been demonstrated in strawberry (Sondheimer and Kertesz 1952) and sour cherry juices (Özkan and others 2000). Johnson and Toledo (1975) reported that the half-life of ascorbic acid in orange juice concentrate at 24 °C was only 21 d when the aseptic chamber was presterilized with H_2O_2 and 42 d when presterilized with steam.

The characteristic bright red color of pomegranate, sour cherry, and strawberry juices is due to their rich anthocyanin contents which vary between 271 to 316 mg.L⁻¹ (Bodur and Yurdagel 1986; Cemeroglu and Artik 1990), 267 to 688 mg.L⁻¹ (Erbas and Cemeroglu 1992), and 176 - 445 mg.L⁻¹ (Pilando and others 1985), respectively. The color loss in aseptically packaged sour cherry juice has been brought to our attention by one of the major juice producers. Thus, we have recently conducted a study on the susceptibility of sour cherry anthocyanins to H_2O_2 and found that a rapid degradation of sour cherry anthocyanins may occur even at H_2O_2 concentrations as low as the FDA limit and the degradation rate is highly dependent on temperature (Özkan and others 2000). No published data have been found in the literature to compare the effects of H_2O_2 on the degradation of anthocyanins from various fruit juices under the same experimental conditions. Therefore, this study was conducted to show whether there are any differences in the susceptibilities of anthocyanins to H_2O_2 in sour cherry, pomegranate, and strawberry juices.

Materials and Methods

Materials

Sour cherries (*Prunus cerasus L.*) were obtained from the Çubuk region of Ankara and brought to the fruit juice pilot plant of the University's Dept. 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 rack (Bucher-Gyer, Niederweningen, Switzerland) and cloth press. The juice was depectinized (Pectinex Ultra SP-L; Novo Nordisk, Dittingen, Switzerland), clarified, and filtered. The filtered juice was then pasteurized in a plate heat exchanger at 90 °C, hot-filled into glass bottles, and stored at room temperature after cooling in the dark.

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 and pressed on the same rack and cloth press. The extracted juice was kept frozen at -30 °C in glass bottles. Before use, the juice was clarified with gelatin at 4 °C overnight and then filtered.

Fully ripened strawberries (*Fragaria ananasia L.*) were purchased from a local market in Ankara. Fruits were washed in cold tap water and homogenized in a high-speed Osterizer (Sunbeam, Hattiesburg, U.S.A.) blender. The homogenate was depectinized and filtered through 7 layers of muslin cloth and then filter paper. The juice was kept frozen at -30 °C in glass bottles until used for analysis.

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 the degradation studies. These absorbance values were achieved by diluting 100 mL of sour cherry, strawberry, and pomegranate juices with 200, 300, and 350 mL distilled water, respectively. The absorption spectra were scanned from 350 to 700 nm. 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. Spectrophotometric measurements were carried out using a Unicam UV2-100 spectrophotometer (Unicam, Cambridge, England).

Degradation studies

The effects of various H₂O₂ concentrations on the antho-

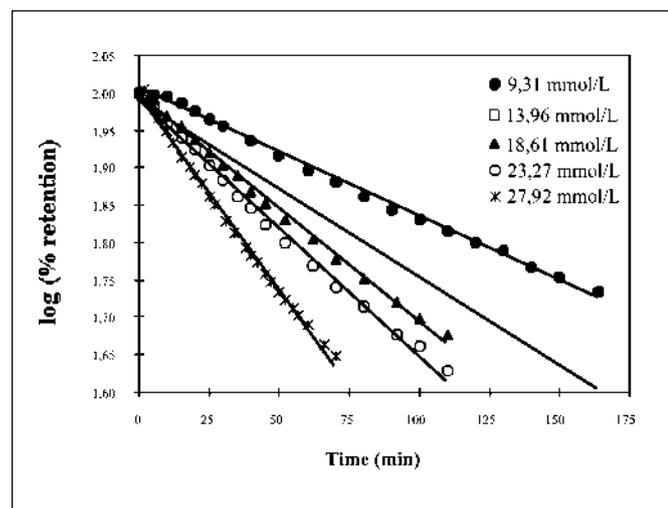


Figure 1—Degradation of pomegranate juice anthocyanins at various H₂O₂ concentrations and 20 °C.

cyanins 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 (Sanyo, Gunma, Japan) refrigerated incubator. Then the predetermined amounts 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, 18.6, 23.27, and 27.92 mmol.L⁻¹. The high H₂O₂ concentrations were selected to compare easily the anthocyanin degradation rates from these 3 fruit juices.

