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Effects of hydrogen peroxide on the stability of ascorbic acid during storage in various fruit juices

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Abstract

Ascorbic acid degradation in orange, grape and pomegranate juices, and sour cherry nectar was studied at 20, 30 and 40 °C, with or without the addition of hydrogen peroxide (H_2O_2). Analysis of kinetic data suggested that the degradation fitted better to a zero-order model than a first-order model. Rate constants increased slightly in the presence of 0.5 ppm H_2O_2. However, increasing H_2O_2 concentration from 0.5 to 5 ppm caused a substantial increase in the degradation rates of ascorbic acid. Anthocyanins markedly accelerated the degradation of ascorbic acid in sour cherry nectar and pomegranate juice, especially at 5 ppm H_2O_2 concentration. Degradation was slowest in orange juice, with or without the addition of H_2O_2. Activation energies were lowest for grape juice (26.2 kJ mol^{-1}) and highest for pomegranate juice (71.0 kJ mol^{-1}) in the presence of 0.5 ppm H_2O_2.

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

1. Introduction

Ascorbic acid and its derivatives are used in many foods for various purposes. They are added to foods, including fruit juices, to improve the nutritional quality and to prevent enzymatic browning reactions (Freedman & Francis, 1984; Starr & Francis, 1968). Ascorbic acid has a potent antioxidant capacity by acting as a singlet oxygen quencher (Elliott, 1999). Aside from these applications, 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 hydrogen peroxide (H_2O_2) residues from H_2O_2-treated fruits and vegetables.

Ascorbic acid is also used as an index of the nutrient quality of fruit and vegetable products. This is because, as compared to other nutrients, it is much more sensitive to various modes of degradation in food processing and subsequent storage. Therefore, it is assumed that, if the ascorbic acid is well retained during processing and storage, other nutrients would be as well. Ascorbic acid degradation in packaged fruit juices depends mainly on storage temperature, dissolved oxygen level, residual H_2O_2 left after the sterilization of packaging material and trace metal ions.

Aseptic packaging technology has been widely accepted by the fruit juice industry for the production of shelf-life stable fruit juices. H_2O_2 is the primary chemical for the sterilization of plastic packaging material used in aseptic systems. An FDA regulation currently limits residual H_2O_2 to 0.5 ppm, leached into distilled water, in finished food packages (Code of Federal Regulations, 2000). However, during the sterilization of aseptic chambers or packaging materials with H_2O_2, residues left on the packaging material or vapours generated during drying may get trapped inside the package upon sealing (Stannard & Wood, 1983; Toledeo, 1986). Therefore, residues left inside packages may occasionally be over the legal limit and particularly cause the degradation of ascorbic acid (Johnson & Toledo, 1975) as well as anthocyanin pigments (Özkan, Yemenicioğlu, Çıtak, & Cemeroğlu, 2000; Özkan, Yemenicioğlu, Asefi, & Cemeroğlu, 2002; Sondheimer & Kertesz, 1952).

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Beverages and fruit juices are currently fortified with ascorbic acid for both nutritional purposes and potential health benefits (Elliott, 1999). It is generally believed that fruit juices should contain high amounts of ascorbic acid, in spite of the fact that ascorbic acid itself participates in non-enzymatic browning reactions which, in turn, result in the degradation of colour (Starr & Francis, 1968). In this study, orange, sour cherry, grape and pomegranate juices were selected. Orange juice is an important source of ascorbic acid in the human diet. The recommended daily allowance (RDA) for ascorbic acid is 60 mg day^{-1} for a male or female adult (Whitney & Rolfes, 1993). This can be obtained from half a cup (125 ml) of orange juice per day. Grape, pomegranate and sour cherry juices are not good sources of ascorbic acid; therefore, they are good candidates for fortification.

We conducted extensive research on evaluating the degradation of anthocyanin pigments in sour cherry, pomegranate and strawberry juices in the presence of H_2O_2 alone (Özkan et al., 2000, 2002) and in combination with ascorbic acid (Özkan, 2002). There is limited literature dealing with ascorbic acid degradation in the presence of added H_2O_2 (Harper, Morton, & Rolfe, 1969; Johnson & Toledo, 1975). However, no reports have been found on the kinetics of ascorbic acid degradation in the presence of added H_2O_2. Therefore, this study was undertaken to determine the rates of ascorbic acid degradation in various fruit juices with addition of H_2O_2 at various storage temperatures. Ascorbic acid degradation was also studied in orange juice and sour cherry nectar without addition of H_2O_2.

