

Physiological parameters of leaves as a quality parameter of red currant fruits indicating harvest maturity

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Abstract: Red currant (*Ribes rubrum* L.), black currant and raspberry are ones of the most frequently produced soft fruits in Poland because of their characteristic taste and chemical properties, which are widely used in food processing. The possibility of assessing the bushes on plantation during the growing season, including physiological parameters of the leaves and determining the nutritional status of the plants through the use of optical meters, allows growers to react quickly to obtain fruit of high processing quality. In this study, the relationships between selected physiological parameters of leaves and morphological traits and mechanical and chemical properties of the fruits of three red currant cultivars ('Luna', 'Losan' and 'Red Dutch' ('Holenderska czerwona' in Polish) on the day of harvest were assessed. The study showed that the content of flavonoids and chlorophyll in red currant leaves increased with the fruit ripening and was highest on the day of fruit harvest, and the values of FlvM and AnthM for 'Luna' cultivar were significantly higher, which was related to its earlier fruit ripening compared to the other two ('Red Dutch' and 'Losan') cultivars analysed. Correlations between morphological traits, mechanical and chemical properties of fruit and selected physiological parameters of leaves of currant cultivars varied. The data indicated that multi-pigment meter can be useful for an easy, non-destructive evaluation of selected physiological parameters of leaves to assess red currant fruit quality and harvest maturity.

Keywords: fluorescence, leaves, mechanical and chemical properties of fruits, morphological features of fruits, multi-pigment meter, red currant, transmittance

INTRODUCTION

Red currant (*Ribes rubrum* L.) bushes are widely grown in Europe (Russia, Ukraine, Poland, Germany, France) on commercial plantations and in home gardens (Kuźniar *et al.*, 2022). The currant species cultivated in Poland differ by colour; *Ribes rubrum* L. is red, *Ribes nigrum* L. is black, and *Ribes niveum* L. characterised white-cream skin (Jurgiel-Małecka and Buchwał, 2016). The ease of establishment and maintenance of currant plantations contributes to its high popularity (Djordjević *et al.*, 2020). Red currant yield depends on many factors; hence, the selection of an appropriate cultivar, as well as the bush size and shoot age, ripening time, cultivation system or agroclimatic conditions during the growing season are important (Ersoy *et al.*, 2018; Heijerman and Gessel, 2020). The abiotic stress of red currant bushes caused, for example, by water shortages or too low

or high average daily air temperature during the growing season significantly affects growth and development of currant bushes, e.g. status parameters of individual aboveground parts of the plant or chemical composition of the fruit (Panfilova *et al.*, 2021).

Selected physiological parameters of leaves can be determined during the growing season using, among other methods, spectral methods measuring the properties of VIS and NIR light (405–1100 nm) using an LED at a specific wavelength with chlorophyll meters, flavonoid meters or plant nitrogen meters. Modern meters are cheap, accurate and easy to use and have a high repeatability of results (Zlatev *et al.*, 2023). The degree of leaf development, optical properties, leaf coverage, agrotechnical conditions or the severity of stress factors have a significant influence on the readings. In addition to water and minerals, the chemical composition of leaves includes various pigments responsible for colour, among other things. The pigments

contained in leaves are carotenoids, anthocyanins and chlorophyll, which are responsible for photosynthesis and therefore for carbon supply and energy source (Brown, Williams and Dash, 2022). The chlorophyll contained in leaves occurs as chlorophyll a and chlorophyll b. Emerging abiotic stresses, e.g. water shortage, result in a decrease in the proportion of chlorophyll a in leaves, while the proportion of carotenoids, which are responsible for the characteristic yellow-orange colour of leaves, increases (Jarocińska and Zagajewski, 2008). Reduced chlorophyll content negatively affects photosynthesis and thus results in reduced development of the whole plant, including reduced fruit yield (Richardson, Duigan and Berlyn, 2002; Ciganda, Gitelson and Schepers, 2009).

Red currant, like other berry bushes, is cultivated for its fruit production, while the leaves are also a valuable source of compounds with biologically active properties which can be used as functional foods, nutraceuticals or dietary supplements (Ferlemi and Lamari, 2016). Red currant leaves contain polyphenolic compounds such as flavonoids, of which anthocyanins are a subgroup. Anthocyanins can be found in various parts of plants, including leaves, stems, fruits and flowers; in the cellular part in vacuoles, while they are not found in the parenchyma tissues or cell wall. Anthocyanins are natural pigments characterised by colours ranging from orange-red to violet-blue, depending on the chemical structure of the predominant anthocyanin in a given part of the plant (Piątkowska, Kopeć and Leszczyńska, 2011). In optimally fertilised red currant leaves, the colour of anthocyanins is masked by the chlorophyll colour, while any unfavourable external factors such as UV radiation, water deficiency or sudden temperature reduction increase the plant's secretion of anthocyanins, causing leaf discolouration (Piątkowska, Kopeć and Leszczyńska, 2011).

The physical properties of agricultural products are important in food design and processing. Features such as colour, mechanical and rheological properties, thermal and electrical resistance, water content and other physical quantities describe the quality of raw materials and products. Measuring these properties can provide information about the impact of processing and storage conditions on food. Four basic values can be obtained from mechanical property tests: force (load), strain (distance, displacement, penetration), slope (ratio of force to strain), and area under the force/strain curve (energy). The engineering parameters based on these measurements are stress, strain, modulus, and energy, respectively. Stress is the force per unit area, contact, or cross-section. Strain (relative deformation) is the deformation expressed as a percentage of the initial height or length of the specimen subjected to loading. Modulus of elasticity is a measure of stiffness based on the stress/strain ratio (Dobrzański and Rybczyński, 2011).

Antioxidant polyphenol compounds contained in various parts of plants play a very important role in the human body because they protect against the harmful effects of free radicals, the excess of which in the body can cause neurodegenerative diseases, cancer and cardiovascular diseases (Kasprzak-Drozd *et al.*, 2022).

