Relationship of colour with the phytocompounds present in *Cornus mas* cultivars

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**ABSTRACT**

Cornelian cherry (*Cornus mas* L.) fruits are a well-known source of antioxidants and other biologically active compounds, and these compounds depend on maturity. Ripeness is recognized by means of a visual assay of the growing fruit. The study aimed to determine the relationship between the color of the tested cultivars of *Cornus mas* and their content of the predominant compounds (i.e., flavonoids, anthocyanins, vitamin C, carotenoids and chlorophyll). The studied deviation between cultivars is based on the tested parameters and compared with the genetic profile of Cornelian cherry cultivars. Cornelian is a rich source of anthocyanins and flavonoids. Particularly valuable is the cv. Szafer variety, which color is the darkest and the richest in phenols, flavonoids and anthocyanins. However, the correlation between colorimetric and chemical parameters is found to be low for most variables. Genetic polymorphism analysis showed different distances between the cultivars than the values resulting from chemical analyses. The part of fruit, which was subjected to colorimetric measurement, did not affect the distance projection. Colorimetric parameters were poorly correlated with spectroscopic results, but did not change the distances between the samples. Differences in fruit color and composition, and genetic relationship can be drawn from the adaptation of different cultivars to the current study.

**INTRODUCTION**

Cornelian cherry is a plant growing in Eastern Europe, where fruits are used in traditional cuisine as a component of fruit preserves and liquors. Cornelian cherry fruits are rich in vitamin C and phenolic compounds, i.e., flavonoids, phenolic acids, and anthocyanins.\(^{[1]}\) The latter are mainly responsible for the anti-proliferative and antioxidant properties of Cornelian cherry juice\(^{[2,3]}\). The maturity stage of Cornelian cherry fruits affects the levels of phytocompounds they contain, especially plant secondary metabolites (e.g., anthocyanins) (Szczepaniak et al. 2019a; Yarlgag et al. 2019). Because Cornelian cherry is seasonal, there is a need to control the stage of ripeness. Chemical analyses are time-consuming and expensive, therefore the aim of our study was to determine the relationship between the color of the ripe fruit and its chemical constituents.
In recent years, many authors have paid special attention to Cornelian cherry fruits, describing both sensory and qualitative attributes and pro-health qualities. However, depending on the cultivar and growth conditions, these fruits differ not only visually but also in terms of their chemical and antioxidant properties [1,4]. *Cornus mas* fruits possess a high content of dry substance, which in approx. 85% contain compounds soluble in water. Organic acids, such as malic acid and quinic acid, are responsible for the tart and sour flavor of Cornelian cherry fruits [1]. However, these compounds do not affect the fruit color. The key substances identified and determined in Cornelian cherry, responsible for its biological activity, are vitamin C, anthocyanins, flavonoids and ursolic acid. Among anthocyanins, *Cornus mas* contains mono- and diglycosides of pelargonidin, cyanidin and delphinine. Among flavonoids, quercetin glycosides, as well as kaempferol galactoside and aromadendrin glucoside have been identified [1,5]. Flavonoids, like anthocyanins, possess biological activity and affect fruit color. They have, among others, the ability to modulate the activity of several enzymes, thus preventing enzymatic browning reactions.[6-7]

**MATERIALS AND METHODS**

**Studied material**

The ripe fruits of Cornelian cherry (*Cornus mas* L.) were collected from the orchard farm Szynsad in Dąbrówka Nowa, Błędów, Mazowieckie, Poland (51°47′01″N 20°43′04″E) in 2018. The fruit was harvested from the third year of plant cultivation on soils characterized by pH 6.1 and the content of humus 1.1%. Soil samples for chemical analysis were collected with a specialized soil auger. Nutrient contents (pH, humus) in the soil were determined at the District Chemical and Agricultural Station in Poznań.

The average amount of precipitation in the growing season equaled 320 mm/m², with an average temperature of approx. 14.9°C. Characteristics of climatic conditions which prevailed during the field research were based on data from the meteorological station belonging to the farm (51°47′01″N 20°43′04″E), which the fruits for the study originated from. The fruits of five cultivars grown in Poland were used: Szafer, Jolico, Florianka, Słowińan, and P5. The fruits were stored in cooling conditions (4°C) until the extracts were prepared.

**Extraction**

Cornelian cherry extracts comprised fresh fruits according to the methodology described by Szczepaniak et al.[4]. The fruit skin was cut to allow proper maceration of the plant material. The extract used – 40% (v/v) ethanol and the entire maturation process were chosen to simulate the tincture production process. The fruit matured into extractants at a ratio of 1 g fruit: 5 ml extractant. The average mass of the fruit used to obtain one extract approximated 26 g. Samples were encoded as follows: S-cv. Szafer extract P-cv. P5 extract; V-cv. Słowińan extract; F-cv. Florianka extract; and C-cv. Jolico extract.

