horticulturae

Article

Effects of Ripening Phase and Cultivar under Sustainable Management on Fruit Quality and Antioxidants of Sweet Cherry

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Abstract: Sweet cherry grown under sustainable management produces highly valuable fruits, whose quality shows important biochemical and morphological changes during ripening. Research was carried out in Iasi (Romania), with the aim to assess the quality characteristics of the sweet cherry fruits of three cultivars (Van, Andreiaș, Margonia), grown in an inner or outer position inside the tree crown, at the pre-ripening or full ripeness phase. In 2022, the colour component a* showed higher values in cv. Van and Andreias red fruits and in an inner position, whereas the components L* and b* at the full ripeness phase were highest in cv. Margonia. The dry matter and total soluble solids contents increased from the pre-ripening to the full ripeness phase and were highest in cv. Van sweet cherry fruits; the DM of fruit from the outer part of crown was higher than that of fruit from the inner part at the pre-ripening phase. The content of phenolics was the highest in cv. Margonia fruits at the pre-ripening stage and in cv. Van at the full ripeness phase and higher in the inner tree crown zones. The cultivar Margonia generally showed the highest vitamin C content in both years and development phases. The yellow fruit cv. Margonia mostly showed the highest values of chlorophyll a and b. The fruit's content of carotene, lycopene, and anthocyanins was generally the highest in the red fruits of cv. Andreias. The examined sweet cherry cultivars showed a high variability in fruit nutritional quality and proved to be a rich source of bioactive compounds with antioxidant potential.

Keywords: *Prunus avium* L.; organic farming; vitamin C; polyphenols; carotenoids; chlorophylls

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Citation: Mineață, I.; Murariu, O.C.; Sîrbu, S.; Tallarita, A.V.; Caruso, G.; Jităreanu, C.D. Effects of Ripening Phase and Cultivar under Sustainable Management on Fruit Quality and Antioxidants of Sweet Cherry. *Horticulturae* **2024**, *10*, 720. https://doi.org/10.3390/horticulturae10070720

Academic Editors: Simona Fabroni, Francesco Montemurro and Luana Bontempo

Received: 11 June 2024
Revised: 30 June 2024
Accepted: 3 July 2024
Published: 8 July 2024

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1. Introduction

Sweet cherry (*Prunus avium* L.) is a fruit tree species belonging to the family Rosaceae, particularly appreciated for its fruits which are the first fresh fruits of the year [1] and show premium quality characteristics in terms of colour, sweetness, sourness, firmness, and nutrients that are beneficial to health [2].

The consumer preference for sweet cherries is mainly influenced by taste and colour, but in recent years also by their nutritional value and the content of bioactive compounds such as anthocyanins, polyphenols, carotenoids, vitamins C and E, and antioxidants [3,4]. Moreover, an increasing percentage of consumers have been paying attention to aspects related to the whole production chain's sustainability in terms of the balance between resource input and yield output, impact on the environment, nutritional value of the products, and respect of workers' rights.

There are important factors, i.e., the cultivar, climate conditions, ripening stage, and crop management, that influence the content of bioactive compounds, starting from the growth stage of the fruit until the moment of harvesting [3].

Physiologically, sweet cherry is a non-climacteric fruit and goes through three stages of development before ripening: stage I, which begins after flowering and is followed by a rapid growth and division of the mesocarp cells; stage II, with the volume of the fruit augmenting slowly and the endocarp becoming lignified; and stage III, when the fruit's volume increases quickly again, and ripening occurs with biochemical changes [5,6].

In the last phenological stage of development, i.e., the full ripening, sweet cherry fruits undergo important biochemical and morphological changes, including the enhancement of colour and sugar content [7], which are the main indicators of maturity for commercial harvesting. However, fruit maturity varies within and between single trees and depends on the cultivar, meteorological conditions [8], agronomic practices [9], canopy management, fruit load [10], and fertilization [11]. The mentioned factors need to be examined from the perspective of crop system sustainability, mainly from the technical, nutritional, and environmental points of view.

Physicochemical studies are crucial to appropriately manage pre-harvest and harvest technology for sweet cherry production worldwide [12].

According to previous works related to some *Prunus* species, variations in quality parameters and fruit size reportedly depend on the position of the fruit within the crown of the tree [13,14]. Particularly, the location should be taken into account of both the fruit inside the tree, i.e., the sunny or shady side, and the tree within the orchard [15].

Previous studies [9,16–18] highlighted that the ripening stage influences the quality of sweet cherries by visibly changing the fruit colour and skin intensification, measurable by the parameters L* (luminosity), a* (red–green), and b* (yellow–blue), which turn from green to red or yellow depending on the cultivar, as chlorophyll pigments are degraded and anthocyanins accumulate.

