Article

Not peer-reviewed version

Red Cabbage Anthocyanin-Based Colorimetric Sensor for Visual and Quantitative Detection of pH Variations and Contamination in Cell Culture

Omer Umut Gunes *

Posted Date: 24 September 2025

doi: 10.20944/preprints202509.1999.v1

Keywords: Anthocyanin; Colorimetric sensor; pH detection; Contamination; Red cabbage extract

Article

Red Cabbage Anthocyanin-Based Colorimetric Sensor for Visual and Quantitative Detection of pH Variations and Contamination in Cell Culture

Omer Umut Gunes

Department of Bioengineering, Gebze Technical University, Kocaeli, Turkey; omerumutgunes2026@gmail.com

Abstract

Anthocyanins are natural pigments known for their pronounced pH-dependent color changes, making them promising candidates for rapid, visual detection systems. In this study, anthocyanin extract from red cabbage (*Brassica oleracea var. capitata f. rubra*) was prepared using a 1:2 (w/v) ratio in 25% ethanol, followed by vacuum evaporation and application to two experimental systems: (i) a pH series ranging from 4.0 to 12.0, and (ii) Dulbecco's Modified Eagle Medium (DMEM) under different sterility conditions (sterile, contaminated, and cell-containing). Color changes were quantified using spectrophotometry (570–630 nm) and image analysis with ImageJ. Results indicated a strong correlation between pH increase and changes in RGB mean values, with a marked shift in hue under alkaline conditions. In DMEM samples, contamination resulted in noticeable reductions in red, green, and blue intensities compared to sterile media, indicating potential for detecting contamination. This work demonstrates that anthocyanin-based sensors can visually and quantitatively detect pH variations and contamination in cell culture media, offering a rapid, low-cost, and sustainable approach for routine laboratory monitoring.

Keywords: anthocyanin; colorimetric sensor; pH detection; contamination; red cabbage extract

---

Introductıon

Anthocyanins are water-soluble flavonoid pigments responsible for the red, purple, and blue coloration of many fruits and vegetables. Their chromatic properties vary significantly with pH due to structural transformations between flavylium cations, quinoidal bases, and chalcone forms (Castañeda-Ovando et al., 2009). These compounds are not only valued as natural food colorants but also investigated for their potential in analytical sensing applications, particularly as environmentally friendly pH indicators.

Recent studies have demonstrated that anthocyanin extracts from botanical sources such as red cabbage (*Brassica oleracea* var. *capitata f. rubra*) can serve as cost-effective and biodegradable sensing agents for detecting pH changes across a wide range (pH 2–12) with visible color transitions. The colorimetric shifts are easily perceived by the naked eye and quantitative analysis can be performed using spectrophotometry or digital image processing.

In cell culture systems, pH changes in the growth medium can be an early indicator of contamination by bacteria, fungi or yeast; as microbial metabolism often alters the acid–base balance of the medium (Dervisevic et al., 2022). Conventional contamination detection relies on microscopy, microbial culturing or metabolic assays, which can be time-consuming, require specialized equipment, and may not allow real-time observation. Therefore, the development of a rapid, low-cost, and sustainable colorimetric method for both pH monitoring and contamination detection could significantly improve laboratory efficiency and biosafety practices.

This study investigates the use of red cabbage anthocyanin extract as a colorimetric sensor for visual and quantitative detection of pH variations and contamination in cell culture media. By combining spectrophotometric measurements with digital image analysis (ImageJ), we aim to evaluate the sensor's performance in differentiating between sterile and contaminated media samples, with potential applications in routine laboratory monitoring.

Materıals and Methods

Materials

Red cabbage (Brassica oleracea var. capitata f. rubra) was obtained from a local market in Beyoglu, Turkey. Ethanol (analytical grade), sodium hydroxide (NaOH, analytical grade), Dulbecco's Modified Eagle Medium (DMEM), Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) strains, and filter paper were supplied by Gebze Technical University laboratories. pH test solutions (pH 4.0, 5.5, 7.0, 8.0, 9.0, 10.0, and 12.0) were prepared in laboratory; using distilled water, pH meter calibration buffers (pH 4.01, 7.01, 10.01 at 25 °C), and diluted NaOH solution. All chemicals and reagents were of analytical grade and used without further purification.