**Color measurements**

The color parameters of Cornelian cherry’s flesh (M), skin and these two anatomical parts together (M + O) were investigated. Fruit skins were measured both in the frozen (Z) and thawed (O) state. Color measurement was made in the L* a* b* CEN unit system using spectrometer CM-5 (Konica Minolta, Japan), accordingly to the methodology described by the device manufacturer. As a source of light, D 65 was applied, and the color temperature was 6504 K. The observation angle of the standard colorimetric observer was 10°. Measurements for each anatomical part and for each cultivar were repeated five times. The instrument was calibrated using a black pattern.
**Total phenolic content**

Total phenolic content (TPC) was determined using own spectroscopy methodology [4]. The total amount of 5 mmol of the tested extract and the volume of Folin–Ciocalteu reagent (Chempur, Piekary Śląskie, Poland) were added to a 50 ml volumetric flask. After mixing the mixture for 5 s, 5 ml of supersaturated solution of Na₂CO₃ and NaHCO₃ were added. Before the absorbance measurement, the sample was incubated in a dark room for 90 min. The analysis was carried out with a Specord 40 spectrophotometer (Analytic Jena, Germany), and the absorbance was measured at $\lambda = 765$ nm. Each sample was measured in a six-fold repetition and for each tested extract the whole analytical procedure was repeated three times. The calibration curve was based on gallic acid (Sigma-Aldrich, Germany) at five different concentrations: 22.21 mg/ml; 26.65 mg/ml; 31.09 mg/ml; 35.54 mg/ml and 44.42 mg/ml. Each standard solution was measured six times. The $r^2$ value of the calibration curve was 0.9523, and the curve was validated using internal cross validation. The total average recovery observed was 102.9%. The results are related to the fresh weight of the extract and expressed in milligrams of gallic acid equivalent (GAE)/100 g fresh weight (f.w.).

**Total anthocyanin content**

The content of anthocyanins was determined using the spectroscopic method described by Lee et al.[8] with slight modifications. The assay was based on measuring the difference in absorbance between the two wavelengths and buffer media in which the 0.4 ml test sample was diluted 10 times, i.e., either 25 mM KCl solution in 0.1 M HCl (pH = 1.0) or sodium acetate buffer (pH 4.5). Total anthocyanin content (TAC) was calculated using the following equation. The results were given in mg cyaniding-3’-glucoside equivalent (CGE) per 100 g of fruit.

$$
TAC = \left( \frac{(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}}{26.9} \right) \times 4492 \times 5 \left[ \frac{\text{CGE}}{100 \text{ g f.w.}} \right]
$$

Where: $A_{510}$ is the absorbance at $\lambda = 510$ nm; $A_{700}$ is the absorbance at $\lambda = 700$ nm. The measurement was repeated three times for each sample using the Specord S40 (Analytic Jena, Germany) spectrophotometer. Buffer solutions were used as blank samples.

**Total flavonoid content**

The total flavonoid content (TFC) was determined by a modified method of Meda et al.[9] based on the colorimetric reaction between flavonoids in the tested extract and aluminum ions. A 2% AlCl₃ solution was prepared in methanol and mixed in a test tube with the tested extract at a ratio of 1:1 (v/v). Then, after 10 minute conditioning in the dark place, absorbance was read at 415 nm. All extracts were analyzed in triplicate. A mixture of pure methanol (Honeywell, UK) and the tested extract (1:1 v/v) was used as a negative sample. The absorbance of the negative sample was subtracted from the Cornelian cherry extract. The standard curve was prepared using standard quercetin solutions (Sigma-Aldrich, Germany) ($r^2$ 0.9989). The final results are given in mg quercetin equivalent (QE)/100 g f.w.

**Total carotenoid and chlorophyll content**

The assay was conducted according to the Abou-Arab et al. method.[10] The obtained extracts were subjected to multi-wavelength absorbance measurement in the SPECORD S40 apparatus (Analytic Jena, Germany). Absorbance was read for the following wavelengths: 440 nm ($A_{440}$); 644 nm ($A_{644}$) and 662 nm ($A_{662}$). Each extract was analyzed in triplicate. The content of chlorophyll A (CA), chlorophyll B (CB), and total carotenoids (TC) was given in mg/100 g f.w. and calculated according to equations (2–4).
\[
CA = 0.5 \times (9.784 \times A_{662} - 0.99 \times A_{440}) \\
CB = 0.5 \times (21.426 \times A_{664} - 4.65 \times A_{662}) \\
TC = 0.5 \times [4.695 \times A_{440} - 0.369 \times (CA + CB)]
\]

The measurements were made in fourfold repetitions.

**Vitamin C determination**

The content of vitamin C (AA) was determined according to Omaye et al. spectroscopy method,\textsuperscript{[11]} modified independently in the following way. The whole volume of 600 μl of the tested extract was added to the test tube and then 300 μl of 0.85 M trisodium citrate (POCH, Poland) solution was added. Next, 590 μM of dichlorophenolphindophenol (DPIP, Sigma-Aldrich, Germany) was added, and after 30 s, absorbance \((A_1)\) was measured at a wavelength of 520 nm (Metertek, Taiwan). After that, the absorbance of the tested solution was scavenged by adding ascorbic acid (AA, Chempur, Poland) to the cuvette. The sample with AA overdose was measured \((A_2)\). The measurement was made in threefold repetition. In the negative control sample, 600 μl of 40% ethanol was used instead of the tested extract. The blank sample was the chosen extractant – 40% ethanol. The absorbance drop \((X)\) was calculated according to the following equation.

\[
X = (A_{n1} - A_{n2}) - (A_1 - A_2)
\]

Where \(A_{n1}\) is the absorbance of the negative control; \(A_{n2}\) is the absorbance of the negative control after scavenging by ascorbic acid additive. The calibration curve was made using AA standard solutions. The linearity of the curve coefficient \((r^2)\) equaled 0.98. The final results are given in mg AA/100 g f.w.