Dry matter and total soluble solids contents increase during the development of fruits [18,19], which become much sweeter during ripening due to the sugar concentration increase, while acids (malic acid) remain relatively constant or decrease [1].

The purpose of this study was to evaluate the effects of the cultivar and position of the fruits inside the tree crown, at the pre-ripening or full ripeness phases, on the quality parameters of organically grown sweet cherry fruits.

2. Materials and Methods

2.1. Experimental Protocol and Growing Conditions

Research was carried out in 2022 and 2023 at the experimental field of the Research Station for Fruit Growing (RSFG) in Iași, North-East Romania (47°20′ N; 27°60′ E and 165 m altitude), in a sweet cherry orchard planted in a meso-relief with a gently sloping plateau and predominantly cambic chernozem soil with good natural drainage [20], pH 7.3, 4.0% humus content, and 0.19% N.

The meteorological conditions at the experimental field during the research years (2022–2023) were monitored by an Agroexpert system and are presented in Table 1.

Table 1. Average monthly values of temperature, rainfall, and relative humidity during the experimental period (RSFG Iași, Romania, 2022–2023).

Month	Air Temperature (°C) a*		Precipitation (mm) L*		Relative Humidity (%)	
	2022	2023	2022	2023	2022	2023
March	3.2	6.6	56.9	5.8	60.0	58.0
April	10.0	9.7	58.0	2.0	59.1	66.0
May	16.6	16.2	17.4	5.0	50.5	54.0
June	21.9	20.7	26.6	10.0	53.6	53.0
July	23.2	23.3	27.8	91.5	50.0	51.0

The experimental protocol was based on the factorial combination between three cultivars (Van, Andreiaș, Margonia) and two fruit positions inside the tree crown (inner, outer). A split plot design was used for the treatment distribution in the field, with three replicates, with a density of 500 plants ha^{-1}.

Sweet cherry samples were harvested from the sampled trees at two different fruit development phases: pre-ripening, with fruit at about 90% of their final size and beginning colouring; and full ripeness.

The three cultivars of sweet cherry (Van, Andreiaș, and Margonia) were provided by the Experimental Orchard Fields of RSFG, Iași. The trees of the mentioned cultivars were reared on high-vigour rootstocks of *Prunus mahaleb* L., spaced 5 m along the rows, which were 4 m apart, and trained as a free palmette. The soil was ploughed using a rotary tiller along the rows and covered by grass between the rows.

The cultivar Van originated from Canada and is an early and very productive cultivar, with medium to large fruits (7.5–8.5 g), a globular shaped, bright red colour, stony pink-red flesh, and a sweet acidic taste [21].

Andreiaș and Margonia are two new cultivars of sweet cherry, created and certified at RSFG Iași. Andreiaș has large (8.5–9.5 g), cordiform, red-brown fruit, almost black when fully ripe, and the cultivar Margonia has yellow, cordiform, slightly flattened fruit, elongated at the tip, with an average weight of 8.0–9.0 g [22].

The crops were organically managed [23], using the formulations permitted under the European regulation both for fertilization and plant protection.

2.2. Sampling Procedures

Fruit samples were harvested in all the plots, put in biodegradable bags, and immediately transported under appropriate conditions to the laboratory for analysis (Figure 1).

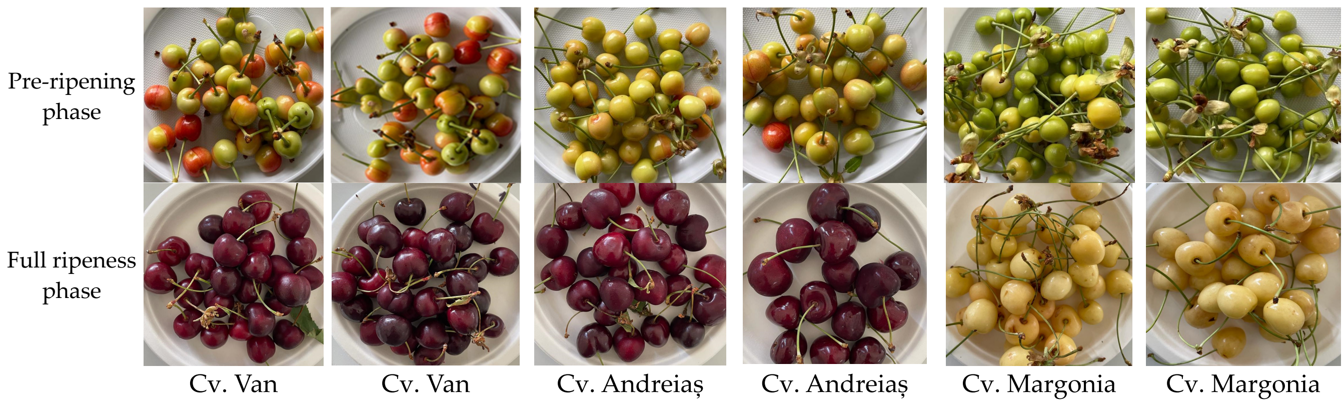

Figure 1. Sweet cherry cultivars at the two fruit development phases examined.

