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Article

Degradation Kinetics of Anthocyanins in Sour Cherry Cloudy Juices at Different Storage Temperature

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Abstract: The aim of this study was to characterize in detail 25 sour cherry cultivars and provide data on their industrial processing into high-quality sour cherry cloudy juices (ScCJ). Anthocyanin composition was identified and quantified by LC-PDA-ESI-MS QToF, UPLC-PDA. Kinetic degradation ($k \times 10^3$, $t_{1/2}$, D value) and color (CIE $L^*a^*b^*$) were measured before and after 190 days of storage at 4 °C and 30 °C. A total of five anthocyanins, four cyanidins (-3-O-sophoroside, -3-O-glucosyl-rutinoside, -3-O-glucoside, and -3-O-rutinoside) and one peonidin-3-O-rutinoside were detected across all investigated juices. Total anthocyanin content ranged from ~590 to ~1160 mg/L of juice, with the highest levels in Skierka, Nagy Erdigymulscu, Wilena, Wiblek, and Safir cvs., and the lowest in Dradem and Nanaones. During 190 days of storage a significant change was observed in the content of anthocyanins. Their degradation depended rather on the storage conditions (time and temperature) than on the type of anthocyanin compounds present in the ScCJ. Half-life values of ScCJ ranged from 64.7 to 188.5 days at 4 °C and from 45.9 to 112.40 days at 30 °C. Sample redness changed more rapidly than yellowness or lightness and Chroma or hue angle. These results may be useful for the juice industry and serve as a starting point for the development of tasty sour cherry juices with high levels of bioactive compounds.

Keywords: *Prunus cerasus* L.; cloudy juices; LC-PDA-ESI-MS QToF; stability; anthocyanins; half-life periods; color

1. Introduction

Sour cherry fruits are primarily cultivated in Europe (80%) and over 65% of total world production (1,215,748,000 t) is located there. Major growers are Poland, with a share of 16% (201,681,000 t), Turkey 15% (185,435,000 t), Russian Federation 13% (157,000,000 t), Ukraine, Iran, and the United States. In Poland, sour cherry production represents more than 50% of all stone fruits, and it is the second most popular fruit grown after apple [1]. Sour cherries are utilized principally by the processing industry as frozen fruits, concentrates, clear juices, jams and marmalades, alcoholic beverages and soft drinks, and especially nectars. So far, the most popular type of liquid product obtained from sour cherry fruits has been a clear juice. Majority of clear juices available at retail sellers contain only minor amounts of polyphenols. During production of clear juices some process i.e., pulp enzymation, clarification, filtration makes a reduction of serum viscosity and eliminates the cloudiness factors then follows the removal of pectin, polysaccharides, and especially phenolic compounds causes significant deterioration in the pro-healthy properties of the clear juice. Therefore, for an alternative to the clear juice production is a natural cloudy juice, rich in phenolic compounds and fiber. In the manufacturing

process there is no pulp enzymation, filtration, or clarification therefore followed significant behavior of polyphenol compounds and other active substances.

It is pressed directly without an addition of enzymes during mash and clarification steps. Previous studies demonstrated that cloudy juices made from apples, strawberries, and quince contained much more bioactive compounds i.e., polyphenols, vitamins, minerals, and pectins than clear juices [2–4].

Sour cherries are a rich source of health-promoting compounds such as secondary metabolites known as phytochemicals, and of natural antioxidant substances, such as anthocyanins [4–6]. Anthocyanins may also exhibit antioxidant, anti-inflammatory, antiallergic, antiviral, antiproliferative, cardioprotective, anticarcinogenic, and blood-lipid lowering properties [7]. Total content of anthocyanins in different cultivars of sour cherry ranges from 90 to 1000 mg/100 g of fruits [4]. Daily consumption of anthocyanins was recently reported to be 12.5 mg/day in the United States [8] and 82 mg/day in Finland [9]. Such huge differences in estimates of the daily intake were claimed to result from different food intake data [8]. With diverse availability and potential sources of phytochemical modifications throughout processing and storage, a thorough evaluation of changes in anthocyanins and red color due to processing and storage is important for consumers, particularly with regard to recommended daily servings of specific products.

Additionally, red color is one of the most crucial sensory properties of food; therefore, minimization of pigment loss during processing and storage is the most important quality parameter. Many works [5,10–12] showed that anthocyanin stability, apart from food processing, depended on numerous factors including storage temperature, pH, light, the presence of metal ions, oxygen, enzymes, ascorbic acid, sugars, sulfur dioxide or sulfites, and copigments belonging to various compound classes. Also, earlier studies [3,4] indicated significant influence of cultivars on the content of phytochemicals, particularly of phenolic compounds. Despite that, information on the changes in main compounds of sour cherry cloudy juices (ScCJ) being 100% fruit products is still limited. Kim et al. [12] reported the effects of sour cherry fruit to jam processing on phenolic content and antioxidant activity but no data on the changes in anthocyanins after storage were provided. Toydemir et al. [5] studied the quality of industrially prepared sour cherry nectars. Damar et al. [11] investigated antioxidant capacity and anthocyanin profile of clear sour cherry juices after an enzymatic maceration. However, to the best of our knowledge there have been no reports in the literature about cloudy juices made from sour cherry. Still, data are lacking on the quality and composition of anthocyanins in ScCJ before and after storage. Additionally, red color stability of ScCJ is of great interest to food industry and scientists. Considering the above, we decided to investigate the possibilities of cloudy juice production from sour cherry fruits.

Therefore, the aim of this work was to characterize the anthocyanins (by LC-PDA-ESI-MS, UPLC-PDA) of different 25 ScCJs and to determine their stability during 90 and 180 days of storage at different temperatures (4 °C and 30 °C). To this end, changes in their color and kinetic parameters of anthocyanin degradation were investigated.

2. Materials and Methods

2.1. Chemicals

All chemicals and reagents were of analytical grade and were supplied from Sigma–Aldrich (Poznań, Poland). All polyphenolic standards were supplied from Extrasynthese (Lyon, France). Water for UPLC analysis was prepared by using an HPL SMART 1000 s system (Hydrolab, Gdańsk, Poland).

