

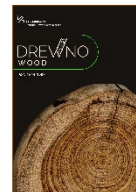
Article citation info:

Kopyciński B., Duda A., Langer E., Kamińska-Bach G. 2024. Color Photostability Assessment of Ultrasound-Assisted Extracts From European Blueberry (*Vaccinium Myrtillus* L.) Obtained with The Use of Non-Toxic Solvents. *Drewno. Prace naukowe. Doniesienia. Komunikaty* 67 (213): 00019. <https://doi.org/10.53502/wood-187625>



## Drewno. Prace naukowe. Doniesienia. Komunikaty Wood. Research papers. Reports. Announcements

Journal website: <https://drewno-wood.pl/>



# Color Photostability Assessment of Ultrasound-Assisted Extracts From European Blueberry (*Vaccinium Myrtillus* L.) Obtained with The Use of Non-Toxic Solvents

Bartosz Kopyciński<sup>a, c, \*</sup>

Alicja Duda<sup>b, c</sup>

Ewa Langer<sup>a</sup>

Grażyna Kamińska-Bach<sup>a</sup>

<sup>a</sup> Łukasiewicz Research Network – Institute for Engineering of Polymer Materials and Dyes, Toruń, Poland

<sup>b</sup> Łukasiewicz Research Network – Institute of Non-Ferrous Metals, Gliwice, Poland

<sup>c</sup> Doctoral School, Silesian University of Technology, Gliwice, Poland

### Article info

Received: 13 February 2023

Accepted: 12 January 2024

Published online:

22 April 2024

### Keywords

European blueberry (*Vaccinium myrtillus* L.)

ultrasound-assisted extraction

non-toxic solvents

weathering test

color photostability

dyeing additives

In recent years, the search for environmentally friendly industrial solutions has been gaining momentum, especially in the areas of materials science and food technology. The aim of this study was to evaluate the color photostability of European blueberry (*Vaccinium myrtillus* L.) extracts obtained with the use of non-toxic solvents. Extracts of lyophilized forest berries were prepared with the use of water, ethanol, glycerin, and their mixtures by means of ultrasound-assisted extraction. ATR-FTIR spectroscopy was used to determine chemical structure. Color measurement in the CIELab color space was performed before, during and after the QUV accelerated weathering test conducted for dyed cotton substrates. The content of anthocyanins expressed as cyanidin 3-glucoside equivalents was determined based on UV-Vis absorbance intensity measurement and a pH-differential method. The highest color photostability was observed for the extract prepared using water and ethanol in the proportion 50/50 (v/v). However, the highest content of anthocyanins was recorded for the mixture of glycerin and ethanol in a ratio of 50/50 (v/v). It is shown that the addition of glycerin supports the extraction of anthocyanins to some extent; however, it results in a reduction of the color photostability of extracts used to dye cotton fabrics.

DOI: 10.53502/wood-187625

Published by Łukasiewicz Research Network – Poznań Institute of Technology. This work is licensed under the Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by/4.0/>

### Introduction

Due to the biodiversity of flora, forests are a source of many valuable raw materials – not only wood, but also plants with a wide spectrum of uses, from food, through medicines to dyes. In recent years, there has

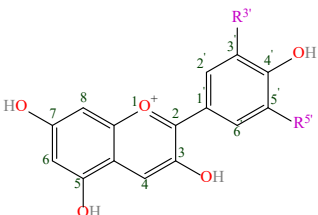
been growing interest in dyes of plant origin, including materials containing anthocyanins. Methods of vegetable and fruit processing that provide a high content of anthocyanins are sought, especially those which align with the trend of Green Chemistry. Nevertheless, acquiring extracts with selective solvents is

\* Corresponding author: [bartosz.kopycinski@impib.lukasiewicz.gov.pl](mailto:bartosz.kopycinski@impib.lukasiewicz.gov.pl)

still the primary method by which they are obtained. The solvents used are mainly polar organic liquids such as methanol, ethanol or acetone. They are usually acidified with organic and inorganic acids, and the use of strong acids may cause hydrolysis of the glycosidic bond in the dye molecule (Silva et al. 2015). Due to the toxicity and high costs associated with the removal of some of the aforementioned compounds, more environmentally friendly solutions are being developed; for example, the use of glycerin in the extraction process (Shehata et al. 2015; Kowalska et al. 2021). Among known improvements in this area, ultrasonically assisted extraction is particularly worthy of note. It has been proven that ultrasonic extraction not only increases the process efficiency, but is also economically and ecologically justified (Chemat et al. 2016). Factors supporting extraction also include the influence of microwaves (Liazid et al. 2011), the presence of an electric field (Puértolas et al. 2013), or the use of supercritical CO<sub>2</sub> (Paes et al. 2014).

Anthocyanins are a group of dyes derived from plants, which among other things control the color of vegetables, fruits and flowers. They are formed from a combination of a sugar residue and anthocyanidin (aglycone) (Kayesh et al. 2013; Quina et al. 2009). They can take shades from blue and purple to red and orange, depending on the presence of hydroxyl groups in the molecule, the number and type of sugar moieties, and the aliphatic or aromatic carboxylates attached to them (Kong et al. 2003). Many research projects relating to the identification of anthocyanins have led to the recognition of several hundred compounds belonging to this group (numbers from 500 to 700 are mentioned in the literature) (Wallace and Giusti 2019). The most common naturally occurring anthocyanidins include cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin, which have chemical structures based on an identical carbon skeleton with various glycosidic substitutions at positions 3 and 5. The general anthocyanidin structure with possible substitutions is shown in Table 1 (Salehi et al. 2020).