The present study aimed to use a multi-pigment meter as a non-destructive method to assess selected leaf physiological parameters as a quality and harvest maturity indicator of fruits of three red currant cultivars grown on an organic farm.

MATERIALS AND METHODS

MATERIALS

The study material consisted of leaves located on the shoot apex and fruits collected from red currant bushes of three cultivars: 'Red Dutch' ('Holenderska czerwona' in Polish), 'Luna' and 'Losan' grown in the organic farm located in Łopuszka Wielka (49°56'12"N 22°23'35"E), Podkarpackie Voivodship, Poland. The bushes of cultivar 'Red Dutch' were 12 years old, and the 'Luna' and 'Losan' were 7 years old.

FLUORESCENCE AND TRANSMITTANCE MEASUREMENTS OF RED CURRANT LEAVES

Observation and measurements were performed in 2022. In the two weeks prior to fruit harvest maturity (on the 15th (23 Jun 2022), 10th (28 Jun 2022), 5th (02 Jul 2022), and day (07 Jul 2022) of red currant fruit harvest), the fluorescence and transmittance of leaves were measured using a MPM-100 multi-pigment meter (ADC BioScientific), determining the parameters NFI (nitrogen-flavonoid index), FlvM (flavonoid content), AnthM (anthocyanin content), ChlM (chlorophyll content) and the relative content of chlorophyll, flavonoid and anthocyanins in the analysed leaves of an individual red currant cultivar by carrying out 10 measurements of the same leaves in growing season 2022. With the MPM-100 multi-pigment meter, we can measure five values: three measurements of fluorescence intensity and two measurements of transmittance values and directly convert them into individual leaf chemical content indices according to following Equations (Cerovic *et al.*, 2008):

$$\text{ChlM} = \frac{T_{850}}{T_{720}} \quad (1)$$

$$\text{FlvM} = \log\left(\frac{F_{660}}{F_{375}}\right) \quad (2)$$

$$\text{AnthM} = \log\left(\frac{F_{660}}{F_{525}}\right) \quad (3)$$

$$\text{NFI} = \frac{T_{850}}{T_{720}} \cdot \frac{F_{375}}{F_{660}} \quad (4)$$

$$\text{RelChlM} = \frac{T_{720}}{T_{850}} \quad (5)$$

$$\text{RelFlvM} = \frac{F_{375}}{F_{660}} \quad (6)$$

$$\text{RelAnthM} = \frac{F_{525}}{F_{660}} \quad (7)$$

where: ChlM = chlorophyll content, FlvM = flavonoid content, AnthM = anthocyanin content, NFI = nitrogen-flavonoid index, RelChlM = relative chlorophyll content, RelFlvM = relative

flavonoid content, RelAnthM = relative anthocyanin content, T = transmittance, F = fluorescence; 375, 525, 660, 720 and 850 nm = wavelengths.

DETERMINATION OF THE MORPHOLOGICAL CHARACTERISTICS AND WATER CONTENT OF RED CURRANT FRUITS

The sample size was 15 fruits for each variant. For individual fruits, the diameter d was determined with an accuracy of 0.01 mm and the weight with an accuracy of 0.001 g. The density of the individual fruits was calculated as the ratio of their weight to the volume of the sphere with diameter d .

The water content in the individual tested red currant fruits was determined using the drying method (105°C), in accordance with PN-A-75101-03:1990, using a laboratory moisture analyzer (Radwag, Poland).

DETERMINATION OF THE MECHANICAL PROPERTIES OF RED CURRANT FRUITS

The mechanical parameters of the currant fruit were measured in a compression test between two horizontal planes using the Brookfield CT3. The destructive force F_D , the absolute deformation λ and the destructive energy E_D were recorded after each measurement. The value of the apparent modulus of elasticity E_C , which is a measure of the effective value of the mechanical resistance of the test material, was calculated from a modified formula (Gorzelay *et al.*, 2022, Kuźniar *et al.*, 2022):

$$E_c = \frac{E_D}{0.26d^2 \cdot \lambda} \quad (8)$$

where: E_C = apparent modulus of elasticity (MPa), E_D = destructive energy (mJ), d = diameter of the fruit (mm), λ = deformation of the fruit in the direction of the load (mm).

Relative deformation was calculated from formula (Kulig, Łysiak and Skonecki, 2015):

$$\varepsilon = \frac{\lambda}{d} 100\% \quad (9)$$

where: ε = relative deformation (%), d = diameter of the fruit (mm), λ = deformation of the fruit in the direction of the load (mm).

DETERMINATION OF BIOACTIVE COMPONENTS OF RED CURRANT FRUITS

The content of ascorbic acid in red currant fruits was determined according to the PN-A-04019:1998 standard. The total content of polyphenols in red currant fruits was determined using the Folin-Ciocalteu method (Piechowiak *et al.*, 2019). The free radical scavenging activity (DPPH method) was determined using the method described in Djordjević *et al.* (2010) and expressed as IC50 (mg·cm⁻¹). The antioxidant activity was determined using the ABTS method according to the methodology described by Jakobek *et al.* (2007), and the result was given in $\mu\text{M TE}\cdot\text{g}^{-1}$ DM of fruit. The iron reduction capacity (FRAP method) was determined using the method given by Chiabrando and Giacalone (2015), and the result was given in mM Fe²⁺·100 g⁻¹ of fruit. All analyses were performed in triplicate.