The absorbance of the sample solutions was measured periodically. Depending on temperature and concentration, the absorbance values were recorded at 15 min time intervals at 10 ° and 20 °C and 5 min at 30 °C for sour cherry juice anthocyanins; 10 min at 10 °C and 5 min at 20 ° and 30 °C for pomegranate juice anthocyanins; and 5 min at 10 °C and 3 min at 20 ° and 30 °C for strawberry anthocyanins. The zero-time absorbance values were determined by preparing the samples with the same amount of distilled water instead of H₂O₂. At 10 ° to 30 °C, the change in absorbance of the sample solution containing no H₂O₂ is insignificant over time. The anthocyanin retention for each time period was calculated as percentage of zero-time absorbance readings, taken as 100% retention.

Results and Discussion

Degradation mechanism of anthocyanins by H₂O₂

In an aqueous solution, H₂O₂ can easily decompose to form very active products: the perhydroxyl anion (HOO⁻), and the hydroxyl (*OH) and perhydroxyl (*OOH) radicals. The dissociation (1) and homolytic cleavage of O-H or O-O bonds (2, 3) of H₂O₂ were summarized by De and others (1999), as follows:

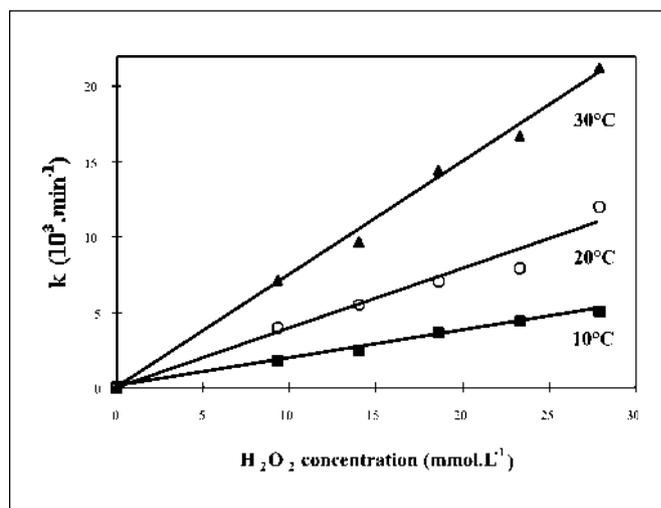


Figure 2—Effect of temperature on the degradation rate constants of pomegranate juice anthocyanins at various H₂O₂ concentrations.

Table 1—Effect of H₂O₂ concentration and temperature on the degradation of strawberry anthocyanins

H ₂ O ₂ conc. (mmol.L ⁻¹)	Temp. (°C)	k (10 ³ .min ⁻¹)	E _a (kcal.mol ⁻¹)	k _o (10 ⁵ .min ⁻¹)	Q ₁₀ 10 to 20 °C	Q ₁₀ 20 to 30 °C	t _{1/2} (h)
9.31	10	2.37 (0.996) ^a	9.4	0.5	1.72	1.76	4.9
	20	4.08 (0.999)					2.8
	30	7.19 (0.996)					1.6
13.96	10	3.11 (0.996) ^a	9.9	1.3	1.84	1.73	3.7
	20	5.71 (0.999)					2.0
	30	9.90 (0.998)					1.2
18.61	10	3.94 (0.997) ^a	10.9	9.6	1.99	1.79	2.9
	20	7.85 (0.999)					1.5
	30	14.07 (0.996)					0.8
23.27	10	4.97 (0.999) ^a	11.1	19.9	1.95	1.90	2.3
	20	9.70 (0.999)					1.2
	30	18.40 (0.999)					0.6
27.92	10	5.50 (0.997) ^a	10.8	12.3	2.22	1.59	2.1
	20	12.23 (0.999)					0.9
	30	19.46 (0.996)					0.6

^aNumbers in parentheses are the determination coefficients.