**Table 1.** Selected anthocyanidin structure and its possible substitutions

General structure	Anthocyanidin	Substituent	
		R <sub>3</sub> '	R <sub>5</sub> '
	Cyanidin	-OH	-H
	Delphinidin	-OH	-OH
	Malvidin	-OCH <sub>3</sub>	-OCH <sub>3</sub>
	Pelargonidin	-H	-H
	Peonidin	-OCH <sub>3</sub>	-H
	Petunidin	-OH	-OCH <sub>3</sub>

Anthocyanins are soluble in water, poorly resistant to high temperatures, and have strong antioxidant properties (Castañeda-Ovando et al. 2009; Turturică et al. 2018). They also change color depending on the pH of the environment: they turn red/pink, purple and green-yellow in acidic, neutral and alkaline solutions respectively (Michaelis et al. 1936). Depending on the pH, anthocyanins undergo structural changes. In an acidic environment they are transformed into carbinol, pseudobase, and quinonoidal structures, and at alkaline pH they are transformed into chalcone (Castañeda-Ovando et al. 2009). Their main source is fruit berries, including black chokeberry (*Aronia melanocarpa* Michx. Elliott), elderberry (*Sambucus nigra* L.), and European blueberry (*Vaccinium myrtillus* L.) (Wu et al. 2006).

Widely distributed and readily available, products rich in anthocyanins are used in a variety of applications. An interesting direction in the development of such extracts is their use for staining

cellulose-based materials (Phan et al. 2020) or fibers made of cotton and silk (Vankar and Shukla 2011; Wang et al. 2016). Apart from being components of the human diet and environmentally friendly alternatives to synthetic food dyes (Coultate and Blackburn 2018), they can also be used to obtain intelligent packaging films. The most commonly used matrices include chitosan (Halász and Csóka 2018), cassava (tapioca) starch (Luchese et al. 2018), calcium alginate (Al-Qahtani et al. 2021), bacterial cellulose (Mohammadinejad et al. 2020), gelatin (Musso et al. 2018) and poly(vinyl alcohol) composites (Ma et al. 2017). They have also been found to improve the health-promoting properties of food (Mattioli et al. 2020). Another interesting potential application of anthocyanins is as inhibitors of metal corrosion processes (Kopyciński and Duda 2022).

This paper describes research concerning the color photostability of anthocyanin-rich extracts from European blueberry (*Vaccinium myrtillus* L.).

Water, glycerin, ethanol, and their mixtures were used as environmentally friendly solvents. The color photostability of the analyzed extracts was determined by exposure to fluorescent lamps. The content of anthocyanins was assessed by comparison of three methods – two related to absorbance measurements, and one based on the colorimetric technique.

## Materials and methods

### Materials

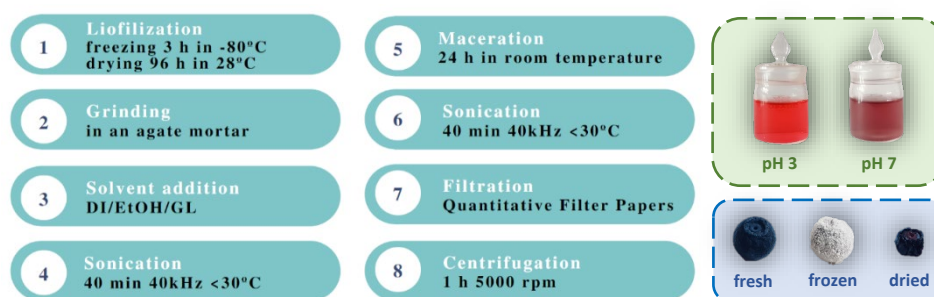
Fruits of European blueberry (*Vaccinium myrtillus* L.), hereafter referred to as VM, were used to prepare liquid extracts. The berries were picked by hand in the Lubliniec Forests (50°39'54.9"N 18°37'58.7"E) in July 2021. The solvents used were deionized water (DI), ethanol (EtOH) (96% pure p.a.), and glycerin (GL) (anhydrous pure p.a.). Potassium chloride (pure p.a.) and sodium acetate (trihydrate pure p.a.) were used to obtain buffers at pH 1.0 and pH 4.5 respectively. The pH of both solutions was adjusted with hydrochloric acid addition. Undyed and unbleached raw cotton fabric, rich in cellulose, was used as a substrate for dyeing. All of the above reagents were purchased from Chempur (Poland).

### Preparation of extracts

After harvesting, the berries were rinsed under running water, then air-dried and stored in cool surroundings before extraction. The preparation of liquid extracts began with the lyophilization of the VM fruits. Fresh berries were frozen at  $-80\text{ }^{\circ}\text{C}$  for 3 hours (ULUF 65, Arctiko). Each of the frozen batches was then placed into the lyophilizer chamber and dried under reduced pressure at  $28\text{ }^{\circ}\text{C}$  for 96 hours (Alpha 2-4 LDplus, Christ). A reduction of approximately 88% in the weight of the fruits was recorded. The prepared lyophilizates were ground in an agate mortar, and appropriate solutions of the solvents were poured over them in a ratio of 1 ml per 0.1 g of dried VM (Table 2). The 24-hour maceration process of the lyophilizates was preceded and completed by 40-minute sonication of the samples in an ice bath ( $< 30\text{ }^{\circ}\text{C}$ ) (Sonic-6, Polsonic). The macerates were centrifuged in a laboratory centrifuge (MPW 351/R/RH, MPW Med. Instruments). Each of the separated supernatants was filtered through quantitative cellulose filters (type 388, Ahlstrom Munksjö). The pH of the prepared solutions was measured (Delta 320, Mettler Toledo). The steps for preparation of the extracts as described above (as well as pH indicators) are shown in Fig. 1.