2.2. Plant Material

Twenty-five different sour cherry (*Prunus cerasus* L.) cultivars: ('Agat', 'Dradem', 'Erdi botermo', 'Erdi Nagygyümöscu', 'Groniasta', 'Karneol', 'Kelleris 14', 'Lucyna', 'Łutówka', 'Morina', 'Nana', 'North star', 'Pandy 103', 'Sabina', 'Safir', 'Topas', 'Turgieniewka', 'Wanda', 'Wiblek', 'Wifor', 'Wilena', 'Wilga', 'Winer', 'Wisok', and 'Wołyńska', were obtained from 8-years-old tree and hand-harvested in June and July 2016 at the Research Station for Cultivar Testing in Zybyszów near Wrocław (51°3'51.11" N

16°54′43.56″ E). Fruits were selected from both the interior and exterior of trees to obtain a representative sample for analysis.

2.3. Preparation of Cloudy Sour Cherry Juices on a Laboratory Scale

Each cvs. of sour cherry fruits (2 kg) were ground using Thermomix (Wuppertal, Vorwerk, Germany) laboratory mill for 20 s with an oxidation inhibitor (ascorbic acid; 1%, *v/v*). After grounding, the mash was pressed in a laboratory hydraulic press (SRSE; Warsaw, Poland), and the juice was heated in the Thermomix from 20 up to 90 °C during 4 min, hot poured into 0.08 L glass jars, immediately inverted for 10 min to sterilize the lids, and cooled to 20 °C. Two separately technological replicates of ScCJ preparation were carried out. The juices directly after processing, and after 90 and 180 days of storage at 4 °C and 30 °C were subjected to analyses.

2.4. Identification and Quantification of Anthocyanins by LC-PDA-ESI-MS QToF and UPLC-PDA Method

The ScCJ for identification (LC-PDA-ESI-MS QToF; Waters Corporation; Manchester, UK) and quantitative (UPLC-PDA; Waters Corporation; Milford, USA) analysis of anthocyanin compounds were performed as described previously by Wojdyło et al. [4]. Separations of anthocyanins were carried out using an Aquity BEH C18 column (1.7 µm, 2.1 by 100 mm; Waters Corporation, Milford, MA, USA) at 30 °C and injection was 5 µL. The mobile phase was flow at 0.42 mL/min and composed of solvent A (0.1% formic acid for identification analysis by LC-MS and 4.5% formic acid for quantification by UPLC-PDA analysis) and solvent B (100% of acetonitrile with 0.1% of formic acid). The mobile phase which consisted of gradient program was as follows: 99% of A as initial conditions, 75% of A at 12 min, 100% of B at 12.5 min, and up to start at 13.5 min—99% of A. The injection volume of samples was 5 µL. PDA spectra were recorded over the wavelength range of 200–600 nm in steps of 2 nm. Characterization of the single components was carried out via the retention time and the accurate molecular masses. Analyses were carried out with voltage ramping cycles from 0.3 to 2 V, using full scan mode, and data-dependent MS scanning from *m/z* 100 to 1800, with collision induced fragmentation experiments were performed using argon as the collision gas. The capillary and cone voltages were 2500 V and 30 V, respectively. The capillary temperature was set to 300 °C, while the source heater temperature was 100 °C, and desolvation gas (nitrogen) flow rate of 300 L/h. Leucine enkephalin was flow rate of 2 µL/min and was used as the reference compound at a concentration of 500 pg/µL, and *m/z* at 554.2615 and 556.2771 were detected for negative and positive ionization, respectively. The mass spectrometer was operated in a positive ion mode, set to the base peak intensity (BPI) chromatograms. Analysis was carried out three times at 520 nm for anthocyanins, and compared with those of pure standards of cyanidin-3-*O*-sophoroside, -3-*O*-glucoside, -3-*O*-rutinoside, peonidin-3-*O*-rutinoside and pelargonidin-3-*O*-glucoside. Calibration curves at concentrations ranging from 0.05 to 0.5 mg/mL ($r^2 \leq 0.9997$). The results were expressed as mg per L of juices.

2.5. Kinetics of Phytochemical Degradation

The degradation kinetics of anthocyanins follows the first-order equation,

$$C_t = C_0 \exp(\pm kt) \quad (1)$$

where C_t and C_0 are the concentration of bioactive compounds (mg/100 mL) at time t and t_0 , respectively, k is the rate constant, and t is the storage time (days). Half-life value ($t_{1/2}$) was calculated as $t_{1/2} = (\ln 2/k)$. Times required for 90% degradation of bioactive compounds (D value) was also calculated as $D = (1/k)$.

2.6. Color

The CIE $L^*a^*b^*$ calculations represent a suitable method to describe color changes in juices during storage. Color changes during storage were determined colorimetrically by A5 Chroma-Meter (Minolta CR300, Osaka, Japan) in 20 mm pathway cuvettes. The system was controlled by Color-quest software.

Values of L^* , a^* and b^* were measured to describe a three-dimensional color space. The vertical axis L^* is a measure of lightness, where values range from completely opaque (0) to completely transparent (100), a^* is a measure of redness (or $-a^*$ of greenness) and b^* is a measure of yellowness (or $-b^*$ of blueness) on the hue-circle. The hue angle is corresponding to the relative amounts of redness and yellowness where $0^\circ/360^\circ$ is defined for magenta color, 90° for yellow, 180° for green and 270° for blue. Chroma gives further information on the saturation or intensity of color.

Chroma (C^*) and hue angle (h°) were calculated from a^* and b^* coordinates with the following equations:

$$C^* = (a^{*2} + b^{*2}) \quad (2)$$

$$h^\circ = \arctan \frac{b^*}{a^*} \quad (3)$$

2.7. Statistical Analysis

Results were presented a mean \pm standard deviation of two independent determinations. All statistical analyses were performed with Statistica version 12.0 (StatSoft, Krakow, Poland). One-way analysis of variance (ANOVA) by Duncan's test was used to compare the mean values. Differences were considered to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Identification of Phenolic Compounds of Sour Cherry Cloudy Juices

LC-PDA-MS QToF of analysis of ScCJ revealed the presence of anthocyanins (Table 1, Figure 1). All compounds were tentatively identified based on their UV adsorption value, m/z value, retention time and elution order as compared with standards and published data [4,6].