2.3. Qualitative Analyses

2.3.1. Measurement of Epidermis Colour

The fruit's epidermis colour was measured using a Minolta CR 400/410C portable colorimeter (Konica Minolta Measuring Instruments, München, Germany), referring to the CIE *L** *a** *b** uniform colour space. The value of *L** reflects the colour lightness, *a** the proportions of red (positive values) and green (negative values), and *b** the proportion of yellow (positive values) and blue (negative values). The parameter *L** defines the colour intensity, while *a** and *b** define the colour chromaticity [24,25].

2.3.2. Total Soluble Solids (TSS)

TSS content was determined by the refractive index of the fruit juice, measured using a Zeiss precision portable refractometer (Jena, Germany), and reported in °Brix.

2.3.3. Dry Matter

The total dry matter content was measured by drying 5 g of fruit samples (without stone) in an oven at 103 ± 2 °C until constant weight [26].

2.3.4. pH and Titratable Acidity

The pH value of the liquid samples was determined using the potentiometric method using a Hanna Instruments (Laval, QC, Canada) pH meter at 20 °C. Due to the similarity of the liquid fruit sample preparation, pH and titratable acidity (TA) were measured simultaneously [27].

To determine the titratable acidity (TA), the samples were homogenized with distilled water and titrated with 0.1 N NaOH until reaching pH 8.1. The results were expressed as percentage of malic acid content and calculated using Formula (1) below:

$$\text{TA (\% malic acid)} = (V \times N \times 100 \times 0.0067)/m \quad (1)$$

where N is the normality of NaOH; V is the volume of NaOH (mL) used in the titration; m is the weight of the sweet cherry sample used (g); and 0.0067 is the conversion factor for malic acid [12]. TA has been calculated in terms of the most common acid in the sample, which for different cultivars of sweet cherry is usually malic acid [15].

2.3.5. Carotene and Lycopene

The identification of carotene and lycopene in sweet cherry fruits (both peel and pulp) was carried out spectrophotometrically with petroleum ether. The ether extract was read by a UV/Vis Specord 210 Plus spectrophotometer (Analytik Jena, Jena, Germany) at 472 nm for lycopene and 452 nm for carotene. The obtained values were integrated into the following formulas [28].

$$\begin{gathered}\text{Carotene} = \text{A452} \times 19.96, \text{ in mg } 100 \text{ g}^{-1} \\ \text{Lycopene} = \text{A472} \times 14.495, \text{ in mg } 100 \text{ g}^{-1}\end{gathered} \quad (2)$$

2.3.6. Anthocyanin Content

The anthocyanin content was determined using a spectrophotometric version method based on the differential pH of 1.0 to 4.5 [29,30] with an acidulated alcoholic solution and measurement at a 515 nm wavelength by a Specord 210 PLUS UV-VIS spectrophotometer (Analytik Jena, Germany) [31].

2.3.7. Chlorophyll Pigments

The content of chlorophyll pigments (chlorophyll *a* and *b*) in the sweet cherry fruits was determined by the spectrophotometric method described by Jităreanu et al. [32], based on the light absorption capacity of the acetonic extract (1%) and analysed by a computer-aided spectrophotometer at 647 and 663 nm absorption based on Lichtenthaler's equations [33,34]:

$$\begin{gathered}Ca = 12.25 \times A663 - 2.79 \times A647 \\ Cb = 21.50 \times A647 - 5.10 \times A663 \\ Ca + b = 7.15 \times A663 - 18.71 \times A647\end{gathered} \quad (3)$$

2.3.8. Total Phenolic Content

The determination of the content of total phenolic compounds was carried out according to the Folin–Ciocalteu [35] by reading values on a spectrophotometer at a 750 nm wavelength. The results reveal the antioxidant activity and are expressed as mg of gallic acid equivalents (GAEs) per 100 g of sample [36].

2.3.9. Vitamin C

In order to determine the vitamin C content of the sweet cherry samples, the 2.6-dichloroindophenol titrimetric method of juice analysis was used (AOAC Method 967.21) [37]. Samples were fused with 40 mL of 2% oxalic acid, and after titration (2.6-dichloroindophenol), the results were expressed in mg ascorbic acid 100 g^{-1} fruit [38].