STATISTICAL ANALYSIS

The Statistica 13.3 software (TIBCO Software Inc, Tulsa, OK, USA) was used to perform statistical analysis of the obtained results including analysis of variance (ANOVA) and *NIR* significance test at the significance level $\alpha = 0.05$. Pearson correlation was also performed between fluorescence and transmittance parameters of leaves and morphological traits, water content and mechanical properties of fruit of tested currant cultivars on the day of harvest. In addition, the correlation between fluorescence and transmittance parameters of leaves and selected chemical properties of fruit on the day of the harvest. The trend lines and best fit equations described changes of the tested parameters depending on the day before fruits harvest maturity and coefficient of determination (R^2) were obtained using Microsoft Excel 2019 MSO (Jamil and Mujeebu, 2018).

RESULTS AND DISCUSSION

Currently, non-destructive methods are used to assess the development and plant status on plantations by measuring individual leaf traits, such as chlorophyll content at a specific wavelength. Results of fluorescence and transmittance parameters of leaves of red currant cultivars grown on an organic farm during the two weeks before fruit ripeness are presented in the Table 1.

The NFI parameter of the leaves on bushes of red currant on the 15th day before the fruit harvest maturity was the lowest among the measurement dates analysed. From the 10th day before harvest maturity to the day of fruit harvest, the NFI parameter was at a similar level of 0.52 on average (Tab. 1). It is known that flavonols provide photoprotection in the UV light spectrum and remove reactive oxygen species to protect plant photosynthesis. Flavonols are also a good indicator of nitrogen content in leaf plants.

The FlvM parameter increased at successive measurement dates; the closer to the optimum of the red currant fruit harvest date, the higher the determined leaf status parameter was, with an average of 1.27 (Tab. 1). On the other hand, the obtained results of the RelFlvM parameter were opposite to those of the FlvM parameter in the analysed days before harvest maturity of fruit (Tab. 1). In the study by Vagiri *et al.* (2015), it was observed that the timing of black currant leaf measurement influenced the content of phenolic compounds; as the plant grew, the content of phenolic compounds increased, and then the closer to the fruit harvest date there were differences in the content of the different groups of compounds included in the total polyphenol content. Changes in the content of phenolic compounds in leaves that occur during the growing season may be due to, among other things, their transport to lower parts of the plant, biosynthesis and conversion to other compounds, e.g. lignins or tannins, or transition to ripening fruit (Vagiri *et al.*, 2015).

The ChlM parameter increased at successive measurement dates; the closer to the optimum of the red currant fruit harvest date, the higher the determined leaf status parameter was, with an average of 0.60 (Tab. 1). However, the obtained results of the RelChlM parameter were opposite to those of the ChlM parameter in the evaluated days before harvest maturity of red currant fruit (Tab. 1). Chlorophyll and flavonols are good

Table 1. Fluorescence and transmittance parameters of red currant leaves depending on fruit harvest maturity and the cultivar

Specification		Parameter						
		NFI	FlvM	AnthM	ChlM	RelChlM	RelFlvM	RelAnthM
Days before harvest maturity	15	0.34 ^a ±0.09	1.11 ^a ±0.23	0.08 ^b ±0.03	0.37 ^a ±0.05	0.73 ^c ±0.03	0.09 ^c ±0.04	0.83 ^a ±0.06
	10	0.52 ^b ±0.08	1.21 ^{ab} ±0.12	0.03 ^a ±0.06	0.62 ^b ±0.08	0.62 ^b ±0.03	0.07 ^b ±0.02	0.93 ^b ±0.11
	5	0.53 ^b ±0.14	1.31 ^b ±0.15	0.03 ^a ±0.06	0.66 ^b ±0.14	0.61 ^b ±0.06	0.05 ^b ±0.02	0.94 ^b ±0.13
	0	0.51 ^b ±0.12	1.46 ^c ±0.12	0.09 ^b ±0.05	0.74 ^c ±0.14	0.58 ^a ±0.05	0.04 ^a ±0.01	0.81 ^a ±0.10
Cultivar	'Red Dutch'	0.48 ^b ±0.19	1.23 ^a ±0.13	0.05 ^a ±0.07	0.59 ^a ±0.21	0.64 ^a ±0.08	0.06 ^b ±0.02	0.90 ^b ±0.13
	'Losan'	0.50 ^b ±0.11	1.22 ^a ±0.18	0.05 ^a ±0.05	0.61 ^a ±0.15	0.63 ^a ±0.06	0.07 ^b ±0.03	0.90 ^b ±0.11
	'Luna'	0.45 ^a ±0.10	1.35 ^b ±0.19	0.08 ^b ±0.06	0.60 ^a ±0.15	0.63 ^a ±0.06	0.05 ^a ±0.02	0.84 ^a ±0.11
Average		0.48 ±0.14	1.27 ±0.18	0.06 ±0.06	0.60 ±0.17	0.63 ±0.07	0.06 ±0.03	0.88 ±0.13

Explanations: NFI = nitrogen-flavonol index; FlvM = flavonoid content; AnthM = anthocyanin content; ChlM = chlorophyll content; RelChlM = relative chlorophyll content; RelFlvM = relative flavonoid content; RelAnthM = relative anthocyanin content. Data are expressed as a mean value ($n = 15$) \pm SD; SD = standard deviation. Mean values within a row with different letters are significantly different ($p < 0.05$).

Source: own study.

indicators of a plant's N status. With N deficiency, plants produce more flavonoids or carbon-based compounds. The nitrogen in leaves is the main component of chlorophyll. Therefore, by measuring the amount of chlorophyll, the nutritional status of the plant in this element can be determined (Richardson, Duigan and Berlyn, 2002; Pérez-Patricio *et al.*, 2018). The content of chlorophyll a and b in red currant leaves decreases during drought thus reducing photosynthesis, while the proportion of carotenoids in the leaves increases (Richardson, Duigan and Berlyn, 2002; Panfilova and Golyaeva, 2017). Chlorophyll content in leaves decreases during plant growth and development (Richardson, Duigan and Berlyn, 2002; Ciganda, Gitelson and Schepers, 2009).