**Phenolic acids and flavonoids quantitative determination**

Phenolic compounds in samples were analyzed after performing alkaline and acidic hydrolysis.\textsuperscript{[12]} The analysis was carried out using the Acquity H class UPLC system equipped with the Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed on the Acquity UPLC\textsuperscript{*} BEH C18 column (100 mm×2.1 mm, particle size 1.7 μm) (Waters, Ireland). The elution was carried out in a gradient mode using the following mobile phase composition: A: acetonitrile with 0.1% formic acid, B: 1% aqueous formic acid mixture (pH = 2). Concentrations of phenolic compounds were determined using an internal standard at wavelengths \(λ = 320\) nm and 280 nm and finally given at mg/100 g f.w. of Cornelian cherry. Compounds were identified based on a comparison of retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of the standard to the analyzed samples and repeating the analysis. Detection level was 1 μg/g. Retention times for phenolic acids were protocatechuic acid 1.56 min, gallic acid 4.85 min, p-coumaric acid 8.06 min, 2,5-dihydroxybenzoic acid 9.55 min, 4-hydroxybenzoic acid 9.89 min, chlorogenic acid 12.00 min, caffeic acid 15.20 min, syringic acid 15.60 min, vanillic acid 16.80 min, sinapic acid 17.10 min, ferulic acid 17.50 min, salicylic acid 17.85 min, and t-cinnamic acid 19.50 min. Retention times for flavonoids were apigenin 1.10 min, vitexin 8.00 min, kaempferol 11.00 min, luteoline 16.90 min, quercetin 17.00 min, naringenin 17.50 min, rutin 17.90 min, and catechin 19.50 min (S1-S2 Figs.).

**Genetic relationship analysis**

The tested fruits were frozen in liquid nitrogen and pounded into powder in a porcelain mortar. DNA was isolated using the Junghans and Metzlfaff's methods.\textsuperscript{[13]} The quantity and quality of the
isolated nucleic material were validated by the Nanodrop (ThermoFischer, UK) spectrometer at wavelengths 260, 280 and 230 nm. For the analyses, 14 pairs of SSR (Simple Sequence Repeats) markers and 34 ISSR (Inter Simple Sequence Repeat) markers (Table 1) were used.

The SSR reacting mixture was composed as follows: 6.0 µl H₂O, 1.5 µl 10xHypernova reagent buffer (DNA Gdańsk – Blirt, Poland), 2.0 µl MgCl₂ (50 mM, DNA Gdańsk – Blirt, Poland), 1.0 µl dNTP (8.0, DNA Gdańsk – Blirt, Poland), 0.8 µl Forward starter (10 µM), and 0.8 µl Reverse starter (10 µM), 1.1 µl polymerase (Hypernova, DNA Gdańsk – Blirt, Poland), 1.8 µl genome DNA (30 ng/µl). The ISSR reacting mixture contained 7.0 µl H₂O, 2.0 µl reacting buffer 10xHypernova (DNA Gdańsk – Blirt, Poland), 2.5 µl MgCl₂ (50 mM, DNA Gdańsk – Blirt, Poland), 1.0 µl dNTP (8.0, DNA Gdańsk – Blirt, Poland), 1.1 µl starter (10 µM), 1.0 µl polymerase (Hypernova, DNA Gdańsk – Blirt, Poland), and 1.5 µl genome DNA (30 ng/µl). The polymerase chain reaction (PCR) was conducted in the Biometra thermocycler. PCR for SSR was conducted in 35 cycles and involved the stages: initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, attaching of starters at 52°C for 30 s, amplification at 72°C for 30 s, final amplification at 72°C for 10 min. PCR for ISSR was conducted in 40 cycles and involved: initial denaturation at 95°C for 5 min, denaturation at 94°C for 1 min, binding of starters at 52°C for 30 s, amplification at 72°C for 30 s, final amplification at 72°C for 10 min. Amplification results were checked using capillary electrophoresis in the Qiaxcel apparatus (Qiagen, Hilden, Germany). The binary matrix was composed in the Qiaxcel ScreenGel software using the Binary Peak (Qiagen) function.

**Statistical analysis**

Statistical analysis was conducted using the Statistica 13 software (StatSoft, Poland). We treat color parameters (L*, a* and b*) as the former quantitative dataset. The latter comprised the other parameters discussed in this study. A cultivar was treated as a qualitative variable. We performed the principal component analysis of the studied parameters for the tested cultivars. Moreover, we showed correlations between the tested cultivars using the Euclid’s tree projection. To construct an Euclid’s tree projection for all tested parameters, the results of color measurements for different anatomical parts and cultivars were treated as separate variables.

The results of genetic polymorphism were analyzed statistically in the R software. To illustrate the genetic distance between the tested cultivars, a distance tree chart was used based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMAM).