2.4. Statistical Analysis

The data were statistically processed by two-way analysis of variance, and Duncan's multiple range test was performed for mean separation at $p \leq 0.05$ using the SPSS software version 29. The standard deviation (STDEV) and the Pearson coefficient of variation (COVAR) were also determined [39].

3. Results and Discussion

As no significant interactions arose between the two experimental factors applied, i.e., cultivar and fruit position inside the tree crown, only their simple effects are presented.

The colour of fresh sweet cherry fruits is among the primary factors influencing consumer choice, because it is an important quality attribute, also serving to estimate the maturity and harvesting stage [40].

With regard to the fruit's chromatic parameters (*L**, *a**, *b**), in 2022, the component *a** showed significantly higher values in the red fruits of the cultivars Van and Andreias and in the inner position inside the tree crown, both at the pre-ripening and full ripeness phase (Table 2). In the same year, the components *L** and *b** were significantly affected by cultivar only at the full ripeness phase, with the highest levels recorded in the yellow fruits of the cultivar Margonia. In 2023 (Table 3), the cultivars Van and Margonia attained higher values of the three colour components at the pre-ripening phase, whereas similar trends to the previous year were recorded at the full ripeness phase. No colour components were significantly influenced by the fruit's position inside the tree crown.

Table 2. Fruit skin colour of three sweet cherry cultivars at two development phases, examined in 2022.

Treatment	Pre-Ripening Phase			Full Ripeness Phase		
	L*	a*	b*	L*	a*	b*
Cultivar						
Van	56.7	0.57 a	38.1	20.4 b	17.1 a	1.7 b
Andreiaș	56.7	0.43 b	39.9	19.8 b	15.7 a	1.5 b
Margonia	57.0	0.32 c	40.7	60.8 a	0.9 b	35.3 a
	n.s.		n.s.			
Fruit position						
Inner	58.7	0.59	41.2	33.6	12.0	13.3
Outer	54.9	0.28	37.9	33.8	10.4	12.4
	n.s.	*	n.s.	n.s.	*	n.s.
Av.	56.79	0.44	39.55	33.67	11.22	12.83
STDEV	2.15	0.25	2.25	21.01	8.20	17.43
COVAR s%	3.79	56.66	5.68	62.39	73.06	135.82
Min.	54.32	0.11	37.18	19.75	0.14	1.00
Max.	59.09	0.74	42.55	60.79	19.30	36.26

n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

Compared to the year 2022, in the meteorological conditions recorded in 2023, there was generally a decrease in the values of colour parameters of Andreias fruits at the pre-ripening phase; moreover, the component *a** was not affected by the fruit's position inside the crown.

As for the comparison between the two development phases, the yellow fruits of the cultivar Margonia showed a stability of values, whereas the red fruits of cv. Van and Andreias exhibited a decrease in *L** and *b** components and an increase in the *a** component during the ripening process.

The differences between the two phenological phases or between the inner and outer crown area can be useful in evaluating the fruit ripening process to compare the quality of cultivars and the influence of climatic conditions and to determine the time required to reach full maturity. In this respect, compared to the year 2022, in 2023, the time interval from the pre-ripening stage to full ripening was longer due to the lower average temperature, which caused a slower but more efficient accumulation of anthocyanin pigments in the red fruits, associated with higher values of the *a** parameter.

Table 3. Fruit skin colour of three sweet cherry cultivars at two development phases, examined in 2023.

Treatment	Pre-Ripening Phase			Full Ripeness Phase		
	L*	a*	b*	L*	a*	b*
Cultivar						
Van	57.5 a	0.67 a	38.5 a	20.5 b	17.5 a	1.6 b
Andreiaș	51.8 b	0.35 b	30.4 b	20.7 b	17.8 a	2.0 b
Margonia	58.2 a	0.72 a	38.3 a	59.2 a	1.9 b	35.4 a
Fruit position						
Inner	56.3	0.59	36.0	33.6	12.4	13.4
Outer	55.3	0.56	35.4	33.3	12.3	12.6
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Av.	55.8	0.6	35.7	33.4	12.3	13.0
STDEV	3.3	0.3	4.2	19.5	8.1	17.3
COVAR s%	6.0	43.4	11.8	58.2	65.6	133.6
Min.	51.3	0.1	30.2	20.0	1.9	0.9
Max.	59.3	0.8	39.8	60.3	18.1	36.9

n.s.: not significant. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

The chromatic values recorded in our investigation are consistent with those detected by Goncalves et al. [41] in sweet cherry fruits of the cultivar Van at the partial and full ripeness phases. The results of the present research are also similar to those obtained by Girad and Kopp [42], who stated that darker sweet cherries (lower *L** values) tended to be less red (lower *a** value) and less yellow (lower *b** value). The maximum values of the colour component *L** corresponded to the minimum values of the hue parameters *a** and *b**. Similar colour variations during fruit ripening also occurred in sour cherries, with the skin colour parameters *L**and *b** decreasing uniformly [40,43].