2. Materials and methods

2.1. Materials

Oranges and pomegranates were purchased from a local market in Ankara. Grapes were obtained from the Horticulture Department' s experimental orchard in the Kalecik region. Sour cherry nectar was obtained directly from the fruit juice pilot plant of the Food Engineering Department.

Orange juice was extracted using a household centrifugal extractor (Moulinex T574, France) and filtered through cheesecloth to remove the pulpy section. The outer skins of pomegranates were hand-peeled. The juicy sacs from the fruit pericarp were separated and pressed through cheesecloth. The juice was then clarified with only 50 ml of 0.5% gelatin per litre at 4 °C overnight. Grapes were pressed on a Bucher model rack and cloth press. The resulting juice was depectinized with Pectinex 3XL (Novo Nordisk, Dittingen, Switzerland) at 50 °C for 1 h. The depectinized grape juice was clarified with 12 ml of 5% gelatin, 0.5 ml of 15% (v/v) kizelsol and 10 ml of 5% bentonite per litre at 50 °C for 1 h.

The clarified pomegranate and grape juices were then filtered. All juice samples were bottled in glass and stored at −30 °C until used for analysis. Before use, the grape juice was filtered to remove the tartrate precipitates formed during storage.

2.2. Methods

2.2.1. Sample preparation

The juice samples were thawed at room temperature and sodium benzoate (5 g l^{-1} juice) was added to prevent spoilage. Ascorbic acid (300 mg l^{-1} juice) was added to sour cherry nectar, and grape and pomegranate juices. Sodium benzoate, ascorbic acid and hydrogen peroxide were obtained from Merck Co. (Darmstad, Germany).

2.2.2. Degradation studies

Ascorbic acid degradation was studied in sour cherry nectar, and orange, pomegranate and grape juices at 0.5 ppm H_2O_2 concentration at 20, 30 and 40 °C, and also at 5 ppm H_2O_2 concentration at 40 °C. The degradation studies were carried out in a Memmert BE 400 model incubator (Memmert, Schwabach, Germany) at 20 and 30 °C, and a Memmert WB 14 model thermostatic water bath (Memmert, Schwabach, Germany) at 40 °C. The juice samples were allowed to reach the required temperature, and then the predetermined amounts of diluted H_2O_2 solution were added. For 0.5 and 5 ppm H_2O_2 concentrations, 1 ml of 0.0045% H_2O_2 and 1.3 ml of 0.035% H_2O_2 per 100 ml juice were added, respectively. At regular time intervals, samples were removed from the water bath or incubator, and the predetermined amounts of diluted SO_2 solution were added rapidly to the samples to halt the reaction between H_2O_2 and ascorbic acid. The samples were then rapidly cooled by plunging into an ice water bath and held at −30 °C until analysed for ascorbic acid content.

Ascorbic acid degradation was also studied in the absence of H_2O_2 in orange juice and sour cherry nectar at 20, 30 and 40 °C. The juice samples were prepared by adding the same amounts of distilled water in place of H_2O_2.

2.2.3. Ascorbic acid measurement

Ascorbic acid concentration was measured, following the HPLC method outlined by Gökmen, Kahraman, Demir, and Acar (2000). This method was modified by blending the juice samples with 3% metaphosphoric acid (HPO_3) to stabilize ascorbic acid. The samples were then filtered through a 0.45-μl membrane filter (Sartorius, Göttingen, Germany). A 20-μl aliquot of samples was injected into the HPLC. A Shimadzu LC-10AD pump and a Shimadzu SPD-M10A photodiode array detector (Shimadzu, Kyoto, Japan) were used. Chromatograms

were recorded and processed on Shimadzu Class-VP software. Separation was carried out using a Nucleosil 100-C18 column (250×4.6 mm, particle size 5 μm) (HICHROM, Theale, Reading). The detector was set to 254 nm. A 0.2 M potassium dihydrogen phosphate (KH_2PO_4) solution (adjusted to pH 2.4 with H_3PO_4) was used as mobile phase at a flow rate of 0.5 ml min^{-1}.