**Table 2.** Types of solvents used for preparation of VM extracts

Sample	Solvent
VM_E1	DI
VM_E2	EtOH
VM_E3	GL
VM_E4	EtOH:DI 50/50 (v/v)
VM_E5	GL:DI 50/50 (v/v)
VM_E6	GL:EtOH 50/50 (v/v)
VM_E7	GL:EtOH:DI 50/25/25 (v/v/v)
VM_E8	GL:EtOH:DI 33/33/33 (v/v/v)
VM_E9	GL:EtOH:DI 15/42.5/42.5 (v/v/v)



**Fig 1.** Steps for the preparation of VM extracts

### ATR-FTIR spectroscopy characterization

Infrared spectra of the prepared samples were acquired using a spectrometer equipped with a crystal diamond adapter (Nicolet iS 10, Thermo Fisher Scientific) in attenuated total reflectance (ATR) mode with Fourier transformation (FT). Before analysis, liquid extracts were dropped on a glass plate and evaporated in a drying oven at 28 °C for 4 hours. The spectra were collected in the wavenumber range 4000–400 cm<sup>-1</sup> at a scan number of 32 and a resolution of 4 cm<sup>-1</sup>. To facilitate interpretation of the spectra (to suppress signals originating from the solvents used), before analysis the samples were dried at 36 °C for 2 and 4 hours respectively for DI/EtOH- and GL-based extracts.

### Weathering test and colorimetric measurement

A UV-accelerated weathering tester (Q-Panel, Q-Lab) was used to determine the impact of laboratory light exposure on the color photostability of the prepared extracts. The study was performed according to the ISO 16474-3:2021 standard. Method B (“daylight behind window glass”) was applied, with the following parameters: cycle number – 3, exposure period – 24 h dry, lamp type – UVA-351, irradiance – 0.76 W/m<sup>2</sup>/nm at 340 nm, temperature – 40±3 °C. Fragments of undyed, unbleached raw cotton fabric measuring 10 x 15 cm were dyed with each of the prepared extracts. The fabrics were dyed by dropping the extracts onto their surface and then applying them with a roller on both sides until the cotton surface was fully soaked. After drying (48 h, 25±2 °C, without access to sunlight) samples were fixed in the holders of the Q-Panel and tested.

The spectral photometric quantities in the visible range were determined, and the trichromatic components were described using a portable, handheld sphere spectrophotometer (Sp62, X rite) with a measuring geometry of d/8 (according to the PN-ISO 7724-2:2003 standard). The color coordinates in the CIE Lab color space were calculated (L\* – brightness, a\* – color from green to magenta, b\* – color from blue to yellow). The color difference ΔE was determined using the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

For the determination of the color change of the samples, measurements were performed before and after the cycle in Q-Panel. The degree of dyeing was assessed primarily based on the value of L\*.

### UV – Vis spectroscopy analysis and determination of total monomeric anthocyanin pigment content

To estimate the content of anthocyanins in the prepared extracts, quantitative analysis was performed by means of UV-Vis spectroscopy. First, the optimal dilution was selected by establishing the standard curve of sample VM\_E1. Optimal absorbance at wavelength 520 nm was obtained for 32-fold dilution. Afterwards, absorbance at the same dilution was measured for the other VM extracts in order to determine the concentration of anthocyanins. Measurements were made with the use of a spectrophotometer (DU 640, Beckman) in polystyrene cuvettes (Ratiolab, 45 x 12.5 x 12.5 mm, inside width 10 mm). The absorbance intensities on the spectra obtained for the analyzed extracts were compared.

To calculate the total monomeric anthocyanin pigment content (TMAPC) of the prepared extracts, AOAC Official Method 2005.02 was used. Absorbance measurements of extracts in pH 1.0 and pH 4.5 buffers were made at wavelength 520 nm and 700 nm (DU 640, Beckman). Anthocyanin pigment concentration was expressed as cyanidin 3-glucoside (cyd-3-glu) equivalents corresponding to the absorbance difference, as follows:

$$\text{TMAPC} = \frac{A \cdot \text{MW} \cdot \text{DF} \cdot 10^3}{\epsilon \cdot l} \quad (2)$$

where A (absorbance) = (A<sub>520nm</sub> – A<sub>700nm</sub>)pH 1.0 – (A<sub>520nm</sub> – A<sub>700nm</sub>)pH 4.5, MW (molecular weight) = 449.2 g/mol for cyd-3-glu, DF (dilution factor) = 100, l = pathlength in cm, ε = molar extinction coefficient 26900 in L·mol<sup>-1</sup>·cm<sup>-1</sup> for cyd-3-glu, and 10<sup>3</sup> = factor for conversion from g to mg. Buffers were prepared using the following procedures:

- Buffer at pH 1.0 (0.025 M potassium chloride) – 0.186 g of KCl was weighed in a volumetric flask and 90 ml of DI was added. The pH was adjusted to 1.0 by adding around 0.6 ml of HCl. The solution was diluted to a volume of 100 ml with DI.
- Buffer at pH 4.5 (0.4 M sodium acetate) – 90 ml of DI was added to 5.44 g of CH<sub>3</sub>CO<sub>2</sub>Na·3H<sub>2</sub>O in a volumetric flask. The pH was adjusted to 4.5 by adding around 2 ml of HCl. The mixture was then diluted with DI to a volume of 100 ml.