Both in 2022 (Table 4) and in 2023 (Table 5), at the two development phases examined, the dry matter (DM) and total soluble solids (TSS) contents of cv. Van sweet cherry fruits were highest (on average, 17.7% and 12.9 °Brix, respectively), except in 2022, when at the full ripeness phase, the DM of Van did not significantly differ from that of Andreias. Moreover, the DM of the fruit in the outer brighter part of crown was significantly higher than in the inner part in both years but only at the pre-ripening phase.

Table 4. Dry matter and total soluble solids content of fruits from three sweet cherry cultivars at two development phases, examined in 2022.

Treatment	Pre-Ripening Phase		Full Ripeness Phase	
	DM (%)	TSS (°Brix)	DM (%)	TSS (°Brix)
Cultivar				
Van	13.1 a	10.5 a	21.4 a	14.8 a
Andreiaș	11.5 b	9.5 b	20.9 a	12.4 b
Margonia	11.9 b	9.4 b	18.0 b	12.5 b
Fruit position inside the crown				
Inner	11.6	9.5	19.9	13.5
Outer	12.8	10.1	20.2	13.0
	*	n.s.	n.s.	n.s.
Av.	12.17	9.80	20.06	13.23
STDEV	1.07	0.66	1.66	1.29
COVAR s%	8.81	6.77	8.29	9.71
Min.	10.78	9.03	17.93	11.90
Max.	13.30	10.81	21.80	14.80

TSS: total soluble solids; DM: dry matter content. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

Table 5. Dry matter and total soluble solids content of fruits from three sweet cherry cultivars at two development phases, examined in 2023.

Treatment	Pre-Ripening Phase		Full Ripeness Phase	
	DM (%)	TSS (°Brix)	DM (%)	TSS (°Brix)
Cultivar				
Van	13.5 a	10.8 a	22.7 a	15.3 a
Andreiaș	11.5 b	9.2 b	20.5 b	12.5 b
Margonia	11.7 b	9.5 b	19.7 b	12.7 b
Fruit position inside the crown				
Inner	11.5	9.6	20.4	13.2
Outer	12.9	10.1	21.6	13.8
	*	n.s.	n.s.	n.s.
Av.	12.20	9.85	20.97	13.50
STDEV	1.27	1.12	1.53	1.58
COVAR s%	10.37	11.39	7.29	11.73
Min.	10.69	8.00	19.20	12.08
Max.	13.90	10.93	23.53	16.33

TSS: total soluble solids; DM: dry matter content. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

Either the dry matter or the soluble solids content of sweet cherry fruits of the three cultivars examined always increased from the pre-ripening to the full ripeness phase, independently of the year, with the top values being those of cv. Van in 2023 (22.7% and 15.3 °Brix).

Total soluble solids (TSS), predominantly estimating the level of dissolved sugars (sucrose, glucose, and fructose) in fruit juice but also including a small number of other compounds such as organic acids, soluble amino acids, and minerals [15], are an important quality parameter that has a remarkable influence on the fruit taste.

Consistently with our results, in previous research carried out on sweet cherry cultivars, both the DM and TSS contents increased during the fruit ripening, in the same agricultural area and with the same cultivars as in the present experiment [44] or in other locations [17,45–47].

Many scientific investigations have shown that the TSS vary between 11 °Brix and 25 °Brix in sweet cherries in several areas of the world. In the Spanish cultivars Sweetheart and Picota, a TSS range of 14.0–23.2 °Brix was reported by Serradilla et al. [48] and Gonzalez-Gomez et al. [49]. In the cultivars Bing and Summit, average TSS values of 13.3 and 19.6 °Brix were recorded, respectively [12]. Moreover, Wen et al. [50] detected TSS values between 17.8 and 20.0 °Brix for the Chinese sweet cherry cultivars Rainier, Hongyan, and Hongdeng.

In previous research, the meteorological trend in the last month of fruit formation exerted the greatest influence on TSS accumulation in sweet cherry fruit, regardless of the ripening phase [51,52], which has an important impact on the flavour evaluation [53].

Important physicochemical parameters, such as pH, titratable acidity (TA), and nutraceuticals (total polyphenols, ascorbic acid, and pigments) influence the consumer's perception of fruit quality [18,44].

Neither the pH or titratable acidity values in the sweet cherry fruits (on average, 3.87 and 1.04 g 100 g^{-1} malic acid, respectively) were significantly influenced by the cultivar and ripening process, both in 2022 and 2023 (Tables 6 and 7). In previous research, sweet cherry juice had a higher pH than the sour one [54,55], and the levels of both the juice's pH and total acidity were proven to also be genotype-dependant [56].