A total of five anthocyanins, four cyanidins, i.e., -3-*O*-sophoroside ($R_t = 3.69$ min, $\lambda_{\max} = 515$) with $[M + H]^+$ at $m/z = 611.1564$ and main MS/MS fragment at $m/z = 287.0536$), -3-*O*-glucosyl-rutinoside ($R_t = 3.89$ min, $\lambda_{\max} = 517$) with $[M + H]^+$ at $m/z = 757.2184$ and main MS/MS fragment at $m/z = 287.0536$), -3-*O*-glucoside ($R_t = 4.01$ min, $\lambda_{\max} = 516$) with $[M + H]^+$ at $m/z = 449.1063$ and main MS/MS fragment at $m/z = 287.0571$), and -3-*O*-rutinoside ($R_t = 4.31$ min, $\lambda_{\max} = 517$) with $[M + H]^+$ at $m/z = 595.1664$ and main MS/MS fragment at $m/z = 287.0571$), and one peonidin-3-*O*-rutinoside ($R_t = 5.45$ min, $\lambda_{\max} = 517$) with $[M + H]^+$ at $m/z = 609.1857$ and main MS/MS fragment at $m/z = 301.0730$) were detected in all investigated juices. These results corroborated other findings published for sour cherry fruits [4,6,13].

Table 1. Identification of anthocyanins in CScJ by LC-PDA-MS QToF (Positive Mode).

Compound	Rt (min)	λ_{\max} (nm)	$[M] + (m/z)$	MS Ion Fragments (m/z)
Cyanidin-3- <i>O</i> -sophoroside	3.69	242/278/515	611.1564+	449.1063/287.0536
Cyanidin-3- <i>O</i> -glucosyl-rutinoside	3.89	242/278/517	757.2184+	611.1614/433.1125/287.0536
Cyanidin-3- <i>O</i> -glucoside	4.01	242/516	449.1063+	287.0571
Cyanidin-3- <i>O</i> -rutinoside	4.31	242/279/517	595.1664+	499.1063/287.0571
Peonidin-3- <i>O</i> -rutinoside	5.45	241/279/517	609.1857+	301.0730/463.0341

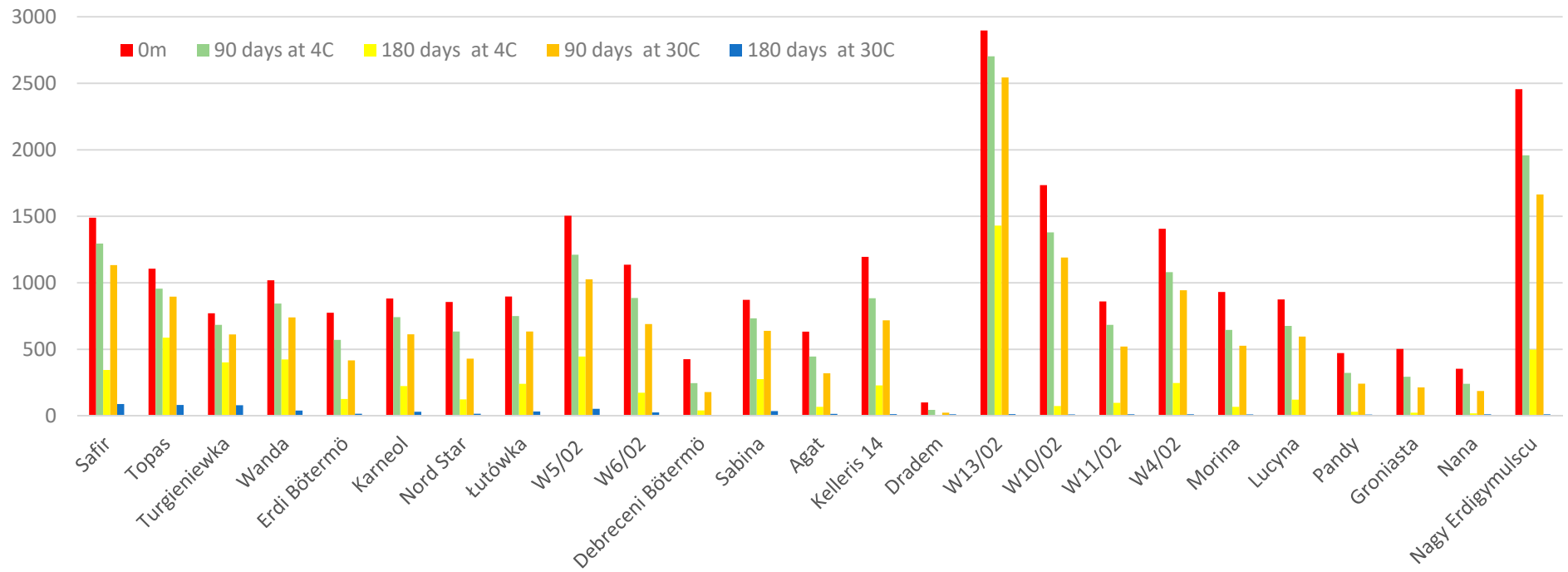


Figure 1. Sum of anthocyanins compounds content [mg/L] in cloudy sour cherry juices after 90 and 180 days of storage time at 4 and 30 °C.

3.2. Anthocyanins of Sour Cherry Cloudy Juices

Anthocyanins are one of the most important phenolic compounds responsible for attractive red color of ScCJ. The color and appearance of food is the first sensory experience of consumers. Processing and especially storage time may affect color properties and appeal of food. Our study demonstrated that sour cherry juices had the same profile of anthocyanins but each compound was detected in different amount.

The results obtained for total anthocyanin content revealed significant differences among juices, with values varying from ~590 to ~1160 mg/L (Table 2). The highest content of anthocyanins was found in Skierka, Nagy Erdigymulscu, Wilena, Wiblek, and Safir (up to 590 mg/L), and the lowest in Dradem and Nana (below 100 mg/L). Dradem and Nana juices were made from typically pink cv., and therefore the content of anthocyanins was exceptionally low. Our findings showed that total amount of anthocyanins in ScCJ was largely affected by the cultivar, as also reported by other authors [4,6,13].