## Results and discussion

### ATR-FTIR characteristics

The spectra collected for the tested extracts were all found to be similar to each other (Fig. 2). Importantly, the absorption bands present at the same wavenumbers

for individual extracts differ in intensity. This effect is probably due to the extraction of several different compounds from the VM fruits, and may indicate that these compounds are extracted in different proportions depending on the composition of the extraction mixture and the proportions of the components used (DI, EtOH, and/or GL). For the analyzed samples, fundamental spectral signals in the wavenumber range 4000–400  $\text{cm}^{-1}$  were characterized. A band at 3359  $\text{cm}^{-1}$  can be ascribed to the stretching of bonds of –OH groups (Haberhauer et al. 1998). The bands at 2936 and 2880  $\text{cm}^{-1}$  indicate the stretching of C–H bonds in alkyl groups (Meissl et al. 2008). The spectral

regions at around 1721–1601  $\text{cm}^{-1}$  correspond to stretching of C=O bonds (carbonyl groups) (Schwaninger et al. 2004). Signals appearing at 1340  $\text{cm}^{-1}$  come from symmetric  $\text{CO}_2$  stretching in carboxylates and/or symmetric COO stretching in oxalate (Nopp-Mayr et al. 2020). Bands at 1112 and 1044  $\text{cm}^{-1}$  indicate stretching of C–O–C bonds (ethers) (Beć et al. 2017). Regions around 818  $\text{cm}^{-1}$  can be associated with primary  $\text{NH}_2$  out-of-plane bending (aromatic) and/or CH bending in alkenes, while those at 781  $\text{cm}^{-1}$  represent symmetric deformation of OCO (oxalate) (Nopp-Mayr et al. 2020).

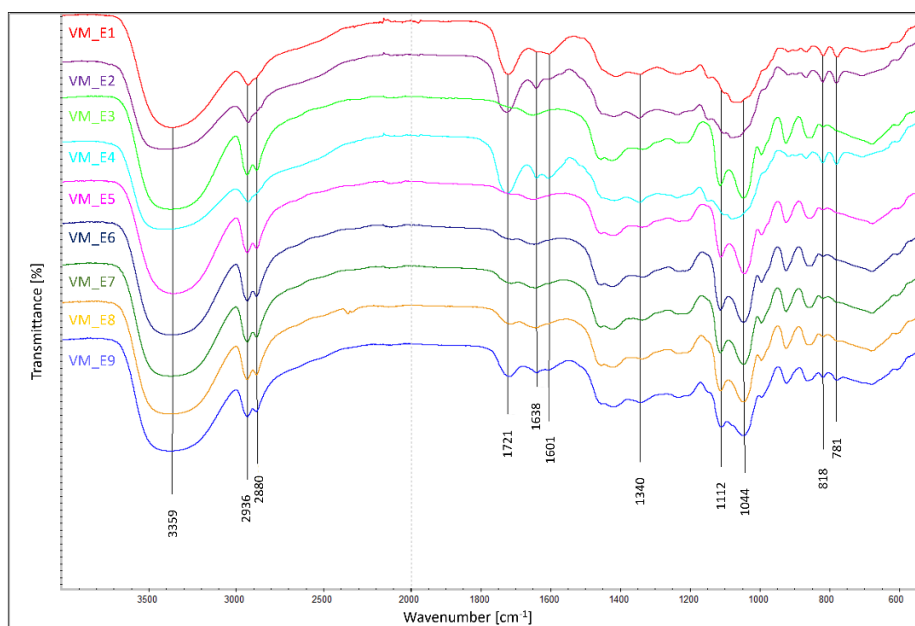


Fig. 2. ATR-FTIR spectra of the analyzed extracts

### Assessment of degree of dyeing and color photostability

Based on the values of the  $L^*$  and  $\Delta E_{\text{tot}}$  parameters, the degree of dyeing and color photostability of the VM extracts were assessed (Table 3). It was observed that the extract with the highest dyeing strength was VM\_E6, obtained with GL:EtOH in the volume ratio 50/50 ( $L^* = 33.08$ ). The lowest degree of dyeing was recorded for VM\_E3, where the solvent was GL ( $L^* = 61.51$ ). For the remaining extracts,  $L^*$  values ranged from 35 to 54. Analysis of the color change of individual fabrics after 24 hours in the UVA weathering tester showed that the extract most resistant to fluorescent lamps is VM\_E4, based on EtOH:DI in the volume ratio 50/50 ( $\Delta E_{\text{tot}} = 11.79$ ). The abovementioned VM\_E3 extract ( $\Delta E_{\text{tot}} = 39.42$ ) proved to be the most susceptible to light degradation. The  $\Delta E_{\text{tot}}$  values for the other analyzed extracts were between 12 and 33.

The dyeing strength of VM extracts can be related to potential anthocyanin content, which is dependent on the type of solvent used. The use of pure glycerin resulted in a low efficiency of dyeing, which may be due to its high viscosity and the probable slowing of the process of penetration of the lyophilizates. On the other hand, glycerin contents of 15% or higher intensify the extraction process, especially a content of 50% in two-component systems with EtOH or in three-component systems with EtOH:DI. Although the presence of glycerin increases the retention of anthocyanins in extracts stored in the dark (Garzón and Wrolstad 2001), it also contributes to their degradation when exposed to sunlight.

The observed relationship corresponds with results on the use of glycerin for the extraction of anthocyanins from chokeberry and elderberry (Kowalska et al. 2021). In that study, anthocyanins were extracted with the use of 30%, 50%, 65% and 80% GL

together with DI and EtOH at 20, 50 and 80 °C. Measurements at 20 °C showed that the extraction efficiency increased with increasing GL content up to 50%, but above that value the efficiency clearly decreased. GL has a higher viscosity (1.412 mPa·s) (Segur and Oberstar 1951) than DI (1.0016 mPa·s) or EtOH (1.074 mPa·s) (Rumble 2018), which is due, among other things, to the presence of three hydroxyl groups that can form hydrogen bonds. When the GL content exceeds 50%, the viscosity of the extraction system increases significantly, which probably limits the release and distribution of anthocyanins in the volume of the solution. Similarly, in another study (Eyiz et al. 2019), a system with 50% GL was found to be the most effective solvent. Despite its advantages, the use of glycerin as a solvent is rarely investigated.