Acidity is essential for the consumer's perception of a good sweet cherry flavour [11].

According to other studies carried out on sweet cherries [45–47], TA widely ranged between 0.39% and 0.87%, but based on Crisosto et al.'s reports [16], the framing limits of TA in ripe sweet cherries vary from 0.5 to 1.0 mg malic acid 100 g^{-1} f.w.

Table 6. Biochemical parameters of fruits of three sweet cherry cultivars at two development phases, examined in 2022.

Treatment	Pre-Ripening Phase				Full Ripeness Phase			
	pH	**TA**	**TP**	**Vit C**	**pH**	**TA**	**TP**	**Vit C**
Cultivar								
Van	3.83	1.12	124.1 c	5.3 c	3.76	0.95	77.2 a	17.5
Andreiaș	4.02	1.05	156.2 b	9.0 b	3.85	0.92	65.6 b	17.5
Margonia	4.03	1.15	186.9 a	9.8 a	3.85	0.87	63.8 b	18.8
	n.s.	n.s.			n.s.	n.s.		n.s.
Fruit position								
Inner	3.96	1.14	156.8	7.7	3.80	0.92	75.4	17.0
Outer	3.95	1.07	154.8	8.3	3.84	0.91	62.3	18.8
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
Av.	3.96	1.11	155.75	8.00	3.82	0.91	68.85	17.92
STDEV	0.10	0.08	28.82	2.28	0.06	0.04	11.70	1.28
COVAR s%	2.60	7.39	18.50	28.50	1.54	4.68	17.00	7.15
Min.	3.82	1.00	122.43	4.50	3.74	0.85	62.11	16.00
Max.	4.04	1.21	193.33	10.50	4.00	0.98	92.13	19.50

TA: titratable acidity, as mg malic acid 100 g^{-1} f.w.; TP: total phenolic content, as mg GAE 100 g^{-1} f.w.; vit C: vitamin C, as mg 100 g^{-1} f.w. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

Table 7. Biochemical parameters of fruits of three sweet cherry cultivars at two development phases, examined in 2023.

Treatment	Pre-Ripening Phase				Full Ripeness Phase			
	pH	**TA**	**TP**	**Vit C**	**pH**	**TA**	**TP**	**Vit C**
Cultivar								
Van	3.85	1.15	145.8 b	5.5 c	3.77	0.97	75.8 a	17.3 b
Andreiaș	3.71	1.12	152.8 b	8.0 b	3.90	1.01	60.0 b	16.5 b
Margonia	4.04	1.21	178.4 a	10.0 a	4.00	0.95	60.1 b	20.3 a
	n.s.	n.s.			n.s.	n.s.		
Fruit position								
Inner	3.88	1.20	164.4	8.0	3.85	0.94	74.5	17.5
Outer	3.85	1.12	153.6	7.7	3.92	1.01	56.2	18.5
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
Av.	3.87	1.16	159.00	7.83	3.89	0.97	65.31	18.00
STDEV	0.16	0.08	17.02	2.75	0.12	0.06	14.33	2.51
COVAR s%	4.08	7.27	10.70	35.12	3.08	5.87	21.94	13.94
Min.	3.66	1.03	142.05	5.00	3.75	0.87	54.68	14.00
Max.	4.08	1.26	182.67	11.50	4.00	1.02	92.99	20.50

TA: titratable acidity, as mg malic acid 100 g^{-1} f.w.; TP: total phenolic content, as mg GAE 100 g^{-1} f.w.; vit C: vitamin C, as mg 100 g^{-1} f.w. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

In both studied years, the contents of phenolic compounds (Tables 6 and 7) were significantly the highest in cv. Margonia fruits at the pre-ripening phase (on average, 182.7 mg GAE 100 g^{-1} f.w.) and cv. Van at the full ripeness phase (on average, 76.5 mg GAE 100 g^{-1} f.w.). Moreover, on average, phenolics showed an over 50% decrease from the pre-ripening to the full ripeness phase in both years.

The polyphenol content was higher in the fruits located in the inner part of the crown but only at the full ripeness phase.

Sweet cherry fruits are considered a very good source of phenolic compounds that are beneficial in a human diet [51]. In the present experiment, the variation in polyphenols contents in fruits mainly depended on the examined cultivars and development phase.

Similar values of TP as in the present research were detected in the ripe fruits of some sweet cherry cultivars by other authors [51,57,58], but in the premature stage, the fruits had a much higher content.

Sweet cherry fruits are appreciated by consumers for their good taste but also for their many healthy nutritional properties, such as the high vitamin C content [51].