Table 2. Content of anthocyanins compounds [mg/L] in cloudy sour cherry juices before storage time.

Sour Cherry Cloudy Juices	0 Days				
	C3s	C3gr	C3r	P3r	dC + C3g
Safir	292.21 ± 9.20 b *	247.87 ± 8.12 c	10.36 ± 0.44 c	44.19 ± 1.31 j	1.20 ± 0.02 d
Topas	118.99 ± 3.75 h	243.89 ± 3.02 c	8.29 ± 0.15 f	69.99 ± 2.07 e,f	1.10 ± 0.04 e
Turgieniewka	81.8 ± 2.58 j	192.52 ± 7.15 f	4.67 ± 0.15 k	28.89 ± 0.86 l	0.29 ± 0.01 j
Wanda	140.14 ± 4.41 g	200.15 ± 4.34 e	9.04 ± 0.43 e	57.45 ± 1.70 h,i	0.94 ± 0.04 f
Erdi Böttermö	81.72 ± 2.57 j	161.9 ± 5.67 g	6.17 ± 0.05 i	59.79 ± 1.77 g,h	0.18 ± 0.01 k
Karneol	81.54 ± 1.57 j	206.84 ± 12.03 e	7.53 ± 0.12 g	56.22 ± 1.67 i	0.79 ± 0.03 g,h
Nord Star	122.76 ± 3.87 h	193.72 ± 10.11 f	3.04 ± 0.10 l	22.36 ± 0.66 m	0.58 ± 0.02 i
Łutówka	96.54 ± 3.04 i	186.86 ± 8.03 f	6.78 ± 0.12 h	67.93 ± 2.01 f	0.85 ± 0.03 f,g
Wiblek	215.48 ± 6.78 d	257.8 ± 9.12 c	19.12 ± 1.20 a,b	108.02 ± 3.20 c	1.49 ± 0.06 c
Wifor	166.05 ± 6.23 f	217.94 ± 4.13 d	8.34 ± 0.33 f	61.27 ± 1.82 g	1.05 ± 0.04 e
Debreceni Böttermö	36.54 ± 1.15 l,m	96.79 ± 5.69 j	2.50 ± 0.07 m	34.13 ± 1.01 k	0.22 ± 0.01 j,k
Sabina	66.06 ± 1.08 k	215.7 ± 6.79 d	5.75 ± 0.65 j	60.03 ± 1.78 g,h	1.05 ± 0.04 e
Agat	41.94 ± 1.32 l	136.85 ± 5.27 h	4.43 ± 0.22 k	69.13 ± 2.05 f	0.56 ± 0.02 i
Kelleris 14	176.23 ± 6.65 e	205.01 ± 7.08 e	9.51 ± 0.11 d	86.4 ± 2.56 d	0.90 ± 0.04 f
Dradem	2.4 ± 0.05 n	29.01 ± 2.06 k	0.38 ± 0.01 r	8.19 ± 0.24 o	0.01 ± 0.00 l
Skierka	631.66 ± 20.89 a	441.11 ± 11.53 a	11.12 ± 0.53 c	69.25 ± 2.05 f	5.82 ± 0.03 a
Wilena	268.09 ± 10.44 c	278.61 ± 13.02 b	21.38 ± 1.12 a	124.05 ± 3.68 b	1.61 ± 0.00 b
Winer	88.96 ± 2.02 i,j	190.18 ± 13.15 f	6.79 ± 0.13 h	57.38 ± 1.70 h,i	0.61 ± 0.02 i
Wisok	159.29 ± 5.01 f	251.86 ± 11.02 c	18.48 ± 1.03 b	131.42 ± 3.89 a	1.46 ± 0.06 c
Morina	96.48 ± 3.04 i	192.62 ± 5.67 f	9.40 ± 0.53 d,e	73.11 ± 2.17 e	0.61 ± 0.02 i
Lucyna	85.76 ± 2.70 j	211.39 ± 10.34 d,e	7.45 ± 0.23 g	44.66 ± 1.32 j	0.72 ± 0.03 h
Pandy	36.26 ± 0.00 l,m	119.22 ± 3.33 i	2.29 ± 0.07 n	30.58 ± 0.91 l	0.30 ± 0.01 j
Groniasta	38.01 ± 1.19 l,m	125.04 ± 7.90 i	2.23 ± 0.02 n	35.49 ± 1.05 k	0.28 ± 0.01 j,k
Nana	1.62 ± 0.00 n	20.82 ± 3.13 l	0.59 ± 0.04 p	0.11 ± 0.00 p	0.05 ± 0.00 l
Nagy Erdigymulscu	31.54 ± 0.93 m	92.86 ± 0.67 j	0.95 ± 0.00 o	16.02 ± 0.47 n	0.24 ± 0.01 j,k

C3s—Cyanidin-3-O-sophoroside; C3gr—Cyanidin-3-O-glucosylrutinoside; C3r—Cyanidin-3-O-rutinoside; P3r—Peonidin-3-O-rutinoside; dC + C3g—sum of derivatives of Cyanidin and Cyanidin-3-O-glucoside. * Values (mean of 3 replications) followed by the same letter, within the same column, were significantly different ($p < 0.05$), according to Duncan's test

Major individual anthocyanins in all sour cherry juices were cyanidin derivatives, particularly cyanidin-3-O-glucosyl-rutinoside (Table 2), and peonidin-3-O-rutinoside. This was consistent with other previously published reports [4–6]. As far as individual anthocyanin concentration was concerned, a significant variation was found between cloudy juices made from different cultivars of sour cherry fruits. Cyanidin-3-O-glucosyl-rutinoside constituted from 25 to 73% of total anthocyanin content. This compound was the most common in Topas, Turgieniewka, Wanda, Erdi Böttermö, Karneol, Nord Star, Łutówka, Wiblek, Wifor, Sabina, Agat, Kelleris 14, Dradem, Wilena, Winer, Wisok, Morina, Lucyna, Pandy, Groniasta, and Nagy Erdigymulscu juices (60–73% of total anthocyanins). The other major anthocyanin compounds identified in ScCJ were cyanidin-3-O-sophoroside (7 to 68% of total anthocyanins) and peonidin-3-O-rutinoside (6 to 27% of total anthocyanins). ScCJ made

from Skierka (631.66 mg/L), Wilena (268.09 mg/L), Safir (292.21 mg/L), and Wiblek (215.48 mg/L) cvs. had a high content of cyanidin-3-O-soforoside, and its lowest levels were determined in Nana (1.62 mg/L) and Dradem (2.40 mg/L) juices. The content of peonidin-3-O-rutinoside was similar to that of cyanidin-3-O-soforoside. The rest of anthocyanins (cyanidin-3-O-glucoside, -3-O-rutinoside and derivatives of cyanidin) were present in ScCJ in low amounts. In general, total content of anthocyanins as well as of individual glycosides was consistent with that reported by other authors [5,13].