3. Results and discussion

3.1. Ascorbic acid degradation

Ascorbic acid degradation was studied at 20, 30 and 40 °C, without addition of H_2O_2, in orange juice and sour cherry nectar. The zero- and first-order rate constants (k) for ascorbic acid degradation were calculated by using the following equations:

$$C_t - C_0 = -kt, \quad (1)$$

$$\ln(C_t/C_0) = -kt. \quad (2)$$

C_0 and C_t are the concentrations of ascorbic acid at the beginning of reaction and after time t heating at a given temperature, respectively. Higher coefficient of determination (R^2) values indicate that a zero-order model fitted the data better than a first-order model (Table 1). Ascorbic acid contents of orange juice were plotted as a function of storage time at various temperatures (Fig. 1).

No study has been found for ascorbic acid degradation during either thermal processing or subsequent storage of the fortified grape and pomegranate juices, and sour cherry nectar. However, ascorbic acid degradation is well documented in orange juice during storage. Our observation agrees well with that of Nielsen, Marcy, and Sadler (1993) and Kaanane, Kane, and Labuza (1988) who described ascorbic acid degradation in orange juice by a zero-order model at 23–45 and 4–45 °C, respectively. Nagy and Smoot (1977) found two different reaction mechanisms for ascorbic acid degradation in canned orange juice stored at 29, 38 and 46 °C. They described the degradation by a zero-order model at 38 and 46 °C, while the degradation pattern fitted

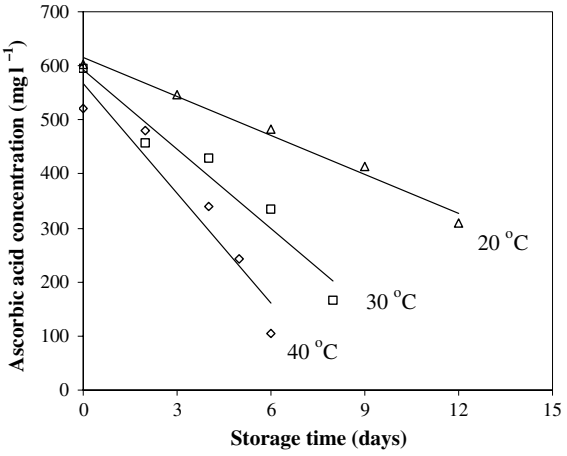

Fig. 1. Ascorbic acid contents in orange juice as a function of storage temperature.

both zero- and first-order kinetics at 29 °C. Ascorbic acid degradation in orange juice or concentrate during storage has also been described by a first-order reaction model (Johnson & Toledo, 1975; Johnson, Braddock, & Chen, 1995; Lee & Labuza, 1975).

The calculated rate constants (k values) varied from 1.00 to 2.83 and 7.43 to 18.80 mg l^{-1} h^{-1} in orange juice and sour cherry nectar without the addition of H_2O_2 at 20–40 °C, respectively (Table 1). The comparison of k values revealed that ascorbic acid was more stable in orange juice than in sour cherry nectar. The different degradation rates can be attributed to the phenolic contents, mainly flavonols and anthocyanins. Flavonols in orange juice may have protected ascorbic acid while anthocyanins in sour cherry nectar may have accelerated the degradation of ascorbic acid. In fact, Martí, Pérez–Vicente, and García-Viguera (2001) found that ascorbic acid in pomegranate juice was completely degraded after 4 days, while no ascorbic acid was detected in a model solution after 12 days of storage at 25 °C.

The protective mechanism of flavonols is mainly due to chelation of metal ions and action as an antioxidant. Harper et al. (1969) reported that flavonols primarily act as antioxidants by donating the hydrogen ions to reactive free radicals which may otherwise cause the

Table 1
Reaction rate constants (k) for ascorbic acid degradation in orange and sour cherry juices during storage

Sample	Temperature (°C)	Zero-order		First-order	
		k (mg l^{-1} h^{-1})	R^2	k (h^{-1})	R^2
Orange juice	20	1.00	0.985	0.0023	0.951
	30	2.04	0.951	0.0060	0.871
	40	2.83	0.916	0.0099	0.779
Sour cherry nectar	20	7.43	0.983	0.0345	0.921
	30	10.1	0.957	0.0668	0.921
	40	18.8	0.994	0.0940	0.944

autoxidation of ascorbic acid. The authors noted that the complex formation between flavonols and copper ions would not be likely in fruit juices where high amounts of chelating fruit acids, such as citric acid, are present. Clegg and Morton (1968) showed that the most effective protection for ascorbic acid was provided by quercetin, which has ortho-hydroxylation, in citrate buffer at pH 2.9. These authors also found that the mixture of quercetin and kaempherol gave a slightly better protection than either compound singly. Similar results were reported by Harper et al. (1969) who found that quercetin and dihydroquercetin, followed by kaempherol (flavonol aglycones), showed the strongest antioxidant activity, while quercetin-3-rutinoside (flavonol glycoside) was the least effective.