**RESULTS**

**Color measurements**

The colorimetric assay showed significant differences between the analyzed Cornelian cherry fruits and their anatomical parts (Table 2). Flesh, considered individually and together with fruit skin, tended to have lower brightness L* values than fruit skin alone. Significant differences between the color of frozen and thawed fruit can be noticed in a* and b* values.

In the context of cultivars, similar values were observed for cultivars Slowianin and P5.

**Total phenolic content**

Table 3 shows that all tested cultivars have similar TPC, as no cultivar is significantly different. The highest value was recorded for cv. Szafer, and the lowest for cv. Slowianin. All five tested cultivars had a high TPC, ranging from 15 to 20 g GAE/100 g f.w.
### Table 1. SSR (Simple Sequence Repeats) and ISSR (Inter Simple Sequence Repeat) characteristics.

| SSR markers | Primer pairs (ID) | Primer sequences (5’-3’) | Repeat motif | Melting point (°C) |
|-------------|-------------------|--------------------------|--------------|-------------------|
| CF48L       | L: gcttgacacaccttcttgcttc  
R: raagagcttccacacacatcagcc | (TG)9 | 52 |
| CF55        | L: tggagtaggggcaaaagatcagag  
R: rtccagggaatgcgcggtagatcagag | (GT)7 | 52 |
| CM007       | L: tcgttaatgtgaaattggaacg  
R: ctccacactgtctggcttacttgg | (CA)12 | 52 |
| CM008       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (GT)24 | 52 |
| CM010       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (GT)11 | 52 |
| CM020       | L: tgccagactagttctgttagc  
R: rtccacactgtctggcttacttgg | (TA)10 | 52 |
| CM026       | L: gaattcatgtaatgttgttgtctgc  
R: cctgcatataattcaggtaaagagc | (CA)14 | 52 |
| CM031       | L: tcgttaatgtgaaattggaacg  
R: ctccacactgtctggcttacttgg | (CA)12 | 52 |
| CM037       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (GT)20 | 52 |
| CM039       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (GT)18 | 52 |
| CM043       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (GT)16 | 52 |
| CF-G17      | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (AT)15 | 52 |
| ccmp2       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (AC)n | 55 |
| ccmp6       | L: gctagcagaagcacagttagcc  
R: tccaacatctaaacccatgatc | (TA)10 | 52 |

| ISSR markers | Primer pairs (ID) | Primer sequences (5’-3’) | Melting point (°C) |
|-------------|-------------------|--------------------------|-------------------|
| UBC 807     | AGAGAGAGAGAGAGAGA  
TUBC 841    | AGAGAGAGAGAGAGAGAC | 45 |
| UBC 808     | AGAGAGAGAGAGAGAGA  
TUBC 842    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 809     | AGAGAGAGAGAGAGAGA  
TUBC 843    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 810     | AGAGAGAGAGAGAGAGA  
TUBC 844    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 813     | AGAGAGAGAGAGAGAGA  
TUBC 845    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 818     | AGAGAGAGAGAGAGAGA  
TUBC 846    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 821     | AGAGAGAGAGAGAGAGA  
TUBC 847    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 822     | AGAGAGAGAGAGAGAGA  
TUBC 848    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 824     | AGAGAGAGAGAGAGAGA  
TUBC 849    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 825     | AGAGAGAGAGAGAGAGA  
TUBC 850    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 826     | AGAGAGAGAGAGAGAGA  
TUBC 851    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 827     | AGAGAGAGAGAGAGAGA  
TUBC 852    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 828     | AGAGAGAGAGAGAGAGA  
TUBC 853    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 829     | AGAGAGAGAGAGAGAGA  
TUBC 854    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 830     | AGAGAGAGAGAGAGAGA  
TUBC 855    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 831     | AGAGAGAGAGAGAGAGA  
TUBC 856    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 832     | AGAGAGAGAGAGAGAGA  
TUBC 857    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 833     | AGAGAGAGAGAGAGAGA  
TUBC 858    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 834     | AGAGAGAGAGAGAGAGA  
TUBC 859    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 835     | AGAGAGAGAGAGAGAGA  
TUBC 860    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 836     | AGAGAGAGAGAGAGAGA  
TUBC 861    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 837     | AGAGAGAGAGAGAGAGA  
TUBC 862    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 838     | AGAGAGAGAGAGAGAGA  
TUBC 863    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 839     | AGAGAGAGAGAGAGAGA  
TUBC 864    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 840     | AGAGAGAGAGAGAGAGA  
TUBC 865    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 841     | AGAGAGAGAGAGAGAGA  
TUBC 866    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 842     | AGAGAGAGAGAGAGAGA  
TUBC 867    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 843     | AGAGAGAGAGAGAGAGA  
TUBC 868    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 844     | AGAGAGAGAGAGAGAGA  
TUBC 869    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 845     | AGAGAGAGAGAGAGAGA  
TUBC 870    | AGAGAGAGAGAGAGAGA | 48 |
| UBC 846     | AGAGAGAGAGAGAGAGA  
TUBC 871    | AGAGAGAGAGAGAGAGA | 48 |

(Continued)
Table 1. (Continued).

| Primer pair (ID) | Primer sequences (5'-3') | Repeat motif | Melting point (°C) |
|------------------|--------------------------|--------------|-------------------|
| UBC 855          | ACACACACACACACAC        |              | 50                |
| UBC 888          | CACACACACACACACA        |              | 50                |