The VM\_E4 variant was selected as exhibiting the best light stability. This is in agreement with the results of previous work on the extraction of anthocyanins with EtOH (Nour et al. 2013; Ćujić et al. 2016). It was confirmed that aqueous solutions containing 50% or 60% EtOH have the highest extraction efficiency. A further increase in the proportion of EtOH results in a decrease in extraction efficiency. In the further part of this work, the sample dyed with VM\_E4 was mounted on the inner glass of a window (southwest wall, 50°17'57.04"N 18°42'23.627"E) and exposed to sunlight for three months in the summer/fall of 2021 (August–October). With direct normal irradiation in this period of about 280 kWh/m<sup>2</sup>, the fabric did not discolor completely after the testing period ( $\Delta E_{\text{tot.}} = 36.95$ ) (Table 4).

**Table 3.** Results of weathering test and colorimetric measurement for VM extracts

Sample	Color before QUV			Color after QUV			$\Delta E_{\text{tot.}}$
	L*	a*	b*	L*	a*	b*	
VM_E1	46.15	30.32	-7.59	46.39	28.88	-7.49	
VM_E2	54.04	21.37	-1.21	54.89	19.03	-3.43	
VM_E3	61.51	16.99	-16.89	77.52	6.05	2.48	
VM_E4	35.67	30.66	-2.39	36.92	29.12	-5.07	
VM_E5	39.88	28.14	-7.4	52.98	20.73	-6.1	
VM_E6	33.08	23.96	-5.31	48.5	19.12	-5.88	
VM_E7	35.18	24.79	-6.55	47.91	19.97	-6.71	
VM_E8	40.42	27.61	-8.22	46.55	22.15	-7.91	
VM_E9	39.94	27.44	-7.08	44.21	23.15	-7.39	
				Color after QUV			
	6 h			24 h			
	L*	a*	b*	L*	a*	b*	
	50.39	25.39	-7.35	56.51	22.43	-4.06	13.49

55.47	18.25	-3.29	64.57	14.83	-0.86	12.4
80.51	4.29	6.98	87.96	0.59	7.31	39.42
37.23	27.56	-5.62	44.78	23.3	-3.77	11.79
58.01	16.33	-2.77	68.04	12.9	4	33.99
55.92	15.26	-3.56	64.56	12.99	3.48	31.94
53.7	16.45	-4.19	62.48	14.78	3.21	30.37
50.93	19.08	-7.14	62.53	11.55	1.6	29.11
45.43	21.5	-6.85	57.38	13.96	-1.61	22.71

**Table 4.** Color change of VM\_E4 extract in three months of sunlight exposure

Sample	Sunlight exposure						$\Delta E_{tot}$
	1 mo.			3 mos.			
	L*	a*	b*	L*	a*	b*	
VM_E4	52.97	14.53	-0.58	63.74	8.99	7.75	36.95

### Absorbance intensity and TMAPC measurement

The absorbance measurement results for the prepared extracts of VM are presented in Fig. 3 and Table 5. A relationship was observed between the intensity of absorbance recorded on the UV-Vis spectra and the type of solvent used. The intensity of the absorbance peak recorded at wavelength 520 nm was analyzed, because its presence in the spectrum statistically corresponds to the group of anthocyanins absorbing light in its vicinity (El Kouari et al. 2015). The highest absorbance intensity was observed for extract VM\_E6, for which the solvent was a mixture of GL and EtOH in a proportion of 50/50 v/v (Abs. int. = 1.2077). The lowest absorbance intensity was recorded for VM\_E3, for which GL alone was used (Abs. int. = 0.6502). For extracts VM\_E5, VM\_E8 and VM\_E9, for which the solvents were mixtures of GL, EtOH and DI, the values of this parameter were practically the same (Abs. int. = ca. 0.84).

The TMAPC measurement results for the VM extracts are presented in Fig. 4 and Table 5. Cyd-3-glu

was used as an equivalent calculation reference because of its widespread occurrence in fruits and vegetables rich in anthocyanins (He and Giusti 2010). Fig. 4 shows one of the UV-Vis spectra, obtained for the VM\_E4 extract solution in 1 and 4.5 pH buffers. Similarly as in the case of absorbance intensity, extract VM\_E6 has the highest TMAPC value (1754.72 mg/L), while VM\_E3 has the lowest (1187.96 mg/L). However, for the remaining extracts, the values did not follow the same order as in the previous case. This discrepancy is due to the fact that in the TMAPC method only one of the anthocyanins is taken into account, representing a certain percentage of all compounds giving color to the fruit and to the solutions of VM. The cyd-3-glu extraction efficiency for the solvents used may vary. The values of TMAPC obtained for VM extracts in our study can be compared to the results recorded in the literature for different sources of anthocyanins. For example, previously reported TMAPC measurements for juice concentrates, including fruits such as raspberry, grape and elderberry, indicated anthocyanin contents of