Anthocynanin Extraction

Fifty grams of fresh red cabbage leaves were weighed and thoroughly macerated using a mortar and pestle to release cell contents. The crushed material was mixed with 100 mL of a 25% (v/v) ethanol–water solution (Araújo et al., 2023) at a 1:2 (w:v) ratio (Chandrasekhar et al., 2012). The mixture was placed on a laboratory shaker for 45 minutes at room temperature, followed by filtration through filter paper to remove solid residues. The resulting extract was transferred into two 50 mL Falcon tubes, wrapped in aluminum foil to prevent light degradation, and stored at +4 °C for 20 hours. After storage, the extract was concentrated by ethanol evaporation in a rotary evaporator at 40 °C, with the vacuum gradually reduced from 1000 to 60 mbar at 100 rpm, yielding anthocyanin-rich aqueous extract.

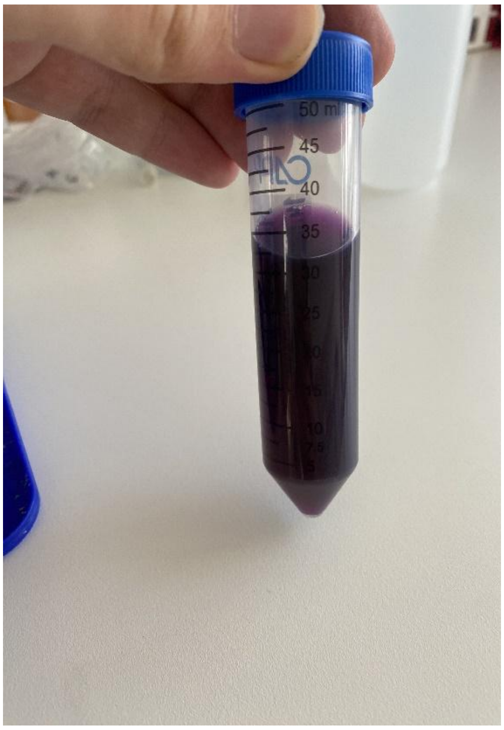

Figure 1. Freshly prepared anthocyanin extract obtained from red cabbage, serving as the pH-sensitive colorimetric indicator in subsequent experiments.

Preparation of pH Test Solutions

pH test solutions at values of 4.0, 5.5, 7.0, 8.0, 9.0, 10.0, and 12.0 were prepared in the laboratory. Initial calibration buffer solutions (pH 4.01, 7.01, and 10.01 at 25 °C) were used as reference points. Intermediate pH values (5.5, 8.0, 9.0) were obtained by adding measured volumes of diluted sodium hydroxide solution (prepared at a 1:10 dilution with distilled water) to the acidic or neutral buffers, as appropriate, while continuously monitoring pH using a calibrated pH meter. The highest pH solution (12.0) was prepared by further addition of diluted NaOH to the alkaline buffer (pH 10.01). All solutions were prepared with distilled water and used immediately after preparation to minimize pH drift.

Colorimetric Measurements

Colorimetric analysis was performed using a 96-well microplate format. For each pH condition, 50 µL of anthocyanin extract was mixed with 50 µL of the corresponding pH test solution (1:1, v/v) directly in the wells. The plate was then placed on a shaker a for 15 min at room temperature to allow complete interaction between the extract and the solution. Absorbance was measured using a microplate spectrophotometer at a primary wavelength of 570 nm and a secondary wavelength of 630 nm, with the anthocyanin extract alone (blank) serving as the reference. The mean absorbance values were recorded. Color changes were also documented photographically.