Table 2. Skin and flesh colors of the tested *Cornus mas* cultivars, given in CIE L*a*b* units, and their visualizations.

| sample | anatomical part | L*   | a*   | b*   | color |
|--------|----------------|------|------|------|-------|
| C      | M*             | 10.69 ± 1.73 | 22.08±2.16 | 8.32±1.69 | red   |
| F      | M*             | 13.14±6.84  | 22.23±2.05 | 9.77±1.88 | red   |
| P      | M*             | 6.79±0.63   | 22.03±0.84 | 7.52±1.99 | red   |
| S      | M*             | 4.85±0.93   | 15.82±2.45 | 4.80±1.05 | brown |
| V      | M*             | 11.00±3.68  | 18.63±1.72 | 6.63±1.57 | purple|
| C      | Z*             | 19.66±0.47  | 8.16±2.45  | 1.90±1.05 | gray  |
| F      | Z*             | 22.55±1.36  | 21.66±6.07 | 8.03±3.10 | pink  |
| P      | Z*             | 21.46±2.83  | 11.14±3.29 | 3.95±2.19 | purple|
| S      | Z*             | 19.60±0.58  | 7.88±1.34  | 1.54±0.42 | gray  |
| V      | Z*             | 21.55±0.66  | 14.88±3.48 | 4.64±1.43 | purple|
| C      | O*             | 21.02±1.79  | 6.58±2.54  | 1.27±1.11 | gray  |
| F      | O*             | 23.79±0.97  | 12.03±1.70 | 3.86±0.99 | purple|
| P      | O*             | 23.52±1.48  | 11.17±5.12 | 3.55±2.36 | purple|
| S      | O*             | 19.92±0.16  | 5.30±0.34  | 0.65±0.31 | gray  |
| V      | O*             | 24.22±1.53  | 5.31±1.11  | 1.39±0.41 | gray  |
| C      | M+O*           | 12.11±2.85  | 18.78±2.33 | 5.64±1.18 | purple|
| F      | M+O*           | 11.36±1.03  | 25.65±0.37 | 11.61±0.49 | red   |
| P      | M+O*           | 9.60±0.87   | 22.91±0.76 | 9.74±0.64 | red   |
| S      | M+O*           | 4.55±0.77   | 15.04±2.16 | 4.27±0.63 | brown |
| V      | M+O*           | 9.05±0.69   | 23.86±1.35 | 9.50±1.43 | red   |

Results are given as mean ± standard deviation; M – Flesh; Z – Frozen skin; O – Thawed skin; † – N = 5; †† – N = 4; ††† – N = 3; a-g – statistically significant differences between samples (P < 0.05).

Table 3. Content of the analyzed phytocompounds in selected *Cornus mas* cultivars.

| sample | TPC* [mg GAE/100 g f.w.] | TFC* [mg QE/100 g f.w.] | TAC* [mg CGE/100 g] | Vit. C* [mg/100 g f.w.] | CA** [mg/100 g f.w.] | CB** [mg/100 g f.w.] | Total chlorophylls** [mg/100 g f.w.] | Total carotenoids** [mg/100 g f.w.] |
|--------|--------------------------|-------------------------|---------------------|------------------------|----------------------|----------------------|-------------------------------------|-------------------------------------|
| C      | 15.59±2.85              | 6.93±0.18               | 7.90±0.11           | 123.83±44.49          | 0.10±0.01            | 0.26±0.02            | 0.36±0.02                          | 0.91±0.01                           |
| F      | 16.82±0.48              | 7.95±0.16               | 8.91±0.07           | 21.95±7.10            | 0.03±0.00            | 0.12±0.01            | 0.16±0.01                          | 1.42±0.01                           |
| P      | 17.05±0.21              | 7.83±0.18               | 2.35±0.03           | 58.26±1.14            | 1.90±0.01            | 3.68±0.02            | 5.58±0.03                          | nd                                  |
| S      | 19.52±0.27              | 16.89±0.57              | 24.25±0.65          | 86.04±7.45            | 2.42±0.06            | 4.79±0.12            | 7.21±0.06                          | 1.31±0.06                           |
| V      | 14.98±1.23              | 1.62±0.46               | 6.65±0.35           | 18.02±6.86            | 0.22±0.13            | 0.46±0.25            | 0.67±0.38                          | 0.16±0.08                           |

Results are given as mean ± standard deviation; *N = 3; **N = 4; TPC – total phenolic content; GAE – gallic acid equivalent; TFC – total flavonoid content; QE – quercetin equivalents; TAC – total anthocyanin content; CGE – cyaniding-3-glucoside equivalent; Vit. C – ascorbic acid; CA – Chlorophyll A; CB – Chlorophyll B. a-e – statistically significant differences between samples (P < 0.05).