The higher degradation rate of ascorbic acid in sour cherry nectar may also be attributed to anthocyanin pigments. Sour cherries contain very high amounts of anthocyanin pigments, 267–688 mg l^{-1} (Erbaş & Cemeroğlu, 1992). The presence of anthocyanins was shown to promote the oxidation of ascorbic acid in pomegranate juice (Martí et al., 2001), in citrate buffer containing blackcurrant anthocyanins (Clegg & Morton, 1968; Harper et al., 1969) and in the same buffer containing cranberry anthocyanins (Shrikhande & Francis, 1974). The percent ascorbic acid losses were 60 and 92 in citrate buffer containing only ascorbic acid, and both anthocyanins and ascorbic acid together at 20 °C for 3 days of storage, respectively (Shrikhande & Francis, 1974).

3.2. Ascorbic acid degradation in the presence of H_2O_2

Ascorbic acid degradation was also studied, at 0.5 ppm H_2O_2 concentration at, 20, 30 and 40 °C and at 5 ppm H_2O_2 concentration at 40 °C in orange, grape and pomegranate juices, and sour cherry nectar. Examples of these results are plotted for orange (Fig. 2) and grape juices (Fig. 3). The zero- and first-order rate constants for ascorbic acid degradation, in the presence of H_2O_2, are presented in Table 2. As indicated by higher R^2, ascorbic acid degradation in the presence of H_2O_2 fitted better to a zero-order model. Comparing rate constants, ascorbic acid breakdown at 0.5 ppm H_2O_2 concentration occurred at a much slower rate in pomegranate and orange juices than in grape juice and sour cherry nectar. The degradation rate at 0.5 ppm H_2O_2 was in descending order: sour cherry nectar > grape > orange and pomegranate juices. At 5 ppm H_2O_2, the degradation of ascorbic acid increased tremendously in all four juices studied, especially in pomegranate juice. The degradation rate at 5 ppm H_2O_2 was in descending order: sour cherry nectar > pomegranate > grape and orange juices.

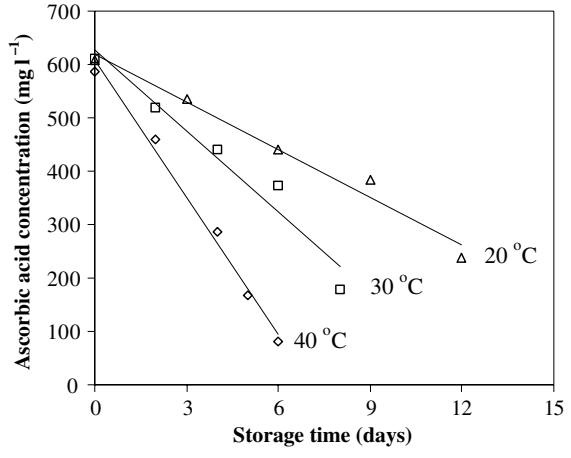

Fig. 2. Ascorbic acid contents in orange juice as a function of storage temperature at 0.5 ppm H_2O_2 concentration.

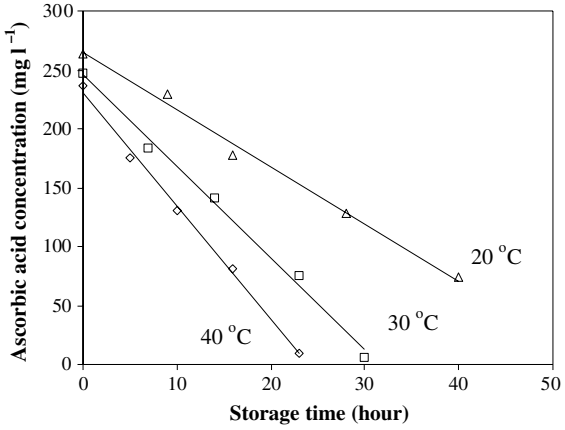

Fig. 3. Ascorbic acid contents in grape juice as a function of storage temperature at 0.5 ppm H_2O_2 concentration.