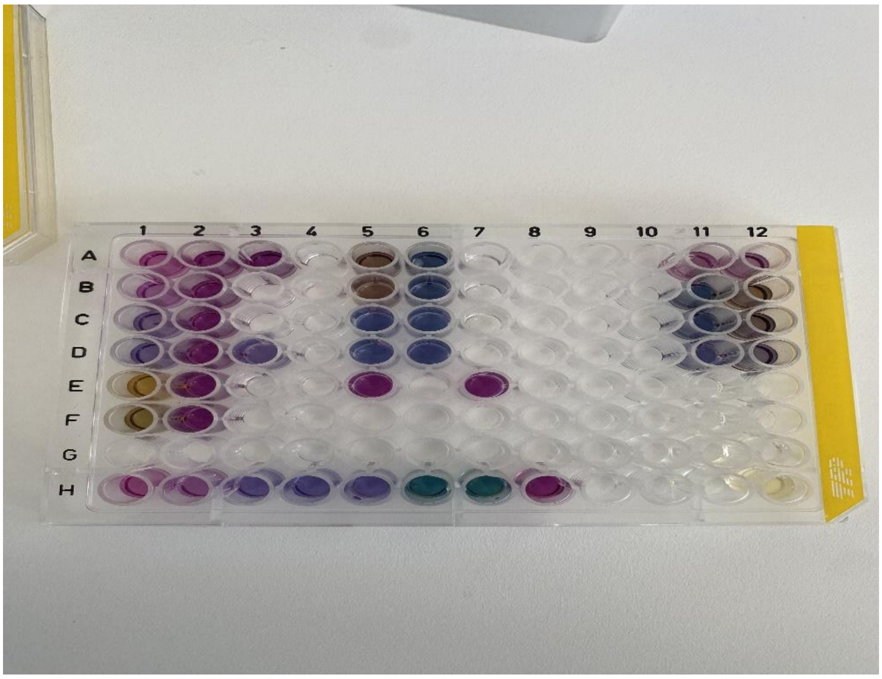

Figure 2. Representative image of a 96-well plate containing anthocyanin extract exposed to solutions of varying pH and cell culture conditions. Distinct color changes are observed across wells, corresponding to pH-dependent spectral shifts of anthocyanin.

DMEM and Bacterial Contamination Tests

Two types of DMEM were tested: sterile DMEM, maintained under aseptic conditions with the container lid closed, and contaminated DMEM, which was exposed to ambient laboratory air for 24 hours with the lid open to allow airborne microbial contamination. In addition, two human dermal fibroblast (HDF) culture media samples were evaluated: one kept sterile and one deliberately exposed to air for 24 hours to induce contamination. Moreover, *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) from laboratory stock cultures are observed. Each condition was prepared in duplicate wells. For analysis, 50 µL of DMEM sample was mixed with 50 µL of anthocyanin extract in a 96-well plate, followed by shaking on shaker for 15 minutes. Absorbance was measured in a spectrophotometer at a primary wavelength of 570 nanometers and a secondary wavelength of 630 nanometers, with the anthocyanin extract alone serving as the blank reference. Color changes were also documented photographically.

Image Analysis

Photographs of the 96-well plates were captured under consistent lighting conditions using a phone camera. Images were analyzed using ImageJ software. Each well was cropped to a standardized region of interest (ROI), and the mean RGB (red, green, blue) values were recorded. Color differences between test samples and the blank reference (anthocyanin extract alone) were quantified.

Results

The colorimetric and spectrophotometric analyses revealed distinct optical responses of the anthocyanin extract to pH variation and bacterial contamination. In the pH experiments (pH 4.0–12.0), RGB measurements from the 96-well plate images showed clear shifts in channel intensities. At acidic pH values (4.0 and 5.5), the red channel intensity was highest (122,627 and 109,745, respectively), producing a reddish-purple coloration, while blue and green channels were comparatively lower. As the pH increased toward alkaline conditions, red intensity decreased sharply (20,512 at pH 12.0), while blue and green channel intensities became more prominent, consistent with the structural transformation of anthocyanins under alkaline conditions. Spectrophotometric absorbance readings (570 nm primary, 630 nm secondary) supported the image-based findings. The highest absorbance was observed at pH 4.0 (0.366), followed by a gradual decrease with increasing pH, reaching negative values at highly alkaline conditions (pH ≥ 10). This decline is associated with the degradation of the colored flavylium cation and conversion into colorless or yellow chalcone forms. In bacterial contamination detection assays, RGB analysis showed that contamination reduced the intensity of all color channels. For DMEM, contamination decreased red from 52,556 to 34,612, blue from 80,704 to 69,767, and green from 43.9 to 33,457, resulting in a visibly duller color. Similarly, HDF samples showed a reduction in red from 64,279 to 41,545, blue from 87,035 to 77,818, and green from 52,336 to 41,289 upon contamination. Absorbance values followed a similar trend. Sterile DMEM and HDF recorded higher absorbances (0.147 and 0.154, respectively) compared to their contaminated counterparts (0.140 and 0.129). The blank anthocyanin extract displayed the highest absorbance (0.645), corresponding to its concentrated pigment content. Overall, the pH-dependent color variation and measurable changes in RGB and absorbance values demonstrate that anthocyanin-based solutions can serve as effective, low-cost optical indicators for both pH detection and bacterial contamination monitoring.

doi:10.20944/preprints202509.1999.v1

Table 1. Mean RGB values obtained from ImageJ analysis of anthocyanin-based pH solutions at different pH levels. Values represent the average intensity for each color channel, with the final column showing the arithmetic mean of R, G, and B values.