3.3. Changes in Anthocyanins during Storage of Sour Cherry Cloudy Juices

Our results showed that the loss of anthocyanins in ScCJ strongly correlated with storage time, temperature, and cultivar but not as strongly with the type of anthocyanin (aglycone type vs. sugar substituent) (Table 2, Tables S1 and S2). Anthocyanins were more stable in the products stored at low temperature. At 4 °C, their average degradation degree was 30–50% after 180 days, while at 30 °C it was up to 92%. The most intense degradation was observed in ScCJ made from Debreceni Bötermő, Wilena, Winer, Morina, Lucyna, and Groniasta cvs., and the least intense in Safir, Topas, Turgieniewka, Wanda, Karneol, Łutówka, Wiblek, and Sabina cvs. As reported previously by other authors [14,15], degradation rate increases along with temperature and this trend was also observed in our study. Wilkes et al. [16] found that anthocyanins decreased in a linear manner for up to five months of storage at 25 °C. Contrary to that, Hellstrom et al. [17] reported no differences in stability of chokeberry anthocyanin glycosides stored at 4 °C, 9 °C, and 21 °C for three months.

An important difference between ScCJ anthocyanins and those found in other fruits (such as blueberry, strawberry, or grape) is their molecular structure [5]. The main anthocyanin in ScCJ is cyanidin-3-glucosyl-rutinoside, which is a tri-glycoside, while in many other fruits, predominant forms of anthocyanins are mono-glucosides [5]. Glycosylation at C-3 and C-5 positions affects the perceived color of the anthocyanin pigment: tri-glucoside derivatives are more intensely colored than di-glucosides and monoglucoside, and acylation of glucose further increases stability of the compound [18]. Anthocyanidin glycosides are generally more stable than the corresponding aglycones [18].

Therefore, as far as structure stability of anthocyanins was concerned, it was observed that their degradation during storage depended rather on the storage conditions (time and temperature) than on the type of anthocyanin compounds present in the sour cherry fruits. Cyanidin-3-O-glucosyl-rutinoside was found to be slightly more stable than the other mentioned anthocyanin compounds. Similar effects were observed by Wilkes et al. [16] in chokeberry juices. They reported that after 180 days of storage, the chokeberry juices lost 75%, 76%, 64%, and 75% of cyanidin-3-galactoside, -3-glucoside, -3-arabinoside, and -3-xyloside, respectively. Mena et al. [15] pointed out that diglucosides of pomegranate juices were more stable than monoglucosides in samples stored at 5 °C (5% and 24% of loss, respectively). However, the same juices stored at 25 °C showed a different trend, and monoglucosides were much more stable anthocyanins (48% of loss for monoglucosides vs. 56% for diglucosides).

Anthocyanins are highly unstable compounds. Their stability, apart from temperature and structure, depends on such factors as pH, light, and the presence of accompanying substances, i.e., vitamin C or monomers of flavan-3-ols [15,19,20]. pH of the analyzed ScCJs was between 3.16 and 3.66 and the content of vitamin C was between 0.4 and 1.5 mg/L (data not presented). At pH higher than 2.0 anthocyanins are known to be thermally degraded through the formation of chalcone structure [21]. This can be associated with different forms of anthocyanin present at different pH, e.g., flavilium cationic form of anthocyanin is common in acidic solutions, whereas in neutral conditions less stable chalcone, carbinol, and quinonoidal forms prevail.

3.4. Kinetics of Anthocyanin Degradation during Storage of Sour Cherry Cloudy Juices

The loss of anthocyanins during storage (Tables 2 and 3) fitted the exponential curves for the two selected temperatures (Table 4). This indicated that the pigment degradation at isothermal conditions followed first-order reaction kinetics, as usually reported for red juices [13,15,22]. Kinetic parameters revealed a significant cultivar effect on the evolution of anthocyanins during storage at both 4 °C and

30 °C (Table 3). Half-life periods of the anthocyanins differed depending on the cultivar and storage conditions. The rate constant (k) increased at higher storage temperature and equaled from 0.662 to 1.929 and from 1.110 to 2.717 for 4 °C and 30 °C, respectively. This demonstrated a considerable effect of temperature on accelerated anthocyanin degradation as suggested by Alighourchi et al. [23] and Mena et al. [15]. Half-life values ($t_{1/2}$) for stored ScCJs ranged from 64.7 to 188.5 days at 4 °C and from 45.9 to 112.40 days at 30 °C. It meant that the long $t_{1/2}$ of anthocyanin degradation at 4 °C in ScCJ were made from Topas, Turgieniewka, Wanda, Dradem, Skierka cvs. (>150 days) and the lowest for Groniasta, Pandy, and Morina cvs. (<80 days). For sample stored at 30 °C the long $t_{1/2}$ of anthocyanin degradation were evaluated for ScCJ made from Turgieniewka cv. (>100 days) and the lowest for Skierka, Wilena, and Lucyna (<80 days). D values denoting the time required for a degradation of 90% of ScCJ anthocyanins ranged between 95.7 and 271.9 days for juices stored at 4 °C and between 66.2 and 158.3 days at 30 °C. The highest D values were noticed for ScCJ made from Topas, Turgieniewka, Dradem, Skierka, Wanda, and Wilena cvs. stored at 4 °C, and Turgieniewka, Topas, Safir, Wanda, Dradem, Karnelo and Sabina cvs. stored at 30 °C. The lowest D values (less than 100 days) were calculated for ScCJ made from Wilena and Groniasta cvs. stored at 4 °C, and Skierka, Kelleris 14, Groniasta, and Lucyna cvs. stored at 30 °C. In general, the time to degrade 90% of anthocyanins was longer during cold storage. The same observation was made in other studies [15,24].