The degradation of ascorbic acid, in all four juices studied, with or without the addition of H_2O_2, may probably be attributed to the formation of H_2O_2 during the aerobic oxidation of ascorbic acid. The addition of 0.5 ppm H_2O_2 did not greatly increase the degradation of ascorbic acid. However, raising H_2O_2 concentration from 0.5 to 5 ppm resulted in a tremendous increase in the degradation rates, especially for the anthocyanin-rich sour cherry nectar and pomegranate juice. We believe that, at 0.5 ppm H_2O_2, the antioxidant substances in fruit juices, i.e., flavonols and anthocyanins, reacted with H_2O_2, thereby preventing the autoxidation of ascorbic acid. Harper et al. (1969) elucidated the reaction mechanism for ascorbic acid degradation. According to these authors, one mole of H_2O_2 is liberated for every mole of ascorbic acid oxidation. The resulting H_2O_2 reacts with Cu^{++} to form HOO* (hydroperoxy radical) which, in turn, feeds the autoxidation reactions of ascorbic acid. The authors also showed that the addition of H_2O_2 (55 ppm), in the absence of Cu^{++}, increased the rate of ascorbic acid degradation. The combination of Cu^{++} and H_2O_2 increased the oxidation tremendously.

Table 2
Reaction rate constants (k) for ascorbic acid degradation in the presence of hydrogen peroxide in various fruit juices during storage

Sample	H_2O_2 concentration (ppm)	Temperature (°C)	Zero-order		First-order	
			k (mg l^{-1} h^{-1})	R^2	k (h^{-1})	R^2
Orange juice	0.5	20	1.24	0.978	0.0029	0.922
	0.5	30	2.1	0.953	0.0058	0.854
	0.5	40	3.5	0.989	0.0131	0.885
	5	40	6.62	0.957	0.0184	0.964
Sour cherry nectar	0.5	20	7.74	0.997	0.0539	0.924
	0.5	30	11.8	0.998	0.2192	0.776
	0.5	40	13.7	0.968	0.1163	0.948
	5	40	22.2	0.986	0.2870	0.810
Grape juice	0.5	20	4.84	0.993	0.0320	0.975
	0.5	30	7.76	0.994	0.1094	0.774
	0.5	40	9.66	0.996	0.1303	0.845
	5	40	16.2	0.980	0.1069	0.973
Pomegranate juice	0.5	20	0.399	0.843	0.0009	0.830
	0.5	30	0.873	0.833	0.0030	0.777
	0.5	40	2.58	0.917	0.0090	0.843
	5	40	17.1	0.989	0.1071	0.978

The adverse effect of H_2O_2 on ascorbic acid was reported in 55° Brix orange juice concentrate packaged in plastic cups containing H_2O_2 in the headspace (Johnson & Toledo, 1975). This report showed that 68% of the ascorbic acid was lost in the one week of storage at 24 °C. Compared to our results, high degradation rate of ascorbic acid in orange juice concentrate may be due to the much higher H_2O_2 concentration. Sondheimer and Kertesz (1952) studied the degradation of ascorbic acid and anthocyanins in both strawberry juice and pure solutions of the major strawberry anthocyanin (pelargonidin-3-glucoside). In both systems, over 90% of the ascorbic acid was lost in 10 h at 30 °C. Addition of thiourea (0.1%), of metal-chelating agent, significantly reduced the degradation of both ascorbic acid and anthocyanins.

Among the anthocyanin-rich fruit juices studied, ascorbic acid in the presence of 0.5 ppm H_2O_2 was much more stable in pomegranate juice than in sour cherry nectar. The different susceptibilities of ascorbic acid to H_2O_2 in these juices may be due to their varying anthocyanin compositions. The major anthocyanins are the 3-glucoside and 3,5-diglucoside of cyanidins, followed by delphinidin and pelargonidins in pomegranate juice (Martí et al., 2001). In sour cherries, the main anthocyanin pigments are cyanidin 3-rutinoside, cyanidin 3-glucosyl-rhamnosyl-glucoside, cyanidin 3-glucoside and cyanidin 3-sophoroside (Dekazos, 1970).

The stability of individual anthocyanidins varies considerably. For instance, Hernández, Melgarejo, Tomás-Barberán, and Artés (1999) reported that delphinidin derivatives are more easily oxidized than the corresponding cyanidin and pelargonidin derivatives. In fact, Clegg and Morton (1968) found a higher protection of ascorbic acid by delphinidin-3-gycoside and delphinidin-3-rhamnoglucoside than cyanidin-3-gycoside and cyanidin-3-rhamnoglucoside. These findings indicate that the loss of delphinidins is favoured for the protection of ascorbic acid. The higher delphinidin content of pomegranate juice may have protected the oxidation of ascorbic acid from the low level of H_2O_2 (0.5 ppm). However, at a high level of H_2O_2 (5 ppm), the concentration of delphinidins may not be adequate to protect the oxidation of ascorbic acid from H_2O_2 in pomegranate juice.