As can be observed in Tables 6 and 7, in our study, the cultivar Margonia showed the highest vitamin C content in both years and development phases (14.7 mg·100 g^{-1} f.w., on average), though no significant differences between the cultivars were found at the full ripeness phase in 2022. Only in the latter case, a significant difference was recorded between the positions inside the crown, because the outer sweet cherry fruits had higher vitamin C contents than the inner ones. The fully ripe fruits showed a more than doubled vitamin C content compared to the unripe ones.

Contrary to the reports by Średnicka-Tober [59], indicating that the fruits of sweet cherry were characterized by a significantly higher content of vitamin C, up to 42.9 mg 100 g^{-1} f.w., in other studies [51,60], depending on the cultivar and the meteorological conditions of the research year, the average content of vitamin C in sweet cherries varied between 5.3 and 20.3 mg 100 g^{-1} f.w.

The phenological phase of full ripeness in sweet cherry fruits influences the content of secondary compounds, such as pigments, which enhance the nutritional and health value of a fruit and, in addition, their evolution visually appeals consumers in terms of fruit colouration [61].

As for the fruit's chlorophyll pigments, in both years (Tables 8 and 9), the yellow fruit cv. Margonia showed the highest values of chlorophyll a and b, either at the pre-ripening (22.0 mg 100 g^{-1} f.w. chlorophyll a and 36.9 mg 100 g^{-1} f.w. chlorophyll b, on average) or at the full ripeness phase (3.9 mg 100 g^{-1} f.w. chlorophyll a and 6.8 mg 100 g^{-1} f.w. chlorophyll b, on average), but in 2023 (Table 9), it did not significantly differ from Andreias at the full ripeness phase. Moreover, only in the latter context was a significant difference recorded between the two fruit positions inside the tree crown, with the outer fruits attaining higher chlorophyll a contents than the inner ones.

Differently from chlorophyll pigments, in both years (Tables 8 and 9), the fruit's contents of carotene and lycopene were highest in the red fruits of cv. Andreias (0.39 mg 100 g^{-1} f.w. carotene and 0.31 mg 100 g^{-1} f.w. lycopene, as an average of the year and development phase), but in 2022 at the full ripeness phase, the carotene level did not significantly differ from that of cv. Margonia. The anthocyanin trend was exactly the same as that of lycopene, but its values were much higher at the full ripeness phase in both years (38.2 mg 100 g^{-1} f.w., on average) compared to the pre-ripening phase (1.5 mg 100 g^{-1} f.w., on average).

Table 8. Pigment content of fruits of three sweet cherry cultivars at two development phases, examined in 2022.

Treatment	Pre-Ripening Phase					Full Ripeness Phase				
	Chl a	**Chl b**	**Car**	**Lyc**	**Anth**	**Chl a**	**Chl b**	**Car**	**Lyc**	**Anth**
	mg 100 g^{-1} f.w.									
Cultivar										
Van	4.9 c	9.3 c	0.23 b	0.06 b	0.73 b	1.45 c	2.55 c	0.11 b	0.25 b	21.6 b
Andreiaș	17.7 b	27.1 b	0.45 a	0.27 a	1.24 a	2.31 b	5.20 b	0.28 a	0.35 a	36.9 a
Margonia	22.2 a	36.5 a	0.09 c	0.09 b	0.34 c	3.11 a	6.41 a	0.28 a	0.07 c	4.7 c
Fruit position										
Inner	14.6	25.2	0.26	0.14	0.74	2.24	4.58	0.22	0.21	22.2
Outer	15.2	23.4	0.25	0.14	0.79	2.33	4.86	0.22	0.22	19.9
	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Av.	14.91	24.30	0.26	0.28	0.77	2,29	4.72	0.22	0.22	21.05
STDEV	8.06	12.63	0.17	0.33	0.44	0.75	1.78	0.09	0.13	14.58
COVAR s%	54.09	51.97	66.68	118.41	56.78	32.58	37.72	40.49	58.07	69.26
Min.	4.84	8.71	0.07	0.05	0.21	1.41	2.24	0.10	0.05	4.50
Max.	23.44	37.35	0.48	0.92	1.47	3.21	6.44	0.29	0.35	40.52

Chl a: chlorophyll a, as; Chl b: chlorophyll b; Car: carotene; Lyc: lycopene; Anth: anthocyanin. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

Table 9. Pigment content of fruits of three sweet cherry cultivars at two development phases, examined in 2023.