Kinetic parameters ($t_{1/2}$ and D value) were clearly higher for Topas, Turgieniewka, Wanda, Wiblek, Sabina, and Łutówka cvs., regardless of storage conditions. This indicated that the stability of anthocyanins in stored ScCJ was closely associated with a cultivar and temperature. Mena et al. [15] reported that half-life values ($t_{1/2}$) for pomegranate juices stored at 5 °C ranged from 98 to 374 days and from 28 to 87 days at 25 °C. D values were between 141 and 539 days for juices stored at 5 °C and between 40 and 125 days for those stored at 25 °C. Bonerz et al. [13] claimed that half-life periods of monomeric anthocyanins varied and depended on their structure and cultivar. Similar effects were described by Mena et al. [15] and Wilkes et al. [16]. In sour cherry, the main anthocyanin is cyanidin-3-O-glucosyl-rutinoside. As for its structure stability, tri- and diglucosides seem to be more stable than monoglucosides and anthocyanin hexosides more stable than pentosides [15,16]. Each compound displayed its own specific decay, related to the sugar binding and the storage temperature. Moreover, it seems that the anthocyanins bound to glucose exhibited a faster degradation rate than those bound to galactose [25]. In regard to conjugated sugars, the ranking order was glucoside > galactoside > arabinoside from the most to the least stable. Bonerz et al. [13] demonstrated that cyanidin-3-O-glucosyl-rutinoside was the most stable pigment in sour cherry juices independent of cultivar, with a half-life period of 72–95 days, with peonidin-3-O-rutinoside showing the fastest degradation rate (29–54 days). Our outcomes confirmed that cyanidin-3-O-glucosyl-rutinoside was more stable than cyanidin-3-O-rutinoside and -3-O-soforoside that were still more stable than peonidin-3-O-rutinoside and cyanidin-3-O-glucoside.

This effect was observed for both types of storage conditions. Fracassetti et al. [25] present results where the reduction anthocyanin content of wild blueberry powder at 80 °C was > 90% after 3 days storage only. Major parameters determining the stability of anthocyanins are water activity (a_w) and water content, temperature, absence/presence of oxygen, light, and relative humidity. Additionally, this could be attributable to a matrix effect and/or a different pH because pH lower than 4.1 maintains anthocyanins stability.

Table 3. Summary of anthocyanins compounds content [mg/L] in cloudy sour cherry juices after 3 and 6 months of storage time at 4 and 30°C.

Sour Cherry Cloudy Juices	Storage Condition				
	0 m	3 m at 4 °C	6 m at 4 °C	3 m at 30 °C	6 m at 30 °C
Safir	595.83 ± 23.61 c	518.17 ± 25.72 b	68.87 ± 3.89 e	453.19 ± 31.55 b	35.55 ± 0.45 a
Topas	442.26 ± 20.52 f	382.34 ± 18.97 e	117.59 ± 7.66 b	358.43 ± 14.96 d	32.60 ± 1.29 b
Turgieniewka	308.18 ± 12.21 i	273.50 ± 13.57 gh	80.27 ± 2.18 d	244.52 ± 19.02 gh	31.70 ± 0.33 b
Wanda	407.73 ± 5.15 g	337.41 ± 26.74 f	84.78 ± 2.35 cd	295.99 ± 20.61 e	15.59 ± 1.51 d
Erdi Bötermö	309.76 ± 3.85 i	228.46 ± 11.33 i	25.30 ± 1.00 i	166.56 ± 9.59 j	6.12 ± 0.24 h
Karneol	352.93 ± 15.98 h	296.72 ± 14.72 g	44.71 ± 1.77 g	245.05 ± 12.06 gh	12.06 ± 0.47 f
Nord Star	342.45 ± 13.57 h	253.37 ± 12.57 hi	24.65 ± 1.65 i	171.82 ± 13.96 ij	6.00 ± 0.13 hi
Łutówka	358.96 ± 4.22 h	299.88 ± 4.88 g	48.13 ± 2.90 g	253.43 ± 7.64 fg	12.96 ± 0.31 f
Wiblek	601.92 ± 34.85 c	484.53 ± 14.04 c	89.15 ± 4.53 c	410.37 ± 28.57 c	20.91 ± 0.02 c
Wifor	454.64 ± 8.18 ef	354.35 ± 17.58 f	34.67 ± 0.37 h	276.07 ± 22.32 efg	10.30 ± 0.41 g
Debreceni Bötermö	170.17 ± 7.74 lm	98.16 ± 4.87 kl	8.02 ± 0.31 l	71.26 ± 7.96 l	2.57 ± 0.00 m
Sabina	348.60 ± 16.81 h	293.29 ± 14.55 g	55.17 ± 5.01 f	255.46 ± 13.78 fg	13.97 ± 0.05 e
Agat	252.92 ± 5.68 j	178.06 ± 5.22 j	13.41 ± 0.53 k	127.76 ± 5.89 k	5.77 ± 0.23 hij
Kelleris 14	478.05 ± 33.94 e	353.48 ± 11.54 f	45.64 ± 0.80 g	287.29 ± 17.18 ef	4.95 ± 0.19 ik
Dradem	39.99 ± 3.58 n	17.91 ± 1.12 m	0.48 ± 0.02 n	9.40 ± 0.65 m	4.34 ± 0.17 l
Skierka	1158.95 ± 33.92 a	1081.28 ± 43.66 a	286.05 ± 3.33 a	1017.49 ± 70.84 a	5.06 ± 0.21 l
Wilena	693.75 ± 44.49 b	551.80 ± 33.38 b	14.64 ± 0.98 k	476.24 ± 13.16 b	4.11 ± 0.09 l
Winer	343.92 ± 23.62 h	273.53 ± 3.57 gh	19.48 ± 0.07 j	208.28 ± 14.50 hi	4.53 ± 0.31 l
Wisok	562.51 ± 12.12 d	431.91 ± 11.45 d	49.36 ± 2.95 g	377.79 ± 29.31 cd	4.50 ± 0.07 l
Morina	372.23 ± 14.75 h	258.45 ± 9.82 hi	13.45 ± 0.44 k	210.86 ± 16.68 hi	4.03 ± 0.16 l
Lucyna	349.98 ± 9.87 h	270.18 ± 11.41 gh	24.38 ± 0.56 i	238.36 ± 11.59 gh	1.90 ± 0.05 n
Pandy	188.66 ± 9.47 kl	128.91 ± 7.39 k	6.01 ± 0.87 l	96.47 ± 6.71 kl	3.65 ± 0.15 l
Groniasta	201.05 ± 8.96 k	117.28 ± 9.03 kl	4.67 ± 0.07 lm	85.20 ± 5.93 l	1.58 ± 0.06 n
Nana	23.18 ± 1.91 n	10.85 ± 0.53 m	0.50 ± 0.10 n	7.74 ± 1.13 m	3.76 ± 0.09 l
Nagy Erdigymulscu	141.61 ± 10.61 m	96.05 ± 4.76 l	3.73 ± 0.33 lm	74.75 ± 7.20 l	4.49 ± 0.21 l