RelAnthM in the plant leaf measurement dates of the red currant cultivars was at similar levels; they increased slightly on the 10th and 5th day before the optimum fruit harvest date to gradually decrease on the harvest day. The mean AnthM and RelAnthM was 0.06 and 0.88, respectively (Tab. 1). Anthocyanins as natural pigments in plants can be red, blue, purple or colourless. In acidic condition, anthocyanin appears as red pigment while blue pigment anthocyanin exists in alkaline conditions. Our studies suggest a role of anthocyanins in protecting plants at extreme temperatures, serving to attract pollinating insects and, due to their colouration, attracting them to distribute seeds.

The main source of bioactive compounds is the fruit, while individual anatomical parts of the currants, e.g. the leaves, are also a valuable raw material with health-promoting potential due to their high content of phenolic compounds (Vagiri *et al.*, 2015; Milenkovic-Andjelkovic *et al.*, 2016; Staszowska-Karkut and Materska, 2020). The content of phenolic compounds in leaves is influenced by many factors, including genotype, environmental conditions during the growing season, harvest time, growth stage or processing and storage conditions (Vagiri *et al.*, 2015; Milenkovic-Andjelkovic *et al.*, 2016; Staszowska-Karkut and Materska, 2020). Phenolic compounds provide the plant with initial protection against pathogens (Raudsepp *et al.*, 2010; Mikulic-Petkovsek *et al.*, 2013). In our studies the NFI and ChlM index values were not statistically significantly different for the tested cultivars, as was the RelChlM. The FlvM and AnthM values

for 'Luna' were significantly higher, which was related to earlier fruit ripening of this cultivar compared to the other tested cultivars. An inverse relationship was observed for the parameters: RelFlvM and RelAnthM (Tab. 1). In the study by Vagiri *et al.* (2015), it was observed that black currant leaves located at the shoot apex had a significantly higher content of phenolic compounds compared to leaves located in the middle and at the base of the shoot. The higher content of phenolic compounds in leaves located at the shoot apex is related to easier access to sunlight, which simultaneously facilitates the rate of operation of the flavonoid pathway. In addition, the cultivar and the age of bushes on the plantation, had a significant effect on the content of phenolic compounds in the leaves (Vagiri *et al.*, 2015).

Two-way analysis of variance (Tab. 2) showed that all determined transmittance and fluorescence parameters depended on the date before harvest maturity ($p < 0.001$). The FlvM, RelFlvM ($p < 0.001$), AnthM and RelAnthM ($p < 0.01$) depended on the cultivar. However, the interaction of term and cultivar was significant at $p < 0.001$ for NFI, FlvM and RelFlvM.

Table 2. Results of two-factor ANOVA of the influence of time before harvest maturity and red currant cultivar on leaf fluorescence and transmittance parameters

Parameter	Time before harvest maturity		Cultivar		Interaction	
	F	P	F	P	F	P
NFI	21.792	0.000	2.288	0.105	4.305	0.000
FlvM	34.660	0.000	15.220	0.000	8.950	0.000
AnthM	15.998	0.000	5.349	0.006	2.076	0.059
ChlM	53.254	0.000	0.204	0.816	1.559	0.162
RelChlM	61.010	0.000	0.530	0.590	1.320	0.252
RelFlvM	31.513	0.000	11.597	0.000	12.945	0.000
RelAnthM	17.190	0.000	5.270	0.006	2.270	0.039

Explanations: F = test value, P = probability, other as in Tab. 1. Source: own study.

The variability of the NFI value of the leaves of the tested red currant varieties depending on the day preceding the harvest ripeness of the fruit is well described by a second-degree polynomial function (Fig. 1). It is characterised by a maximum around the fifth day before harvest maturity.

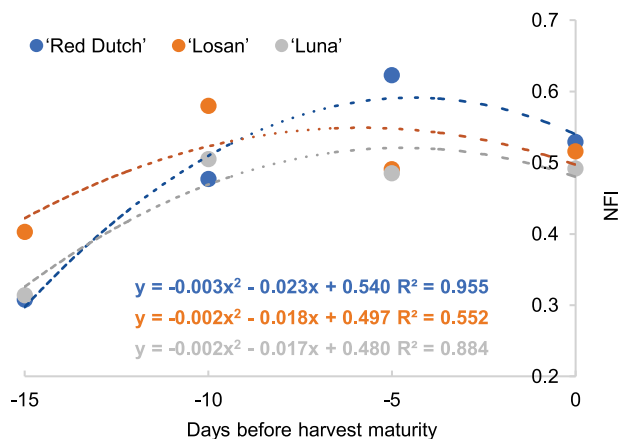


Fig. 1. Changes in leaf nitrogen-flavonoid index (NFI) values of the tested red currant cultivars depending on the day before fruits harvest maturity; source: own study

The value of the leaf NFI of the tested currant cultivars grown on the organic farm varied according to the test date and by cultivar. On the 15th day before the optimum fruit harvest date, the NFI value of leaves of cultivar 'Losan' was characterised by a significantly higher value in relation to the other cultivars. Moreover, this relationship was also observed on the 10th day before harvest maturity (for all tested cultivars, the NFI value on the 10th day before the optimum harvest date was higher in relation to the values obtained on the 15th day before harvest maturity). On day 5 before fruit harvest, the leaf NFI value of the red currant cultivar 'Red Dutch' was significantly higher than that of the other cultivars analysed, and the result obtained was also higher than that obtained on day 10 before fruit harvest maturity (for the other cultivars, slight decreases in leaf NFI values were observed on day 5th compared to day 10th before fruit harvest). On the day of harvest, the leaf NFI value of the tested cultivars was at a similar level (Fig. 1).