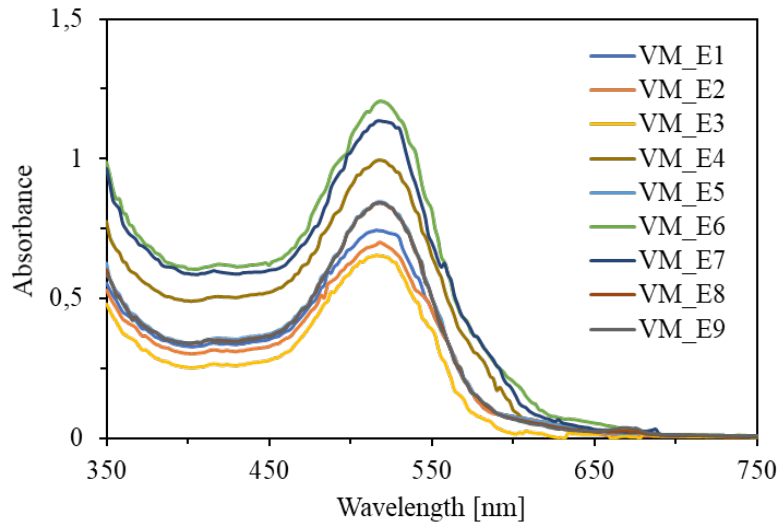


Fig. 3. UV-Vis spectra of obtained VM extracts

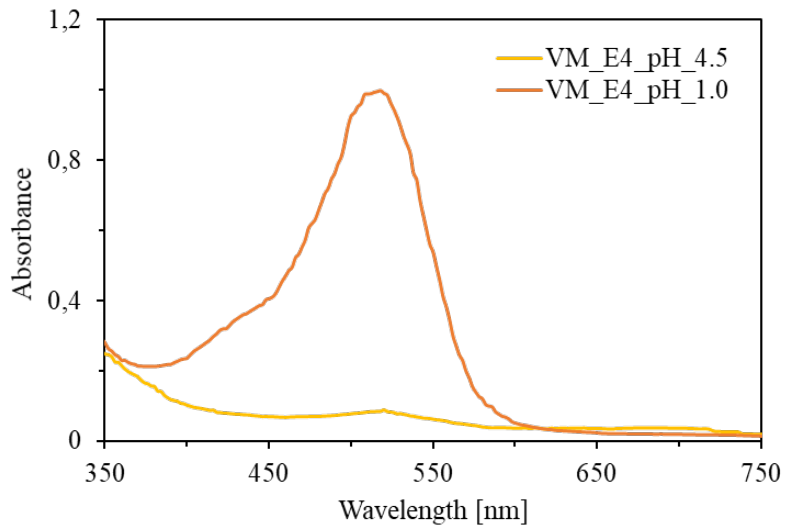


Fig. 4. UV-Vis spectra of VM\_E4 extract in pH 4.5 and 1.0

Table 5. Results of pH, absorbance intensity and TMAPC measurements for VM extracts

Sample	pH	Abs. int. at 520 nm	TMAPC [mg/L]
VM_E1	3.07	0.7403	1246.57
VM_E2	4.71	0.6994	1246.74
VM_E3	4.02	0.6502	1187.96
VM_E4	3.86	0.9971	1536.13
VM_E5	3.25	0.8444	1392.19
VM_E6	4.30	1.2077	1754.72
VM_E7	4.03	1.1346	1727.33
VM_E8	3.76	0.8431	1426.25
VM_E9	3.80	0.8418	1414.06



337 mg/L, 641 mg/L and 3007 mg/L respectively (Lee et al. 2005). In another work, mixtures of water with ethanol or methanol in various proportions were used to obtain VM extracts, and the TMAPC values ranged from 1462 mg/L to 2953 mg/L for water/ethanol solvent and from 1462 mg/L to 3888 mg/L for water/methanol solvent. In that experiment, frozen VM fruits were used in a quantity of 0.25 g per 1 ml of solvent, and the extraction was carried out in an acidic environment (0.1% HCl) in an ultrasonic bath (Căta et al. 2010). It should be remembered that the results obtained by various research groups depend not only on the type of extract used, but also on the method of preparing the fruit and the ratio in which it was mixed with the solvents; the results are therefore often not mutually comparable. The pH of all extracts obtained was acidic (with values within the range 3.07–4.71).

## Conclusion

The emphasis on the development of environmentally friendly solutions is also visible in relation to the processing of resources of natural origin. The potential of this field of research can be confirmed by the considerable number of studies undertaken.

In this study, an investigation was made of the color photostability of ultrasound-assisted extracts from European blueberry (*Vaccinium myrtillus* L.) obtained with the use of water, ethanol and glycerin as solvents. Assessment of the degree of dyeing and color photostability of the prepared extracts was performed in a UV-accelerated weathering tester. On the basis of UV-Vis measurements, the anthocyanin contents in the extracts were also estimated.

European blueberry (*Vaccinium myrtillus* L.) was selected as a raw material due to its fully renewable character and widespread presence throughout Europe, especially in Polish forests. In addition, its characteristics (including natural origin and non-toxicity) make this plant suitable for sensitive applications, including direct contact with food or

cotton fabrics, as well as wood staining. The dyeing strength of all obtained extracts was found to be sufficient without the need to purify their remaining organic components, for example by means of chromatographic techniques. The use of the prepared solvents as a substitute for the traditionally used – and often toxic – liquids reduces the harmfulness of the extracts, thus enhancing their environmentally friendly profile.

The following conclusions can be given on the basis of the measurement results:

1. The extract obtained using ethanol and water in a volume ratio of 50/50 exhibits the highest color photostability, as it undergoes the smallest color change on exposure to UV radiation. This extract, which is also distinguished by a relatively high anthocyanin content, when applied to cotton fabric did not lose its color fully after three months of exposure to sunlight through window glass.
2. The highest content of anthocyanins, as determined using UV-Vis measurements, was recorded for the extract obtained using glycerin and ethanol in a volume ratio of 50/50. In view of a number of advantages, such as low price, easy availability and non-toxicity, glycerin can be used as an additive medium enhancing the process of extraction of anthocyanins from plant materials.