3.3. Temperature dependence

The temperature dependence of the degradation of ascorbic acid in various fruit juices was compared by calculating activation energies (E_a) and temperature quotients (Q_{10}) at 20–40 °C (Table 3) from the following equations:

$$k = k_0 e^{-Ea/RT}, \quad (3)$$

$$Q_{10} = k_{(T+10)}/k_{(T)}. \quad (4)$$

In the absence of H_2O_2, the E_a values were 39.8 and 35.3 kJ mol^{-1} for orange juice and sour cherry nectar, respectively. Our E_a value for ascorbic acid degradation in orange juice was lower than the reported E_a values of 54 kJ mol^{-1} at 23–45 °C (Nielsen et al., 1993) and 56 kJ mol^{-1} at 4–45 °C (Kaanane et al., 1988) in orange juice. Nagy and Smoot (1977) found two distinct Arrhenius profiles for the degradation of ascorbic acid in orange juice at 4.4–49 °C. The respective E_a values were approximately 60 and 96 kJ mol^{-1} at 4.4–24 and 24–49 °C. They concluded that storage of orange juice above the critical temperature, i.e., 22–26.7 °C, caused a significant increase in the rate of ascorbic acid degradation.

Table 3
Effect of temperature on the degradation of ascorbic acid in various fruit juices during storage

Sample	H_2O_2 concentration (ppm)	E_a (kJ mol^{-1})	Q_{10}	
			20–30 °C	30–40 °C
Orange juice	–	39.8 (0.964)[a]	2.04	1.39
	0.5	40.3 (0.999)	1.70	1.69
Sour cherry nectar	–	35.3 (0.957)	1.36	1.86
	0.5	21.8 (0.935)	1.53	1.16
Grape juice	0.5	26.2 (0.962)	1.61	1.24
Pomegranate juice	0.5	71.0 (0.988)	2.19	2.95

[a] Numbers in parentheses are the determination coefficients.

At 0.5 ppm H_2O_2, the E_a values for ascorbic acid degradation were 21.8 kJ mol^{-1} for sour cherry nectar, 26.2 kJ mol^{-1} for grape juice, 40.3 kJ mol^{-1} for orange juice and 71.0 kJ mol^{-1} for pomegranate juice. The Q_{10} values at 0.5 ppm H_2O_2 concentration ranged from 1.36 to 2.04 at 20–30 °C and from 1.16 to 1.86 at 30–40 °C in orange and grape juices, and sour cherry nectar. These results clearly indicate that the rate of ascorbic acid degradation in the presence of H_2O_2 was slower at 30–40 °C than at 20–30 °C. On the contrary, the rate of ascorbic acid degradation in pomegranate juice increased 2.2-fold at 20–30 °C, while the increase was 3.0-fold at 30–40 °C. The highest E_a and Q_{10} values indicate that ascorbic acid in pomegranate juice is more sensitive to temperature increase in the presence of H_2O_2 that in orange and grape juices, or sour cherry nectar. No study has been found reporting the E_a and Q_{10} values for ascorbic acid degradation in fruit juices in the presence of H_2O_2.

4. Conclusion

Ascorbic acid in orange juice showed the greatest stability at the selected H_2O_2 concentrations (0.5 and 5 ppm) and storage temperatures (20–40 °C). The high rate constants indicate that the fortification of sour cherry nectar with ascorbic acid should be avoided. In pomegranate juice, the degradation was slow at 0.5 ppm H_2O_2 concentration, but increasing of the H_2O_2 concentration from 0.5 to 5 ppm accelerated ascorbic acid degradation tremendously. Since the adverse effects of ascorbic acid on anthocyanins have been well known, the addition of ascorbic acid to anthocyanin-rich fruit juices should be avoided, e.g., in sour cherry nectar, or it should be done with great caution, e.g., in pomegranate juice, to protect ascorbic acid as well as anthocyanins. The effect of temperature on the degradation rates of ascorbic acid in juice samples was more pronounced at higher H_2O_2 concentrations. Thus, greater ascorbic acid losses should be expected as residual H_2O_2 concentration and storage temperature increase in aseptically packaged fruit juices.

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