The FlvM values of the leaves of the tested currant cultivars gradually increased from the 15th day before the optimum harvest date to the day of fruit harvest, and the highest values were obtained for the cultivar 'Luna' (except on the 10th day) and the cultivar 'Losan'. The smallest increments in FlvM values on individual test days were observed for the cultivar 'Red Dutch' (Fig. 2). Flavonoids have protective functions in the plant, including neutralising reactive oxygen species associated with plant stress, e.g. due to unfavourable environmental conditions (Staszowska-Karkut and Materska, 2020). Changes in the FlvM value of the leaves of the tested cultivars depending on the day preceding the harvest ripeness of the fruit are very well described by an increasing first-degree polynomial (Fig. 2).

The obtained AnthM values of the leaves of the red currant grown on the organic farm varied depending on the test date and the cultivar analysed. On day 15th before fruit set maturity, the AnthM values of leaves of the cultivar 'Luna' were characterised by the highest value, while the cultivar 'Losan' by the lowest AnthM value. On days 10th and 5th before fruit ripening,

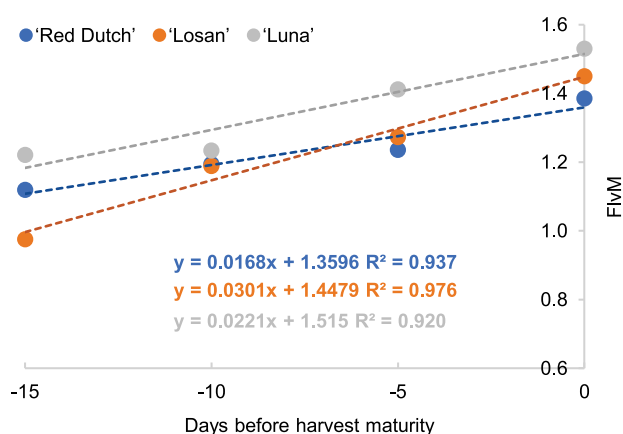


Fig. 2. Changes in leaf flavonoid content (FlvM) values of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

'Luna' had a significantly higher leaf AnthM value, while on day 5th before fruit ripening the leaf AnthM value of 'Red Dutch' was significantly lower than that of the other cultivars analysed. On the day of fruit harvest, leaf AnthM values of the tested cultivars were significantly higher (Fig. 3). The variability of the AnthM value of the leaves of the tested cultivars depending on the day preceding the harvest ripeness of the fruit is well described by the second-degree polynomial function, which is characterised by a minimum around the eighth day before harvest ripeness (Fig. 3).

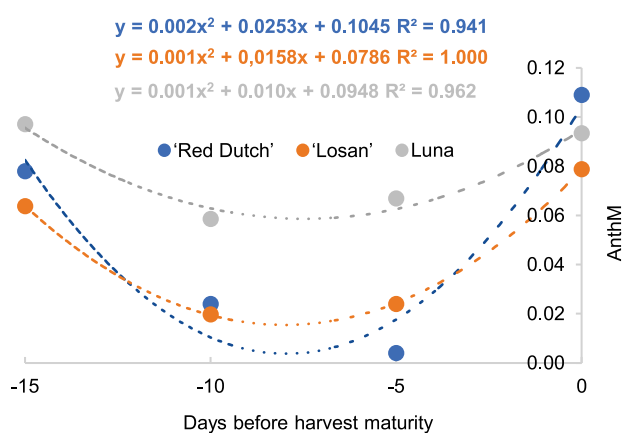


Fig. 3. Changes in leaf anthocyanin content (AnthM) values of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

The ChlM values of leaves gradually increased from the 15th day before fruit harvest maturity to their optimum harvest date, with the greatest differences observed for 'Losan' on the 10th day before the optimum harvest date (significantly higher leaf ChlM value) and on the 5th day before fruit harvest maturity (significantly lower ChlM value) compared to the other cultivars analysed (Fig. 4).

The variability of the ChlM value of the leaves of the tested cultivars depending on the day preceding the harvest maturity of the fruit is well described by the second-degree polynomial function, which is characterised by a maximum around the day of harvest maturity (Fig. 4).

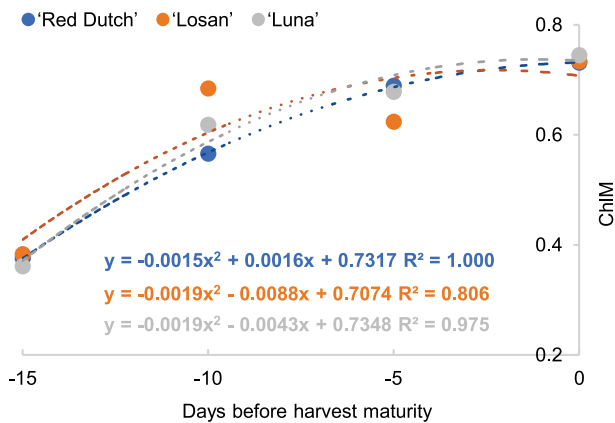


Fig. 4. Changes in leaf chlorophyll content (ChlM) values of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

The changes of the ChlM content of the leaves of the tested red currant varieties depending on the day preceding the harvest ripeness of the fruit is described well, similarly to ChlM, by a second-degree polynomial function. However, it is characterised by the opposite course, i.e. the minimum occurs around the day of harvest maturity (Fig. 5). The functions describing the variability of the RelFlvM (first-degree polynomial) and the RelAnthM (second-degree polynomial) also have the opposite course than for FlvM and AnthM. The RelFlvM is lowest at harvest maturity (Fig. 6), and the RelAnthM is highest approximately on the eighth day before harvest maturity (Fig. 7).