Table 4. Kinetic parameters of anthocyanin degradation in cloudy sour cherry juices during storage at different temperatures.

Sour Cherry Cloudy Juices	Stability of Anthocyanins of ScCJ Storage at 4 °C					Stability of Anthocyanins of ScCJ Storage at 30 °C				
	Variation Kinetics (Concentration in mg/L)	R ²	−k × 10 ³ (Day ^{−1})	t _{1/2} (Day)	D Value (Day)	Variation Kinetics (Concentration in mg/L)	R ²	−k × 10 ³ (Day ^{−1})	t _{1/2} (Day)	D Value (Day)
Safir	2396.794e ^{−1079x}	0.798	1.079	115.7	166.8	3562.000e ^{−1.410x}	0.822	1.410	88.5	127.7
Topas	1018.920e ^{−0.662x}	0.831	0.662	188.5	271.9	2345.462e ^{−1.304x}	0.810	1.304	95.7	138.0
Turgieniewka	726.152e ^{−0.673x}	0.816	0.673	185.4	267.5	1299.585e ^{−1.137x}	0.825	1.137	109.8	158.3
Wanda	1090.651e ^{−0.785x}	0.839	0.785	159.0	229.3	3228.664e ^{−1.632x}	0.823	1.632	76.5	110.3
Erdi Bötermö	1486.698e ^{−1.252x}	0.840	1.252	99.7	143.8	3446.582e ^{−1.962x}	0.865	1.962	63.6	91.7
Karneol	1320.599e ^{−1.033x}	0.812	1.033	120.8	174.2	2967.864e ^{−1.688x}	0.830	1.688	73.9	106.6
Nord Star	1789.955e ^{−1.316x}	0.835	1.316	94.8	136.8	4033.887e ^{−2.022x}	0.874	2.022	61.7	89.0
Łutówka	1290.502e ^{−1.005x}	0.817	1.005	124.2	179.1	2925.961e ^{−1.661x}	0.828	1.661	75.1	108.4
Wiblek	2000.217e ^{−0.955x}	0.834	0.955	130.7	188.5	4975.945e ^{−1.680x}	0.834	1.680	74.3	107.1
Wifor	2326.683e ^{−1.287x}	0.822	1.287	97.0	139.9	4808.504e ^{−1.894x}	0.847	1.894	65.9	95.0
Debreceni Bötermö	1085.732e ^{−1.527x}	0.880	1.527	81.7	117.9	2083.786e ^{−1.096x}	0.898	2.096	59.5	85.9
Sabina	1124.793e ^{−0.922x}	0.820	0.922	135.4	195.2	2683.797e ^{−1.609x}	0.822	1.609	77.6	111.9
Agat	1594.207e ^{−1.469x}	0.838	1.469	85.0	122.5	2504.111e ^{−1.890x}	0.880	1.890	66.0	95.2
Kelleris 14	2069.460e ^{−1.174x}	0.845	1.174	106.3	153.3	8491.887e ^{−2.285x}	0.832	2.285	54.6	78.8
Dradem	74.718e ^{−0.670x}	0.987	0.670	186.3	268.7	108.484e ^{−1.110x}	0.970	1.110	112.4	162.2
Skierka	2878.102e ^{−0.700x}	0.787	0.700	178.3	257.1	41542.963e ^{−2.717x}	0.768	2.717	45.9	66.2
Wilena	8417.307e ^{−1.929x}	0.794	1.929	64.7	93.3	18691.851e ^{−2.564x}	0.805	2.564	48.7	70.2
Winer	2160.469e ^{−1.436x}	0.809	1.436	86.9	125.3	5216.958e ^{−2.165x}	0.836	2.165	57.6	83.1
Wisok	2608.467e ^{−1.217x}	0.830	1.217	102.5	147.9	12315.356e ^{−2.414x}	0.811	2.414	51.7	74.6
Morina	3015.725e ^{−1.660x}	0.831	1.660	75.2	108.4	6293.191e ^{−2.263x}	0.843	2.263	55.1	79.5
Lucyna	1896.350e ^{−1.332x}	0.822	1.332	93.7	135.1	9968.625e ^{−2.608x}	0.805	2.608	47.9	69.0
Pandy	1652.523e ^{−1.723x}	0.832	1.723	72.4	104.5	2093.303e ^{−1.973x}	0.873	1.973	63.3	91.2
Groniasta	2063.496e ^{−1.881x}	0.855	1.881	66.3	95.7	3820.415e ^{−2.423x}	0.878	2.423	51.5	74.3
Nana	60.841e ^{−0.914x}	0.991	0.914	136.5	196.9	207.602e ^{−1.918x}	0.942	1.918	65.1	93.8
Nagy Erdigymulscu	589.538e ^{−1.167x}	0.871	1.167	106.9	154.2	1142.455e ^{−1.726x}	0.883	1.726	72.3	104.3

3.5. Changes in Color Quality During ScCJ Storage

Color of a beverage is of paramount importance as it is the first property to be noticed and one of the main ways of visual assessment of food before consumption. The perceived color provides an indication of the expected taste and quality of food [26]. Each anthocyanin has a particular hue, ranging from red to blue. Cyanidin derivatives, main anthocyanins of sour cherry juice are associated with reddish shades.