pH	*Red(Mean)*	*Blue(Mean)*	*Green(Mean)*	*(R+B+G)/3(Mean)*
4.0	*122,627*	*83,01*	*36,329*	*80,508*
5.5	*109,745*	*91,276*	*46,685*	*82,706*
7.0	*78,289*	*96,887*	*57,624*	*96,887*
8.0	*68,83*	*97,719*	*60,143*	*75,641*
9.0	*70,921*	*96,823*	*58,822*	*75,843*
10.0	*32,575*	*78,816*	*71,602*	*60,99*
12.0	*20,512*	*62,058*	*68,078*	*50,43*

 doi:10.20944/preprints202509.1999.v1

Table 2. Absorbance values of anthocyanin extract at different pH levels measured at the primary wavelength 570 nm and secondary wavelength 630 nm.

pH	*Absorbance*
4.0	*0,366*
5.5	*0,312*
7.0	*0,221*
8.0	*0,189*
9.0	*0,191*
10.0	*-0,04*
12.0	*-0,02*

Table 3. Mean RGB values of sterile and contaminated culture media (DMEM and HDF) compared to the anthocyanin blank reference.

	Red(Mean)	*Blue(Mean)*	*Green(Mean)*	*(R+B+G)/3(Mean)*
DMEM (sterile)	*52,556*	*80,704*	*43,9*	*59,161*
DMEM (contaminated)	*34,612*	*69,767*	*33,457*	*45,991*
HDF (sterile)	*64,279*	*87,035*	*52,336*	*67,876*
HDF (contaminated)	41,545	*77,818*	41,289	*53,641*
Blank	*77,295*	*1,926*	*66,891*	*48,604*

doi:10.20944/preprints202509.1999.v1

Table 4. Absorbance values of anthocyanin extract at sterile and contaminated DMEM and HDF media measured at the primary wavelength 570 nm and secondary wavelength 630 nm.

	Absorbance
DMEM(sterile)	*0,147*
DMEM(contaminated)	*0,14*
HDF(sterile)	*0,154*
HDF(contaminated)	*0,129*
Blank	*0,645*

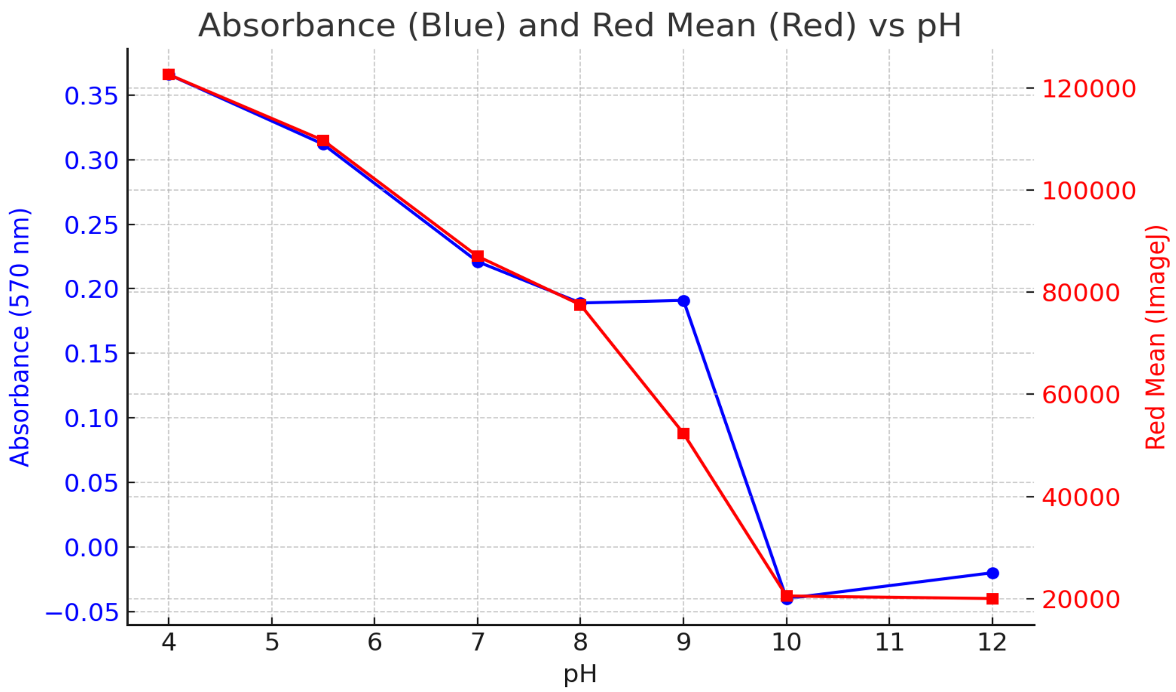

Figure 3. Absorbance and ImageJ Red Mean at different pH levels.