**Total flavonoid content**

TFC ranged from 1.62 to 16.89 mg quercetin equivalent (QE)/100 g f.w. (Table 3). The richest in TFC was cv. *Szafer*, while the poorest was cv. *Słówianin*. The first cultivar had significantly higher TFC values than other samples (approx. 16 times higher than the other one). No statistical difference in the TFC parameter was observed between *Florianka* and *P5* cultivars (Table 3).
**Total anthocyanin content**

Cultivar *Szafer* was found to be the richest in anthocyanins (Table 3), similarly to the TFC test. The content of anthocyanins was approximately 10 times higher than in the P5 cultivar, which had the lowest content of anthocyanins. In contrast to the results observed for flavonoids, the samples for this test varied much stronger.

**Ascorbic acid content**

In contrary to the tests discussed above, the richest in AA was cv. *Jolico*, and the poorest was cv. *Slowianin* (Table 3). As in the case of total anthocyanin content, it found out that cultivars *Slowianin* and *Florianka* had similar values. The remaining cultivars differed significantly both from them and from each other.

**Chlorophyll fractions and total carotenoid content**

The tested Cornelian cherry cultivars differed significantly in the content of total chlorophyll, chlorophyll A, chlorophyll B, and total carotenoids (Table 3). The richest in chlorophylls was cv. *Szafer* – 7.21 ± 0.06 mg/100 g f.w., while cv. *Florianka* had the lowest total chlorophyll content – 0.16 ± 0.01 mg/100 g f.w. Among the chlorophyll fractions, chlorophyll B dominated in all tested samples, and its content was approximately twice as high as that of chlorophyll A. Total carotenoid content ranged from 0.16 to 1.42 mg/100 g f.w. between samples, and no carotenoids were found in cv. *P5* (Table 3).

**HPLC determination of flavonoids and phenolic acids**

The compounds detected in the tested *Cornus mas* extracts, can be considered as two separate groups (Table 4). The first of the dominant compounds comprises chlorogenic acid, gallic acid, quercetin, rutin and naringenin, while the other one involves compounds, whose concentration is low or scarce (e.g., vanillic and salicylic acids). The PCA analysis (Figure 1 a) showed that most phenolic acids and flavonoids are correlated, as they are located in the same upper left corner of the plot. Exceptions are salicylic acid situated near AA and naringin adjacent to the point of cultivar *Slowianin*.

**Correlation analysis**

After including all the above parameters toward a single statistical model, two significant implications appeared. First, the anatomical part variable had no higher statistical significance for several samples than the issue of cultivar (Figure 2a). The Euclid’s tree projection presents a similar arrangement of cultivars to that presented in Figure 1. The PCA analysis has confirmed such significance, as it is presented in the previous points. A close relationship between TFC, cv. *Szafer* and chlorophyll fractions was found. Color measurements did not seem to have a significant relationship with spectroscopic determination, as parameters *a* and *b* are located close to each other in the central plane of the principal scatter diagram. Points representing color measurement variables were placed halfway between the anatomical parts and several of the tested phenolic acids and flavonoids.

The content of individual compounds and their proportions are hardly determinable using color measurements (Table 5). The performed Spearman rank correlation showed strong trends between *L*, *a* and *b* parameters, but none of them related significantly to TFC, AA, TAC, chlorophyll and carotenoid content (Table 5). The correlation matrix shows that there is a low negative relationship between the former and other parameters, except TC, for which the relationship with *L*, *a* and *B* were positive and scarce. For individual phenolic acids and flavonoids, no significant relationship with fruit color was found either.
### Table 4. Concentration of phenolic acids and flavonoids [mg/100 g f.w.] in the tested cultivars of Cornus mas.

| Sample | gallic acid | 2,5-dihydroxybenzoic acid | 4-hydroxybenzoic acid | caffeic acid | syringic acid | p-coumaric acid | ferulic acid | chlorogenic acid | t-cinnamic acid | salicylic acid |
|--------|-------------|-----------------------------|-----------------------|--------------|--------------|----------------|--------------|----------------|----------------|---------------|
| C      | 26.1        | 1.7                         | 1.7                   | 0.6          | 0.4          | 1.0            | 0.9          | 126.2          | 0.3            | 0.2           |
| F      | 16.9        | 2.7                         | 1.7                   | 0.5          | 0.4          | 0.1            | 1.0          | 110.9          | 0.2            | 0.1           |
| P      | 21.1        | 1.6                         | 2.2                   | 0.3          | 0.3          | 0.2            | 0.9          | 76.3           | 0.1            | 0.1           |
| S      | 14.4        | 1.1                         | 1.2                   | 0.3          | 0.2          | 0.3            | 0.6          | 76.3           | 0.1            | 0.1           |
| V      | 12.0        | 2.3                         | 1.2                   | 0.3          | 0.5          | 0.5            | 0.7          | 89.3           | 0.1            | <0.1          |

| Sample | naringenin | vitexin | rutin | quercetin | apigenin | kaempferol | luteoline | sinapic acid | vanillic acid | sinapic acid |
|--------|------------|---------|-------|-----------|----------|------------|-----------|--------------|---------------|--------------|
| C      | 18.1       | 2.9     | 58.5  | 36.6      | 6.1      | 0.2        | 0.4       | 0.5          | 0.4           |
| F      | 26.9       | 4.4     | 45.4  | 30.7      | 1.9      | 0.1        | 0.1       | 0.3          | 0.2           |
| P      | 26.0       | 2.6     | 21.7  | 21.8      | 0.9      | 0.5        | 0.2       | 0.3          | 0.4           |
| S      | 14.8       | 1.0     | 9.3   | 21.7      | 3.3      | 0.1        | 0.1       | 0.2          | <0.1          |
| V      | 45.6       | 5.0     | 42.1  | 18.1      | 4.5      | 0.3        | 0.2       | 0.3          | 0.4           |