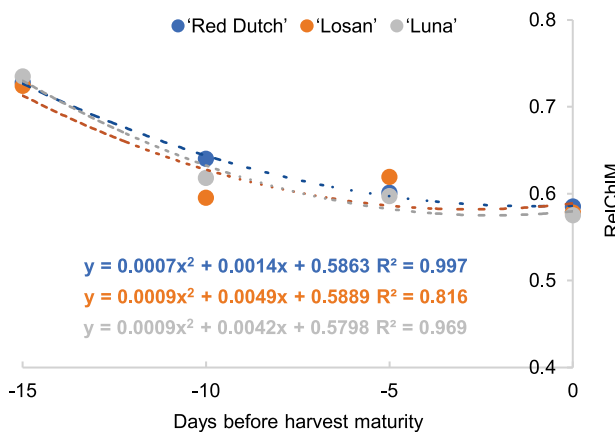


Fig. 5. Changes in relative chlorophyll content (RelChlM) of the leaves of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

Chlorophyll is the green pigment of leaves, plays a key role in photosynthesis, and its content indicates the health of the plant and its resistance to stress (Zlatev *et al.*, 2023). Changes in the RelChlM of the leaves of the tested cultivars in individual days before fruit harvest maturity gradually decreased from the 15th day before fruit harvest maturity to the day of the optimum harvest date. The RelChlM values for the three tested red currant cultivars were at a similar level (Fig. 5).

The RelFlvM of the leaves of the tested red currant cultivars gradually decreased from the 15th day before fruit harvest maturity to the day of harvest. Moreover, on the 15th day before harvest maturity, the value of the RelFlvM of leaves of

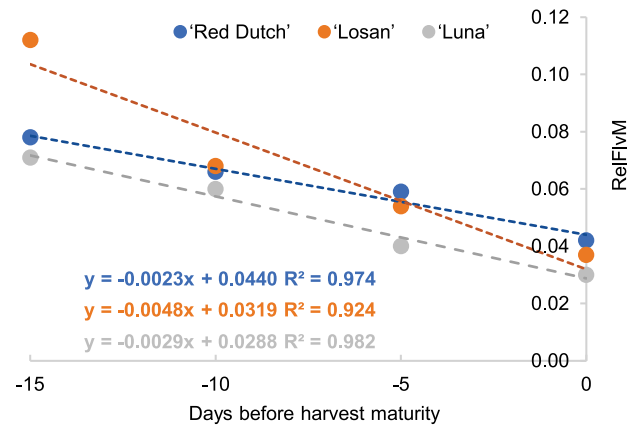


Fig. 6. Changes in relative flavonoid content (RelFlvM) of the leaves of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

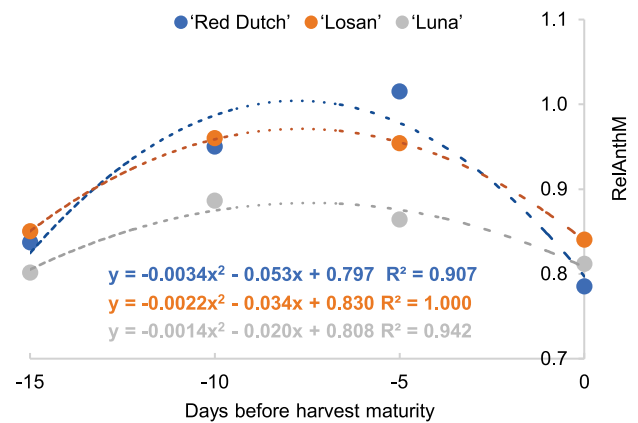


Fig. 7. Changes in the relative anthocyanin content (RelAnthM) of the leaves of the tested red currant cultivars depending on the day before fruit harvest maturity; source: own study

cultivar 'Losan' was significantly higher than the values obtained for the other two cultivars. No statistical differences were observed for the RelFlvM values of the leaves of the individual red currant cultivars at the subsequent test dates (Fig. 6). Currant leaves have the highest content of phenolic compounds when they reach optimum size, i.e. at the full maturity (Tabart *et al.*, 2011). Among the many phenolic compounds found in currant leaves, it can be distinguished: quercetin, rutin, p-coumaric acid, gallic acid, and, in lower concentrations, caffeic acid, chlorogenic acid or ferulic acid (Cyboran *et al.*, 2014; Milenkovic-Andjelkovic *et al.*, 2016; Staszowska-Karkut and Materska, 2020). The phenolic compounds contained in currant leaves have antioxidant, antimicrobial or anti-inflammatory effects (Krzepiłko *et al.*, 2018).

The highest differences for RelAnthM values of leaves of these three cultivars grown on the organic farm were observed on the 10th day before fruit maturity for the cultivar 'Luna' and on the 5th day before fruit maturity for three tested cultivars (including the lowest values again observed for 'Luna' – Fig. 7). Fruits of cultivar 'Luna' ripen earlier than the other two cultivars, hence the highest differences were observed for RelAnthM values. On the day of the fruit harvest, all tested cultivars were characterised by similar RelAnthM values (Fig. 7). Anthocyani-

dins and their glycosidic derivatives called anthocyanins are a group of natural plant pigments ranging in colour from red to blue-violet. In the plant world, they are mainly found in fruit and flowers, but also in leaves or stems (in much lower concentrations). Anthocyanins in leaves absorb UV rays, protecting the plant's DNA structure (trapping free radicals), and have a significant effect on the rate of protein synthesis and cell division (Saluk-Juszczak, 2010).

The fruits of the studied currant cultivars subjected to strength tests were more diverse in terms of mechanical properties than morphological features and moisture content (Tab. 3). The fruits of the 'Red Dutch' cultivar had the largest diameter and weight, and the smallest density and moisture content, while the fruits of the 'Luna' cultivar had the highest density. However, in terms of the determined mechanical properties of the fruits, all the studied currant cultivars differed significantly from each other.

The 'Luna' cultivar was the most resistant to mechanical damage, as it required the greatest destructive force and destructive energy to break, and was characterised by the greatest relative deformation and apparent modulus of elasticity (Tab. 3). On the other hand, the 'Losan' cultivar was the most susceptible

to damage, as it was damaged with the use of significantly the least destructive force and energy and had the lowest apparent modulus of elasticity. The 'Red Dutch' cultivar was significantly the least deformed.