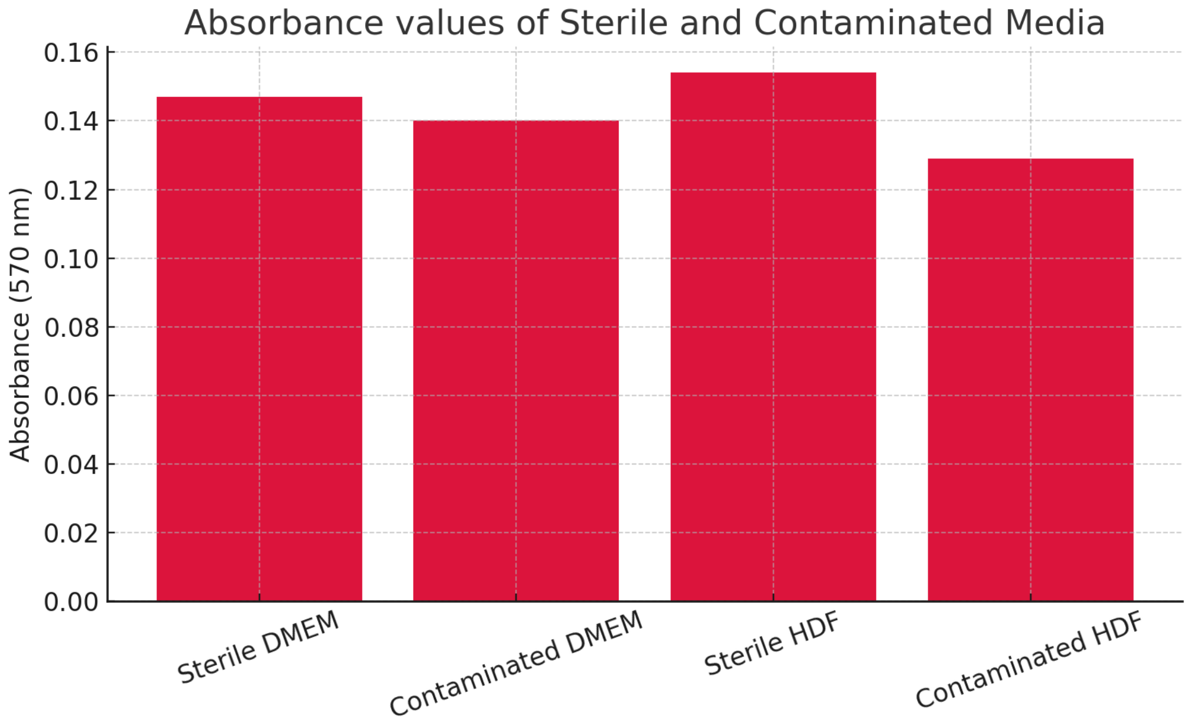

Figure 4. Absorbance values of sterile and contaminated culture media (DMEM and HDF).