Color parameters differed significantly ($p < 0.05$) among ScCJs and storage times (Table S3). In general, a considerable general decrease in redness ($CIEa^*$) was observed for all sour cherry juices during storage. Redness of ScCJ before storage varied from 0.9 to 6.35. The most intense light red color was found in the ScCJ made from Dradem (6.35), Nana (5.48), Nagy Erdigymulscu (5.22), and Karneol (4.56) cvs. and it was the least intense in the juices made from Wiblek (0.9) and Skierka (1.09) cvs. In juices stored at 4 °C, $CIEa^*$ values remained fairly stable, especially after 90 days of storage and a significant change occurred at the end of storage at 30 °C. After 90 days of storage redness slightly ($p > 0.05$) increased in some ScCJ samples. At 30 °C a^* values significantly decreased ($p < 0.05$) and ranged from 3.25 to 0.66 and from 3.54 to 0.18 after three and 180 days, respectively. The change of redness in the samples stored at 4 °C was slower, and amounted from 9.83 to 0.74, and from 6.94 to 0.86 for 90 and 180 days, respectively. ScCJ made from Dradem, Morina and Nana cvs. showed the highest lightness, whereas the juice from Wilena cv. displayed the lowest one. Only a slight change in $CIEL^*$ parameter was observed over the entire storage period. Taking into account both redness ($CIEa^*$) and yellowness ($CIEb^*$), a similar trend was found for all the samples. Contrary to redness, little variation was observed in yellowness ($CIEb^*$). Pilando et al. [27] reported a strong negative correlation of both values with total anthocyanins, and suggested that the increased L^* and b^* resulted from anthocyanin loss. The increase in lightness and decrease in redness were in accordance with the trend followed by red color products made from pomegranate [15,28] maqui [29], and aronia [30] juices or other beverages rich in anthocyanins in a model system [31].

Chroma value and hue angle remained more stable than a^* and b^* in all samples, without notable differences after storage at 4 °C, especially after 90 days. These parameters decreased by half for ScCJ stored at 30 °C. However, the rate of browning, determined by hue angle values (less than 20° difference between initial and end values), was much slower than the rate of anthocyanin degradation, as reported in other red beverages [29]. This could be explained by the fact that regardless of anthocyanin loss during shelf life, red coloration of all ScCJs remained stable during the first 90 days of storage as a result of likely formation of other colored polymers, or copigmentation between anthocyanins and other flavonoids that appreciably preserved color and masked the detrimental changes during storage in the anthocyanins [32]. Despite the loss in total anthocyanin content over the storage period, red color remained stable until the end of the storage as a result of copigmentation between anthocyanins and flavonols that could also modify the color expression by changing tone shift towards purple tonalities over time as previously observed for red wine sample [33], and chokeberry juice [16]. The same effect was repeatedly reported by González-Molina et al. [28,30] and Wojdyło et al. [34] for juices made from aronia, pomegranate and maqui fruits. However, it is also possible that the anthocyanin polymers of flavan-3-ols formed in the juices during processing were more resistant to degradation during storage.

Likewise, to determine a relationship between the anthocyanin levels and color parameters of ScCJ, Person correlation test was carried out. $CIEL^*$, a^* , and b^* negatively correlated with total anthocyanin level ($r = -0.699$, $r = -0.685$ and $r = -0.657$, respectively), as well as with Chroma and hue angle, which showed the strongest negative correlation ($r = -0.672$ and $r = -0.768$). A significant but weaker correlation was determined for anthocyanins in the samples stored at 4 °C ($r = -0.594$; $r = -0.664$, $r = -0.615$ between anthocyanins and L^* , a^* , b^* parameters, respectively). Not significant and the weakest correlation was identified for the samples stored for 180 days. A similar tendency was found in the samples stored at 30 °C but the correlation values were lower. Therefore, color parameters might be taken into account as anthocyanin content indicator in ScCJs, especially for the samples

before storage or stored for 90 days. After 180 days of storage lower correlations suggested that other ingredients of ScCJ than anthocyanin were affected by the color.

4. Conclusions

The investigated ScCJ were characterized by high content of anthocyanins which may have direct implications on juice industry and help to solve browning problems during juice storage. The content of anthocyanins and color parameters load for ScC juices were mainly influenced by cultivar. The dominant anthocyanin was cyanidin-3-glucosyl-rutinoside (25–75%). Total anthocyanin content differed significantly between juices, with values varying from ~590 to ~1160 mg/L. ScCJ made from Skierka, Nagy Erdigymulscu, Wilena, Wiblek, and Safircvs had the highest content of anthocyanins. Degradation of anthocyanins during the juice storage depended rather on the storage conditions (time and temperature) than on the type of anthocyanin compounds present. Kinetic parameters revealed a significant cultivar effect on the evolution of anthocyanins during storage at both 4 °C and 30 °C. Half-life value of the anthocyanins differed and depended on the cultivar and storage conditions. Half-life values of ScCJ ranged from 64.7 to 188.5 days at 4 °C and from 45.9 to 112.40 at 30 °C. Redness of the samples changed more rapidly than yellowness or lightness and Chroma or hue angel. Summing up, the study results may have direct implications for the juice industry as they address problems with deterioration of attractiveness of sour cherry cloudy juices due to loss of anthocyanins and browning during storage. Further studies on bioavailability are necessary to evaluate possible benefits of sour cherry cloudy juices for human health.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2227-9717/7/6/367/s1>, Table S1: Content of anthocyanins compounds [mg/L] in cloudy sour cherry juices after 180 days of storage time. Table S2: Content of anthocyanins compounds [mg/L] in cloudy sour cherry juices after 180 days of storage time. Table S3. Evolution of color parameters in sour cherry cloudy juices through storage time (180 days) at 4 and 30 °C.

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