Among the determined morphological characteristics of red currant fruit, their diameter and weight were very highly positively correlated with the parameters as such the relative chlorophyll content and relative flavonoid content (>0.8) but also highly positively correlated with the parameters of the NFI and AntM (>0.7). The other parameters analysed were highly (ChlM and relative anthocyanin content) or very highly (FlvM) negatively correlated with red currant fruit diameter regardless of the cultivar analysed (Tab. 4). The red currant fruit density was very highly positively correlated with FlvM and ChlM parameters (>-0.9) while very highly negatively correlated with relative flavonoid content, relative chlorophyll content and NFI parameters (>-0.9 - Tab. 4).

The water content of the analysed fruits of the three currant cultivars was very highly positively correlated (>0.9) with the RelAnthM and very highly negatively (>-0.9) correlated with the AnthM. Other correlations between the determined parameters of transmittance and fluorescence of red currant leaves and the

Table 3. Morphological features, moisture content and mechanical properties of red currant fruits depending on the cultivar

Variable		Cultivar		
		'Red Dutch'	'Losan'	'Luna'
Morphological features and moisture content	diameter (mm)	9.1 ^b ±0.8	8.8 ^a ±0.8	8.7 ^a ±0.6
	weight (mg)	434 ^b ±113	404 ^a ±113	400 ^a ±84
	density ($\cdot 10^{-3}$ kg·m ⁻³)	1.08 ^a ±0.05	1.11 ^b ±0.05	1.14 ^c ±0.05
	moisture content (%)	84.9 ^a ±1.8	85.5 ^b ±1.8	85.1 ^b ±2.2
Mechanical properties	destructive force (N)	2.87 ^b ±0.78	2.43 ^a ±0.89	3.62 ^c ±1.16
	relative deformation (%)	35.6 ^a ±6.2	36.4 ^b ±6.5	40.3 ^c ±5.4
	destructive energy (mJ)	5.00 ^b ±1.21	3.88 ^a ±1.26	6.01 ^c ±1.67
	apparent modulus of elasticity ($\cdot 10^{-3}$ MPa)	75.0 ^b ±23.3	62.2 ^a ±19.8	87.3 ^c ±21.1

Explanations: data are expressed as mean values ($n = 15$) ±SD; SD = standard deviation; mean values within rows with different letters are significantly different ($p < 0.05$).

Source: own study.

Table 4. Pearson correlation coefficient of red currant leaf fluorescence and transmittance parameters and morphological characteristics, water content and mechanical properties of red currant fruit

Parameter	Morphological features and moisture content				Mechanical properties			
	diameter	weight	density	moisture content	destructive force	relative deformation	destructive energy	apparent modulus of elasticity
NFI	0.88*	0.84*	-0.99*	-0.16	-0.75*	-0.98*	-0.62*	-0.63*
FlvM	-0.93*	-0.89*	1.00*	0.27	0.67*	0.96*	0.53*	0.54*
AnthM	0.76*	0.82*	-0.52*	-0.98*	0.35	-0.18	0.51*	0.49*
ChlM	-0.80*	-0.74*	0.95*	0.02	0.84*	1.00*	0.73*	0.74*
RelChlM	1.00*	0.98*	-0.97*	-0.53*	-0.43*	-0.83*	-0.27	-0.28
RelFlvM	0.91*	0.87*	-1.00*	-0.24	-0.69*	-0.97*	-0.56*	-0.57*
RelAnthM	-0.74*	-0.80*	0.487*	0.99*	-0.38	0.15	-0.54*	-0.52*

Explanations: * significant ($p < 0.05$), NFI, FlvM AnthM, ChlM, RelChlM, RelFlvM, RelAnthM as in Tab. 1.

Source: own study.

moisture content of the harvested fruits were of medium or low level (Tab. 4).

Among the analysed mechanical traits of red currant fruit, destructive force was highly positively correlated with the ChLM parameter (>0.8), while it was moderately negatively correlated with the NFI and RelFlvM (>-0.7 – Tab. 4). The relative deformation of the analysed fruits was very highly positively correlated with FlvM and ChLM parameters (>0.9) and very highly negatively correlated with RelFlvM and NFI parameters (>0.9 ; Tab. 4). Destructive energy during static compression of red currant fruit was moderately positively correlated with the parameter ChLM and moderately negatively correlated with the parameters NFI, RelFlvM and RelAnthM (Tab. 4). The determined conventional modulus of elasticity of the fruits of the tested cultivars was positively correlated on average with two parameters: FlvM and ChLM and negatively correlated on average with the NFI, RelFlvM and RelAnthM (Tab. 4).

All the tested cultivars differed significantly in terms of ascorbic acid content and total polyphenols content (Tab. 5). The highest number of these bioactive compounds was found in the ‘Losan’ cultivar. The ‘Luna’ cultivar was characterised by the lowest content of ascorbic acid and the ‘Red Dutch’ cultivar had the lowest total polyphenols content.

Varietal differentiation in terms of antioxidant activity of red currant fruit was found only in the case of the cation radical ABTS method, for which the ‘Losan’ cultivar was characterised by significantly the highest antioxidant activity.

The chemical composition of red currant fruit depends not only on the cultivar but also on environmental conditions (Krzepiłko *et al.*, 2018). Among the determined chemical properties in fruit of tested red currant cultivars, ascorbic acid content was highly positively correlated with the ChLM (>0.9) and with the FlvM (>0.7) and highly negatively correlated with the parameters such as the NFI and RelFlvM (Tab. 6). The higher the anthocyanin content in the leaves, the better the ascorbic acid found in red currant fruit is absorbed (Gryszczyńska *et al.*, 2011).