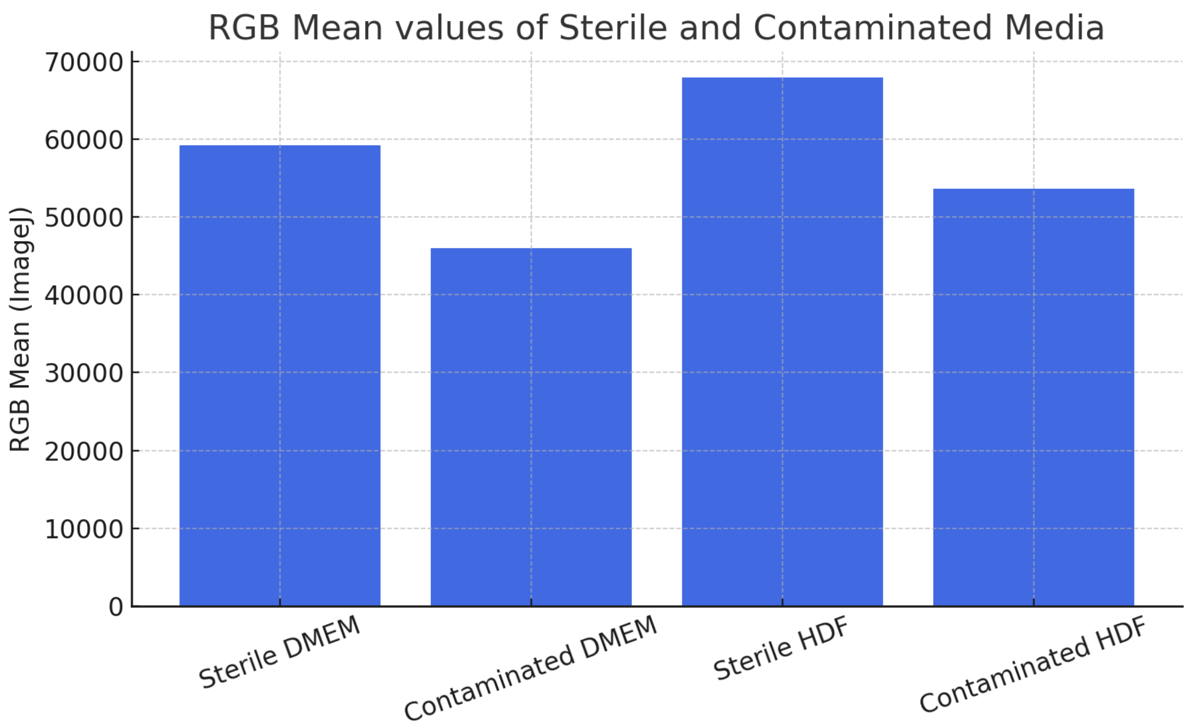

Figure 5. ImageJ RGB Mean values of sterile and contaminated culture media (DMEM and HDF).

Future Work

The present study demonstrated the feasibility of using red cabbage anthocyanin extracts for the visual and quantitative detection of pH changes and microbial contamination in liquid media. In future studies, the developed system can be translated into paper-based test strips, where anthocyanin extracts will be impregnated onto Whatman filter papers or similar substrates. Such strips would allow rapid, point-of-care, and sustainable detection of pH changes and microbial contamination without the need for spectrophotometric equipment. Additionally, anthocyanins are known to possess antimicrobial properties, which may contribute to prolonged stability of the strips during storage by preventing microbial growth on the sensing surface. This dual functionality (serving both as a colorimetric indicator and as a protective antimicrobial agent) could enhance the shelf life and robustness of the sensor, making it more practical for real-world applications in cell culture monitoring, food safety, and environmental testing.

Additionally, further experiments will investigate the capacity of the anthocyanin-based system to differentiate between Gram-positive and Gram-negative bacteria. Preliminary results indicated distinct color responses for *Staphylococcus aureus* (yellow shift) and *Escherichia coli* (green shift), suggesting potential as a bacterial discrimination assay. Quantitative calibration with various bacterial loads and species will be conducted to evaluate sensitivity, specificity, and long-term stability under different storage conditions.

References

1. Castañeda-Ovando, A., de Lourdes Pacheco-Hernández, M., Páez-Hernández, M. E., Rodríguez, J. A., & Galán-Vidal, C. A. (2009). Chemical studies of anthocyanins: A review. Food Chemistry, 113(4), 859–871. https://doi.org/10.1016/j.foodchem.2008.09.001
2. Fotos, P. G., Diaz-Arnold, A. M., & Williams, V. D. (1990). Effect of microbial contamination and pH changes in storage solutions during in vitro assays of bonding agents. Dental Materials, 6(3), 154–157. https://doi.org/10.1016/0109-5641(90)90020-F
3. Araújo, A. C. de, Gomes, J. P., Silva, F. B. da, Nunes, J. S., Santos, F. S. dos, Silva, W. P. da, Ferreira, J. P. de L., Queiroz, A. J. de M., Figueirêdo, R. M. F. de, Lima, G. S. de, Soares, L. A. dos A., Rocha, A. P. T., & Lima, A. G. B. de. (2023). Optimization of extraction method of anthocyanins from red cabbage. Molecules, 28(8), 3549. https://doi.org/10.3390/molecules28083549
4. Chandrasekhar, J., Madhusudhan, M. C., & Raghavarao, K. S. M. S. (2012). Extraction of anthocyanins from red cabbage and purification using adsorption. Food and Bioproducts Processing, 90(4), 615–623. https://doi.org/10.1016/j.fbp.2012.07.004