Table 2—Effect of H₂O₂ concentration temperature on the degradation of pomegranate anthocyanins

H ₂ O ₂ conc. (mmol.L ⁻¹)	Temp. (°C)	k (10 ³ .min ⁻¹)	E _a (kcal.mol ⁻¹)	k _o (10 ⁶ .min ⁻¹)	Q ₁₀ 10 to 20 °C	Q ₁₀ 20 to 30 °C	t _{1/2} (h)
9.31	10	1.80 (0.996) ^a	11.8	2.3	2.18	1.82	6.4
	20	3.92 (0.997)					2.9
	30	7.14 (0.996)					1.6
13.96	10	2.42 (0.993) ^a	11.9	3.7	2.26	1.78	4.8
	20	5.46 (0.994)					2.1
	30	9.72 (0.990)					1.2
18.61	10	3.62 (0.999) ^a	11.8	4.6	1.93	2.07	3.2
	20	7.00 (0.997)					1.7
	30	14.46 (0.997)					0.8
23.27	10	4.38 (0.998) ^a	11.4	2.7	1.80	2.12	2.6
	20	7.88 (0.995)					1.5
	30	16.72 (0.996)					0.7
27.92	10	5.09 (0.996) ^a	12.2	13.4	2.36	1.76	2.3
	20	12.02 (0.995)					1.0
	30	21.19 (0.995)					0.5

^aNumbers in parentheses are the determination coefficients.

Table 3—Effect of H₂O₂ concentration temperature on the degradation of sour cherry anthocyanins

H ₂ O ₂ conc. (mmol.L ⁻¹)	Temp. (°C)	k (10 ³ .min ⁻¹)	E _a (kcal.mol ⁻¹)	k _o (10 ⁵ .min ⁻¹)	Q ₁₀ 10 to 20 °C	Q ₁₀ 20 to 30 °C	t _{1/2} (h)
9.31	10	1.54 (0.995) ^a	10.1	0.9	1.86	1.76	7.5
	20	2.86 (0.992)					4.0
	30	5.02 (0.994)					2.3
13.96	10	2.49 (0.997) ^a	9.9	1.1	1.80	1.76	4.6
	20	4.49 (0.983)					2.6
	30	7.97 (0.991)					1.4
18.61	10	2.72 (0.992) ^a	11.4	17.7	2.05	1.86	4.2
	20	5.57 (0.991)					2.1
	30	10.36 (0.990)					1.1
23.27	10	3.96 (0.998) ^a	10.4	4.4	1.76	1.94	2.9
	20	6.96 (0.975)					1.7
	30	13.50 (0.990)					0.9
27.92	10	5.00 (0.996) ^a	9.5	11.3	1.88	1.62	2.3
	20	9.40 (0.991)					1.2
	30	15.25 (0.990)					0.8

^aNumbers in parentheses are the determination coefficients.

The susceptibility of anthocyanins to H₂O₂ has been known for a long time. Sondheimer and Kertesz (1952) were among the first to investigate the kinetics of anthocyanin degradation by H₂O₂ in both strawberry juice and pure solutions of the major strawberry anthocyanin (pg-3-glucoside). According to these workers, the oxidative degradation of anthocyanins occurs in 2 steps: an initial reversible reaction with the formation of anthocyanin-H₂O₂ adduct, followed

by a slower irreversible one.

The decomposition and dissociation products of H₂O₂ have been shown to be responsible for the oxidation and subsequent degradation of phenolic compounds (Sapers and Simmons 1998; Sapers and others 1999). In fact, De and others (1999) found that *OH radical is the main reactive species to cleave the benzene ring in phenolic compounds and degrade the substrate 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, 2 factors can primarily affect the degradation of anthocyanins by H_2O_2 in fruit juices which generally contain copious amounts of phenolic compounds: (a) the amount of free radicals and HOO^- anion formed by the decomposition and dissociation of H_2O_2 , respectively; and (b) the amount of quinones formed by the H_2O_2 -catalyzed oxidation of phenolic compounds.

Degradation kinetics

The effect of 9.31 to 27.92 mmol.L⁻¹ H_2O_2 concentrations on anthocyanins from sour cherry, strawberry, and pomegranate juices was studied at 10 ° to 30 °C. The degradation process was fitted to a first-order kinetic model (Figure 1). It was assumed that the H_2O_2 concentration was constant at the beginning of reaction. The reaction rate constants (*k*) and half-lives ($t_{1/2}$), the time needed for 50% degradation of anthocyanins at a given H_2O_2 concentration and temperature, were calculated by the following equations:

$$\ln(A_t / A_0) = -kt \quad (4)$$

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

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.