Results are given as mean values (N = 3). <0.1 – less than 0.1 mg/100 g f.w.
Genetic relationship analysis

The results of amplification of DNA markers for the five tested Cornelian cherry cultivars showed a large genetic deviation between the analyzed genotypes. Genetic distance values ranged from 0.72 to 0.89.
The greatest genetic distance was observed between cvs. Florianka and Jolico. The lowest distance value was recorded between cvs. P5 and Szafer. Hierarchical cluster analysis tested with the Figure 2. Euclid's tree projection (a) and principal scatter diagram (b) of Cornus mas samples considered using all testing methods CM – cv. Jolico color measured for flesh, CMO – cv. Jolico color measured for skin and flesh; CZ – cv. Jolico color measured for frozen skin; CO – cv. Jolico color measured for thawed skin; FM – cv. Florianka color measured for thawed flesh, FMO – cv. Florianka color measured for skin and flesh; FZ – cv. Florianka color measured for frozen skin; FO – cv. Florianka color measured for thawed skin; PM – cv. P5 color measured for flesh, PMO – cv. P5 color measured for skin and flesh; PZ – cv. P5 color measured for frozen skin; PO – cv. P5 color measured for thawed skin; SM – cv. Szafer color measured for flesh, SMO – cv. Szafer color measured for skin and flesh; SZ – cv. Szafer color measured for frozen skin; SO – cv. Szafer color measured for thawed skin; VM – cv. Słowianin color measured for flesh, VMO – cv. Słowianin color measured for skin and flesh; VO – cv. Słowianin color measured for frozen skin; VZ – cv. Słowianin color measured for thawed skin. Skin and flesh colors of the tested Cornus mas cultivars, given in CIE L*a*b* units, and their visualizations. M – Flesh; Z – Frozen skin; O – Thawed skin. (Figure 3). The greatest genetic distance was observed between cvs. Florianka and Jolico. The lowest distance value was recorded between cvs. P5 and Szafer. Hierarchical cluster analysis tested with the
Table 5. Correlation (r) matrix of colorimetric and chemical parameters.

| Variable             | L*         | a*         | b*         |
|----------------------|------------|------------|------------|
| L*                   | 1.000000   | 0.644415   | 0.560998   |
| a*                   | 0.644415   | 1.000000   | 0.976364*  |
| b*                   | 0.560998   | 0.976364*  | 1.000000   |
| TFC                  | 0.219190   | 0.246260   | 0.290508   |
| TAC                  | 0.210279   | 0.312569   | 0.368519   |
| CA                   | 0.140474   | 0.316098   | 0.400105   |
| CA                   | 0.237581   | 0.257423   | 0.298193   |
| Total chlorophyll    | 0.238693   | 0.259450   | 0.300661   |
| LC                   | 0.038205   | 0.030222   | 0.037078   |
| gallic acid          | 0.036299   | 0.004366   | 0.017743   |
| 2,5-dihydroxybenzoic acid | 0.257639   | 0.415649   | 0.503472   |
| 4-hydroxybenzoic acid | 0.081154   | 0.216223   | 0.252169   |
| caffeic acid         | 0.133848   | 0.091920   | 0.086847   |
| syringic acid        | 0.244423   | 0.308638   | 0.367858   |
| p-coumaric acid      | 0.004133   | 0.205120   | 0.270752   |
| ferulic acid         | 0.235408   | 0.378548   | 0.443553   |
| chlorogenic acid     | 0.160726   | 0.132449   | 0.138894   |
| sinapic acid         | 0.108001   | 0.017361   | 0.045685   |
| t-cinnamic acid      | 0.053976   | 0.008086   | 0.029700   |
| vanillic acid        | 0.163761   | 0.133639   | 0.146767   |
| salicyclic acid      | 0.016405   | 0.020993   | 0.040499   |
| naringenin           | 0.153763   | 0.209922   | 0.250691   |
| vitexin              | 0.250055   | 0.344286   | 0.414996   |
| rutin                | 0.213729   | 0.193897   | 0.215012   |
| quercetin            | 0.094868   | 0.082484   | 0.078033   |
| apigenin             | 0.008942   | 0.235347   | 0.301048   |
| kaempferol           | 0.030829   | 0.050135   | 0.057892   |
| luteoline            | 0.104711   | 0.023638   | 0.052844   |
| TPC                  | 0.213653   | 0.197192   | 0.225170   |

*→ Significant relationship (P < 0.05) between factors

UPGMAM method allowed us to select three clusters. The most similar were cultivars Slowianin, Szafer and P5. Cultivars Jolico and Florianka were placed on separate roots, thus forming two individual clusters.