The content of total polyphenols in the analysed red currant fruit was very highly positively correlated with the ChLM and FlvM parameters (>0.9) and very highly negatively correlated with the NFI and RelFlvM (Tab. 6). The nitrogen content of the plant significantly affects the amount of polyphenolic compounds in the fruit (Benbrook, 2005). The content of phenolic compounds in red currant leaves depends on the cultivar, as well as the harvest date and the location of the leaves on the bush (Milenkovic-Andjelkovic *et al.*, 2016). The content of polyphenolic compounds and antioxidant activity determined by the

Table 5. The content of ascorbic acid, total polyphenols and antioxidant activity of red currant fruit

Variable	Cultivar			
	‘Red Dutch’	‘Losan’	‘Luna’	
Ascorbic acid content (mg·100 g ⁻¹)	34.1 ^b ±0.50	44.1 ^c ±0.10	31.2 ^a ±0.20	
Total polyphenols content (mg GAE·100 g ⁻¹)	117.4 ^a ±0.6	201.7 ^c ±0.7	119.9 ^b ±0.9	
Antioxidant activity	DPPH IC ₅₀ (mg·cm ⁻³)	3.79 ^a ±0.06	3.78 ^a ±0.08	3.69 ^a ±0.05
	ABTS ⁺ (μM TE·g ⁻¹ DM)	11.27 ^a ±0.27	12.03 ^b ±0.03	11.18 ^a ±0.02
	FRAP (mM Fe ²⁺ ·100 g ⁻¹)	0.59 ^a ±0.06	0.59 ^a ±0.05	0.64 ^a ±0.04

Explanations: DPPH = 2,2-diphenyl-1-picrylhydrazyl antioxidant parameter, ABTS = 2,2-azobis(3-ethylbenzothiazoline-6-sulfonate) antioxidant parameter, FRAP = ferric ion reducing antioxidant parameter, other as in as in Tab. 3.

Source: own study.

Table 6. Pearson’s correlation coefficient of fluorescence and transmittance parameters of red currant leaves and selected chemical properties of red currant fruit

Parameter	Ascorbic acid content	Total polyphenols content	Antioxidant activity		
			DPPH	ABTS ⁺	FRAP
NFI	-0.84*	-0.95*	-0.05	-0.90*	0.23
FlvM	0.78*	0.91*	-0.06	0.85*	-0.12
AnthM	0.10	-0.05	0.91*	0.08	-0.82*
ChLM	0.91*	0.98*	0.20	0.96*	-0.37
RelChLM	-0.57*	-0.75*	0.34	-0.66*	-0.18
RelFlvM	-0.80*	-0.92*	0.02	-0.87*	0.16
RelAnthM	-0.23	0.01	-0.93*	-0.11	0.84*

Explanations: NFI = nitrogen-flavonoid index, FlvM = flavonoid content, AnthM = anthocyanin content, ChLM = chlorophyll content, RelChLM = relative chlorophyll content, RelFlvM = relative flavonoid content, RelAnthM = relative anthocyanin content, DPPH = 2,2-diphenyl-1-picrylhydrazyl antioxidant parameter, ABTS = 2,2-azobis(3-ethylbenzothiazoline-6-sulfonate) antioxidant parameter, FRAP = ferric ion reducing antioxidant parameter, * significant ($p < 0.05$).

Source: own study.

DPPH radical method were highly positively correlated in black currant leaves (Tabart *et al.*, 2007; Milenkovic-Andjelkovic *et al.*, 2016). The content of polyphenolic compounds in black currant leaves is higher than in its fruit but the bioactive profile is different. In addition, a very important role on the total content of phenolic compounds in currant leaves, including mainly flavonoids and anthocyanins, is played by the appropriate choice of soil for currant plantations and its management system (Staszowska-Karkut and Materska, 2020).

Fruits produced on the organic farm are characterised by higher antioxidant activity compared to those collected from conventional cultivation (Rembiałkowska, 2000). The antioxidant activity of red currant fruits determined by the DPPH radical method was very strongly positively correlated with the AnthM parameter due to the selectivity of this method, which allows the determination of only hydrophobic antioxidants such as anthocyanins and very strongly negatively correlated with the parameter of the RelAnthM (inverted relationship). The antioxidant activity of the analysed fruits determined by the ABTS cation radical method showed a very high positive correlation with the ChlM and FlvM parameters (>0.9) and a very high negative correlation with the NFI and RelFlvM (Tab. 6). The ability of red currant fruit to reduce trivalent iron ions using the TPTZ reagent (2,4,6-Tris(2-pyridyl)-s-triazine; FRAP method) was a highly positively correlated with the RelAnthM and highly negatively correlated with the AnthM (Tab. 6). It was reported that younger currant leaves harvested from the shoot apex were characterised by higher antioxidant activity compared to older leaves (Staszowska-Karkut and Materska, 2020). In addition, wild-growing currant bushes had higher antioxidant activity of leaves and fruits compared to those grown on commercial plantations (Milenkovic-Andjelkovic *et al.*, 2016).

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

CONCLUSIONS

Multi-pigment meter can be useful for an easy, non-destructive evaluation of selected physiological parameters of leaves to assess red currant fruit quality and harvest maturity. Based on the results it can be determined not only the current nutritional status of the plants, but also the important influence on the final product, which are morphological characteristics and mechanical and chemical properties of fruit of tested red currant cultivars. The very high positive correlation between selected leaf parameters (RelChlM and RelFlvM) and the diameter and weight of the fruit is obtained. Chlorophyll content in leaves is positively correlated with the fruit destructive power, which is important in processing. While ascorbic acid content and total polyphenols are positively correlated with the content of chlorophyll and flavonoids contained in leaves of tested red currant cultivars. On the basis of the results obtained with using of the multi-pigment meter, it is possible to carry out a preliminary estimation of fruit quality already during the growing season. However, further research is still required to understand the long-term patterns of the findings.

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