Between 9.31 to 27.92 mmol.L⁻¹ H_2O_2 concentrations at 10 to 30 °C, the $t_{1/2}$ values for sour cherry anthocyanins were higher than those for pomegranate and strawberry anthocyanins, respectively (Table 1, 2, and 3). 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. Therefore, 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. Moreover, the susceptibilities of anthocyanins to H_2O_2 may also depend on fruit cultivars.

The different susceptibilities of fruit juice anthocyanins to H_2O_2 may be due to their varying anthocyanidin composition. The cyanidins were reported as the main anthocyanidins in pomegranate seed coats (Du and others 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 H_2O_2 may be attributed to the presence of cyanidins in these juices. Moreover, the quinones, formed by the H_2O_2 -catalyzed oxidation of phenolic compounds, can also contribute to the degradation of anthocyanins. Therefore, the 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. The adverse effects of ascorbic acid on anthocyanins have been shown in strawberry juice (Sondheimer and Kertesz 1952, 1953; Meschter 1953), cranberry juice (Starr and Francis 1968), and black currant nectar (Iversen 1999). Contrary to strawberry juice, both sour cherry (Herrmann 1978) and pomegranate juices (Cemeroglu and others 1992) contain insignificant amounts of

ascorbic acid.

The different susceptibilities of anthocyanins to H_2O_2 were also reported by Sapers and Simmons (1998) who observed the rapid bleaching of raspberry and strawberry anthocyanins by H_2O_2 , whereas they observed higher resistance of sweet cherry anthocyanins to H_2O_2 . Although our results correlate well with those of Sapers and Simmons (1998), these workers 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 may have prevented H_2O_2 to diffuse freely to the interior of fruits. Forney and others (1991) also showed that the treatment of "Red Globe" grapes with H_2O_2 vapor did not affect the grape color. Thus, H_2O_2 can still be used in the surface sterilization of anthocyanin-rich fruits with protective skins.

Temperature dependence

The temperature dependence of the H_2O_2 -catalyzed degradation of various fruit juice anthocyanins was compared by calculating activation energies (E_a) and temperature quotients (Q_{10}) at 10 ° to 30 °C (Table 1, 2, 3) from the following equations:

$$k = k_0 \cdot e^{-E_a/RT} \quad (6)$$

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

Between 9.31 to 27.92 mmol.L⁻¹ H_2O_2 concentrations at 10 ° to 30 °C, the E_a values varied between 9.4 to 11.1 kcal.mol⁻¹ for strawberry, 9.5 to 11.4 kcal.mol⁻¹ for sour cherry, and 11.4 to 12.2 kcal.mol⁻¹ for pomegranate anthocyanins. The Q_{10} values at the same concentration and temperature ranges were between 1.59 to 2.22, 1.62 to 2.05, and 1.76 to 2.36 for strawberry, sour cherry, and pomegranate anthocyanins, respectively. These values clearly indicated the higher temperature dependence for the degradation of pomegranate juice anthocyanins by H_2O_2 .

In long-term storage, the low storage temperatures prevent the degradation of anthocyanins. In fact, Cemeroglu and others (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 to 356 d. Similarly, $t_{1/2}$ values of anthocyanins for aseptically packed cranberry juice cocktail were reported to be 210 d at -18 °C, 112 d at 21 °C, and 86 d 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 ° to 38 °C as compared to cranberry juice anthocyanins with a Q_{10} value of 1.2 at 21 ° to 36 °C (Toledo 1986).

The *k* as compared with H_2O_2 concentrations at different temperatures were also plotted to show the effect of H_2O_2 concentration and temperature on the degradation of pomegranate anthocyanins. As seen in Figure 2, the effect of temperature on the degradation rates of pomegranate juice anthocyanins was more pronounced at higher H_2O_2 concentrations. Similar degradation patterns have also been found for strawberry and sour cherry juice anthocyanins. Thus, greater anthocyanin losses should be expected as residual H_2O_2 concentration and storage temperature increase in aseptically packaged fruit juices.

Conclusion

COLD STORAGE OF ASEPTICALLY PACKED ANTHOCYANIN-RICH fruit juices is strongly recommended to minimize antho-

cyanin degradation by residual H₂O₂ and temperature. Compared to pomegranate and sour cherry juice anthocyanins, strawberry juice anthocyanins were much more susceptible to H₂O₂. Therefore, the removal of H₂O₂ from the packages of aseptically processed strawberry juice should be carefully controlled.

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