Accordingly, due to their good ability to dye cellulosic materials, such extracts have the potential to be applied as dye additives, above all for wood stains. It can be assumed that the most advantageous solution from the point of view of application would be to cover the stained wood with varnish enriched with the addition of appropriate photostabilizers such as UV-absorbers or hindered amine light stabilizers (HALS). This arrangement will extend the color stability of the substrate and extend the life of the coated element. Moreover, they can be used as non-toxic food dyes, and as color concentrates for the preparation of pH-sensitive, biodegradable packaging films for monitoring the freshness of foodstuffs.

## References

- Al-Qahtani S.D., Alzahrani H.K., Azher O.A., Owidah, Z.O., Abualnaja M., Habeebullah T.M., El-Metwaly N.M. [2021]: Immobilization of anthocyanin-based red-cabbage extract onto cellulose fibers toward environmentally friendly biochromic diagnostic biosensor for recognition of urea. *Journal of Environmental Chemical Engineering* 9 [4]: 105493
- Beć K.B., Grabska J., Ozaki Y., Hawranek J.P., Huck C.W. [2017]. Influence of Non-fundamental Modes on Mid-infrared Spectra: Anharmonic DFT Study of Aliphatic Ethers. *The Journal of Physical Chemistry A* 121 [7]: 1412–1424
- Castañeda-Ovando A., Pacheco-Hernández M.L., Páez-Hernández M.E., Rodríguez J.A., Galán-Vidal C.A.

- [2009]: Chemical studies of anthocyanins: A review. *Food Chemistry* 113 [4]: 859–871
- Căta A., Ștefănuț M.N., Tănasie C., Pop R.** [2010]: Comparative analysis of bilberries alcoholic extracts regarding to anthocyanins content, total phenolics and antioxidant activity. *Ovidius University Annals of Chemistry* 21 [1]: 15–19
- Chemat F., Rombaut N., Sicaire A.G., Meullemiestre A., Fabiano-Tixier A.S., Abert-Vian M.** [2016]: Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols, and applications A Review. *Ultrasonics Sonochemistry* 34: 540–560
- Coultate T., Blackburn R.S.** [2018]: Food colorants: their past, present and future. *Coloration Technology* 134 [3]: 165–186
- Ćujić N., Šavikin K., Janković T., Pljevljakušić D., Zdunić G., Ibrić S.** [2016]: Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chemistry* 194: 135–142
- El Kouari Y., Migalska-Zalas A., Arof A.K., Sahraoui B.** [2015]: Computations of absorption spectra and non-linear optical properties of molecules based on anthocyanidin structure. *Optical and Quantum Electronics* 47 [5]: 1091–1099
- Eyiz V., Tontul I., Turker, S.** [2019]: Optimization of green extraction of phytochemicals from red grape pomace by homogenizer assisted extraction. *Journal of Food Measurement and Characterization* 14: 39–47
- Garzón G.A., Wrolstad R.E.** [2001]: The stability of pelargonidin-based anthocyanins at varying water activity. *Food Chemistry* 75 [2]: 185–196
- Haberhauer G., Rafferty B., Strebl F., Gerzabek M.H.** [1998]: Comparison of the composition of forest soil litter derived from three different sites at various decomposition stages using FTIR spectroscopy. *Geoderma* 83: 331–342
- Halász K., Csóka L.** [2018]: Black chokeberry (*Aronia melanocarpa*) pomace extract immobilized in chitosan for colorimetric pH indicator film application. *Food Packaging and Shelf Life* 16: 185–193
- He J., Giusti M.M.** [2010]: Anthocyanins: Natural Colorants with Health-Promoting Properties. *Annual Review of Food Science and Technology* 1: 163–187
- Kayesh E., Shangguan L., Korir K. N., Sun X., Bilkish N., Zhang Y., Han J., Song C., Cheng Z.-M., Fang J.** [2013]: Fruit skin color and the role of anthocyanin. *Acta Physiologiae Plantarum* 35: 2879–2890
- Kopyciński B., Duda A.** [2022]: Anthocyanins – corrosion inhibitors straight from nature. *Ochrona przed Korozją*. 65 [7]: 216–221
- Kong J.M., Chia L.S., Goh N.K., Chia T.F., Brouillard R.** [2003]: Analysis and biological activities of anthocyanin. *Phytochemistry* 64 [5]: 923–933
- Kowalska G., Wyrostek J., Kowalski R., Pankiewicz U.** [2021]: Evaluation of glycerol usage for the extraction of anthocyanins from black chokeberry and elderberry fruits. *Journal of Applied Research on Medicinal and Aromatic Plants* 22: 105493
- Lee J., Durst R.W., Wrolstad R.E.** [2005]: Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International* 88 [5]: 1269–1278
- Liazid A., Guerrero R.F., Cantos E., Palma M., Barroso C.G.** [2011]: Microwave assisted extraction of anthocyanins from grape skins. *Food Chemistry* 124 [3]: 1238–1243
- Luchese C.L., Abdalla V.F., Spada J.C., Tessaro I.C.** [2018]: Evaluation of blueberry residue incorporated cassava starch film as pH indicator in different simulants and foodstuffs. *Food Hydrocolloids* 82: 209–218
- Ma Q., Liang T., Cao L., Wang, L.** [2017]: Intelligent poly(vinyl alcohol)-chitosan nanoparticles-mulberry extracts films capable of monitoring pH variations. *International Journal of Biological Macromolecules* 108: 576–584
- Mattioli R., Francioso A., Mosca L., Silva, P.** [2020]: Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. *Molecules* 25 [17]
- Meissl K., Smidt E., Schwanninger M., Tintner J.** [2008]: Determination of Humic Acids Content in Composts by Means of Near- and Mid-Infrared Spectroscopy and Partial Least Squares Regression Models. *Applied Spectroscopy* 62(8): 873–880
- Michaelis L., Schubert M.P., Smythe C.V.** [1936]: Potentiometric Study of the Flavins. *Journal of Biological Chemistry* 116(2): 587–607
- Mohammadinejhad S., Almasi H., Moradi M.** [2020]: Immobilization of *Echium amoenum* anthocyanins into bacterial cellulose film: A novel colorimetric pH indicator for freshness/spoilage monitoring of shrimp. *Food Control* 113: 105493
- Musso Y.S., Salgado P.R., Mauri A.N.** [2018]: Smart gelatin films prepared using red cabbage (*Brassica oleracea* L.) extracts as solvent. *Food Hydrocolloids* 89: 674–681
- Nopp-Mayr U., Zohmann-Neuberger M., Tintner J., Kriechbaum M., Rosenberger R., Nopp H., Bosa A., Smidt E.** [2020]: From plants to feces: pilot applications of FTIR spectroscopy for studies on the foraging