DISCUSSION

Color is the first determinant of product quality and consumer preferences. Changes in fruit color can be observed not only during storage. Color is also affected by technological processes.\[15,16\] An increase in fruit lightness may be affected by the decay of anthocyanin colorants, due to the relationship of anthocyanin degradation with the formation of yellow colorants and the increase in yellow color fraction. This may be observed as a decrease in the a* parameter.\[5\] A different range of the a* parameter (intense red) observed in the study indicates the various content of colorant substances in the tested Cornus mas fruits (Table 2). A similar effect was also observed in cranberry juice stored and preserved in different conditions, for which it was noted that cranberry color brightening may be the result of anthocyanin transformations, which, as the temperature increases (during heating), first becomes more intense due to cleavage of glycosidic bonds, and then the intensity of the color decreases, as a result of anthocyanin oxidation (Roidoung et al. 2016; 2017). Changes in anthocyanin color can also be affected by enzyme activity, i.e., oxidases and the presence of metal ions. The color of anthocyanins in the presence of metal ions depends not solely on what ions occur in the environment, but also on the plant species (e.g., tin ions change the color of strawberries, raspberries and cherries) into pale-red, and the color of black currant into purple-magenta. Meanwhile, the presence of ferric and cupric ions resulted in a brown color.\[15,16\]

The obtained results of TAC are significantly lower than those reported by De Biaggi et al.,\[17\] who stated that the mean value of total anthocyanin content for Cornelian cherry was over 134 mg cyanine
glycoside equivalent (CGE)/100 g f.w. In 2011, Kucharska et al.\textsuperscript{2} analyzed different Polish cultivars of Cornelian cherry, including \textit{Szafer}, and observed TAC values of 134.57 mg CGE/100 g and 116.68 mg CGE/100 g, for the first and second cultivar, respectively. The differences between the above results and our results may result from different extraction conditions applied. In the cited studies, acidified methanolic solution was used as an extractant, which significantly improved the migration of ionized molecules of anthocyanins.\textsuperscript{2,17} In the study by Popović et al.,\textsuperscript{18} non-acidified 80\% (v/v) ethanol was used. Under these conditions, TAC values ranging from 5.8 to 302.9 mg CGE/100 g fruit were obtained, which are partially consistent with our results.

Vitamin C content in the samples ranged from 18.02 to 123.83 mg/100 g f.w (Table 3). In the reference positions, the authors give a wide range of vitamin C content, which is explained by differences in methodology and extraction procedure. Szczepaniak et al. (2019a) noted that differences in AA levels can be significant if we compare data obtained using different analytical techniques, e.g., spectroscopic and titration ones. In the study by De Biaggi et al.,\textsuperscript{17} the mean AA content amounted to 61.43 mg/100 g f.w., which is similar to that observed in our study for cv. \textit{P5}. Due to its reducing and antioxidant properties, ascorbic acid is commonly used as a food additive. As an antioxidant, it strongly protects food products against discoloration caused both by enzymatic and non-enzymatic browning. In the latter process, vitamin C reduces the production of \textit{ortho}-quinone oxidation, keeping the color of the product unchanged.\textsuperscript{19}

Total carotenoid content ranged from 0.16 to 1.42 mg/100 g f.w. between samples, and no carotenoids were detected in cv. \textit{P5} (Table 3). The cultivation method, climatic and soil conditions and, of course, fruit maturity may have a high impact on the content of bioactive compounds (Szczepaniak et al.\textsuperscript{20}).

Figure 3. Distance tree projection and matrix of genetic distances between the tested \textit{Cornus mas} cultivars 1 – cv. \textit{Jolico}; 2 – cv. \textit{Florianka}; 3 – cv. \textit{P5}; 4 – cv. \textit{Szafer}; 5 – cv. \textit{Słownianin}.
2019b). In the presented study, these conditions were unified; therefore, it should be concluded that the diverse composition of the tested phytocompounds is a matter of cultivar only. Comparing the results of colorimetric study, chemical tests and genetic polymorphism analysis, it must be noted that although the first two results of distance projection were similar, the second one was significantly different. Perhaps this variability was affected by other unknown factors or by the presence of interfering compounds not tested in this study. The tested cultivars could be susceptible to environmental factors in different ways, and therefore their metabolism kinetics and products were significantly different. In their study, Ercisli et al. [20] noted that human selection of different traits and natural selection of local conditions, could produce a wide range of *Cornus mas* genotypes over a long period of time. In another study, [21] several genotypes of the anatomical parts of *Cornus mas* L. and *Cornus officinalis* were tested for their mutual relationship and the results of anatomical measurements showed different and worse relationship between the samples than the genetic polymorphism test. Moreover, a large deviation between plants of the same genus originating from the same region was noted by Kalalagh et al. [22]

The color of the fruit is a complex issue. Its parameters differed whether the color of the skin, flesh, skin and flesh together were measured. In several cases, the anatomical part had a stronger statistical power than the cultivar. Cornelian is a rich source of anthocyanins and flavonoids. Its fruits also contain vitamin C and carotenoids. An attempt to statistically quantify the fruit color as a result of the presence of plant secondary metabolites did not succeed.

The results obtained for colorimetric and spectrophotometric assays were not significantly related, but allowed us to construct a statistical model to present differences between the tested cultivars exactly, analogically to the model based on spectroscopic parameters only. The genetic polymorphism study showed different deviations between the tested cultivars than the first two studies.

**Conflicts of Interest**

The authors declare there are no conflicts of interest.

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