Treatment	Pre-Ripening Phase					Full Ripeness Phase				
	Chl a	**Chl b**	**Car**	**Lyc**	**Anth**	**Chl a**	**Chl b**	**Car**	**Lyc**	**Anth**
	mg 100 g^{-1} f.w.									
Cultivar										
Van	5.9 c	9.1 c	0.29 b	0.11 b	0.87 b	1.74 b	3.65 b	0.11 b	0.08 c	21.6 b
Andreiaș	15.6 b	25.8 b	0.48 a	0.35 a	1.75 a	4.65 a	7.72 a	0.36 a	0.25 a	39.4 a
Margonia	21.8 a	37.3 a	0.10 c	0.07 c	0.46 c	4.70 a	7.12 a	0.11 b	0.16 b	2.3 c
Fruit position										
Inner	15.0	25.1	0.29	0.16	0.90	3.54	5.65	0.19	0.15	22.8
Outer	13.8	23.0	0.29	0.19	1.15	3.85	6.68	0.20	0.17	19.4
	n.s.	n.s.	n.s.	*	*	*	n.s.	n.s.	*	*
Av.	14.42	24.04	0.29	0.17	1.03	3.69	6.16	0.19	0.16	21.09
STDEV	7.52	13.70	0.18	0.14	0.69	1.59	2.11	0.13	0.08	16.85
COVAR s%	52.17	56.98	63.59	78.23	67.18	43.13	34.22	66.94	46.69	79.90
Min.	5.86	8.77	0.08	0.10	0.40	1.01	2.52	0.10	0.10	2.14
Max.	23.27	41.03	0.49	0.35	2.30	4.90	7.73	0.37	0.26	44.19

Chl a: chlorophyll a; Chl b: chlorophyll b; Car: carotene; Lyc: lycopene; Anth: anthocyanin. n.s.: not significant; * significant at $p \leq 0.05$. Within each column, values followed by different letters are statistically different according to Duncan test at $p \leq 0.05$, $n = 3$.

As for the comparison between the two positions inside the tree crown, the content of lycopene was higher in the outer fruits only in 2023 at both the development phases; the anthocyanin level was higher in the outer fruits at the pre-ripening phase only in 2022, and in the inner ones at the full ripeness phase in both years.

The total contents of carotene and lycopene during the ripening period did not univocally change and, according to Abers and Wrolstad's study [62], they can decrease due to the augmentation of the total anthocyanins, which tend to mask the colour of carotenoids and degrade the chlorophyll due to the development of dark pigmented compounds.

Anthocyanins, belonging to the subclass of water-soluble flavonoids, are one of the main phenolic compounds in sweet cherries and is responsible for the red colouring of the fruit epidermis and pulp [15] and associated with the high antioxidant potential of sweet cherry fruits [63].

During the two years of the present study, a remarkable increase in the content of anthocyanins from the pre-ripening to full ripeness phase was recorded in the cultivars with red fruits (Van and Andreiaș), whereas these compounds showed a low accumulation in the yellow fruits of cv. Margonia at the full ripeness phase.

In previous studies regarding conventionally grown sweet cherry fruits, a variability of anthocyanins arose between cultivars, from 12.1 (cultivar Elton Heart) to 91.6 mg 100 g^{-1} f.w. (cv. Kordia) [59]. Ferretti et al. [60] showed that cv. Regina fruits contained 41.0 mg 100 g^{-1} f.w. anthocyanins, and in another study [51], 54.0 mg 100 g^{-1} f.w. anthocyanins were detected in red sweet cherry fruits on average.

4. Conclusions

In the context of sustainable production management carried out in the present research, significant quality differences arose between the organically grown *Prunus avium* L. fruits of the examined cultivars, which proved to be a very good source of biologically active compounds with beneficial nutritional value to human health.

When the fruits ripened and the colour changed, increases were recorded in the values of the quality indices that are highly appreciated by consumers, such as the content of soluble sugars, vitamin C, carotenoids, and anthocyanins, although a higher content of polyphenolic compounds was found at the pre-ripening phase.

The biochemical processes involved in the fruit ripening process led to several changes in quality parameters, mainly influenced by the variation in seasonal meteorological conditions and cultivar, but also by the fruit's position inside the tree crown.

The fruits of the locally created cultivar Andreiaș were the richest in antioxidants, and the latter outcome suggests an opportunity to both valorise and include the mentioned cultivar in breeding strategies under an organic management programme.

Author Contributions: Conceptualization, I.M., O.C.M. and C.D.J.; methodology, I.M. and O.C.M.; software, I.M. and O.C.M.; validation, O.C.M., G.C. and C.D.J.; formal analysis, I.M., A.V.T. and S.S; investigation, I.M., S.S., A.V.T. and O.C.M.; resources, I.M., O.C.M. and C.D.J.; data curation, I.M., O.C.M. and G.C.; writing—original draft preparation, I.M.; writing—review and editing, O.C.M., G.C. and C.D.J.; visualization, O.C.M., G.C. and C.D.J.; supervision, C.D.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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