ecology of an avian herbivore. *Journal of Ornithology* 161: 203–215

- Nour V., Stampar F., Veberic R., Jakopic J.** [2013]: Anthocyanins profile, total phenolics and antioxidant activity of black currant ethanolic extracts as influenced by genotype and ethanol concentration. *Food Chemistry* 141 [2]: 961–966
- Paes J., Dotta R., Barbero G.F., Martínez J.** [2014]: Extraction of phenolic compounds and anthocyanins from blueberry (*Vaccinium myrtillus* L.) residues using supercritical CO<sub>2</sub> and pressurized liquids. *The Journal of Supercritical Fluids* 95: 8–16
- Phan K., Van Den Broeck E., Van Speybroeck V., De Clerck K., Raes K., De Meester S.** [2020]: The potential of anthocyanins from blueberries as a natural dye for cotton: A combined experimental and theoretical study. *Dyes and Pigments* 176:108180
- Puértolas E., Cregenzán O., Luengo E., Álvarez I., Raso, J.** [2013]: Pulsed-electric-field-assisted extraction of anthocyanins from purple-fleshed potato. *Food Chemistry* 136: 1330–1336
- Rumble J.R.** (Ed.) [2018]: *CRC Handbook of Chemistry and Physics*, 99th Edition. CRC Press/Taylor & Francis
- Salehi B., Sharifi-Rad J., Cappellini F., Reiner Z., Zorzan D., Imran M., Sener B., Kilic M., El-Shazly M., Fahmy N.M., Al-Sayed E., Martorell M., Tonelli C., Petroni K., Docea A.O., Calina D., Maroyi A.** [2020]: The Therapeutic Potential of Anthocyanins: Current Approaches Based on Their Molecular Mechanism of Action. *Frontiers in Pharmacology* 11: 1300
- Segur J.B., Oberstar H.E.** [1951]: Viscosity of Glycerol and Its Aqueous Solutions. *Industrial & Engineering Chemistry*. 43 [9]: 2117–2120
- Shehata E., Grigorakis S., Loupassaki S., Makris D.P.** [2015]: Extraction optimisation using water/glycerol for the efficient recovery of polyphenolic antioxidants from two *Artemisia* species. *Separation and Purification Technology* 149: 462–469
- Silva S., Costa E.M., Calhau C., Morais R.M., Pintado M.E.** [2015]: Anthocyanin Extraction from Plant Tissues: A Review. *Critical Reviews in Food Science and Nutrition* 57 [14]: 3072–3083
- Schwanninger M., Rodrigues J.C., Pereira H., Hinterstoisser B.** [2004]: Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vibrational Spectroscopy* 36 [1]: 23–40
- Turturică M., Stănciuc N., Mureşan C., Râpeanu G., Croitoru C.** [2018]: Thermal Degradation of Plum Anthocyanins: Comparison of Kinetics from Simple to Natural Systems. *Journal of Food Quality* 2018: 1–10
- Quina F.H., Moreira P.F., Vautier-Giongo C., Rettori D., Rodrigues R.F., Freitas A.A., Silva P.F., Maçanita A.L.** [2009]: Photochemistry of anthocyanins and their biological role in plant tissues. *Pure and Applied Chemistry* 81[9]: 1687–1694
- Vankar P.S., Shukla D.** [2011]: Natural dyeing with anthocyanins from *Hibiscus rosa sinensis* flowers. *Journal of Applied Polymer Science* 122 [5]: 3361–3368
- Wallace T.C., Giusti, M.M.** [2019]: Anthocyanins – Nature’s Bold, Beautiful, and Health-Promoting Colors. *Foods* 8 [11]: 550
- Wang H., Tang Z., Zhou W.** [2016]: A method for dyeing cotton fabric with anthocyanin dyes extracted from mulberry (*Morus rubra*) fruits. *Coloration Technology* 132 [3]: 222–231
- Wu X., Beecher G.R., Holden J.M., Haytowitz D.B., Gebhardt, S.E., Prior R.L.** [2006]: Concentrations of Anthocyanins in Common Foods in the United States and Estimation of Normal Consumption. *Journal of Agricultural and Food Chemistry* 54 [11]: 4069–4075

#### List of standards

- ISO 16474-3:2021 Paints and varnishes – Methods of exposure to laboratory light sources – Part 3: Fluorescent UV lamps
- PN-ISO 7724-2:2003 Farby i lakiery – Kolorymetria – Część 2: Pomiar barwy