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# Characterization of Red Cabbage Extracts incorporated in Green Based Gelatin Active Films

Aungkana Orsuwan[1*], Wongphaka Wongrat[1], Tarit Apisittiwong[2], Kanvararat Chaiyadech[1] and Poonnawit Jiaranaikhajorn[1]

[1]Department of Food Engineering, Faculty of Engineering at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand
[2]Faculty of Food Technology, College of Agricultural Innovation and Food Technology, Rangsit University, Pathum Thani 12000, Thailand

*Corresponding author; E-mail: aungkana.or@ku.th

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## Abstract

This study aimed to assess red cabbage extracts obtained using solvents with different pH levels and alcohol concentrations, comparing their phytochemical quantities and functional characteristics. Solvents—purified water (PW, pH 7), 70% and 99% ethanol (70%E and 99%E, pH 6.5), and acidified ethanol (AE, pH 2)—were employed for extraction. When the different pH solutions were applied to the red cabbage extracts, they altered the extract's color, ranging from pink to yellow, influenced by various anthocyanin forms responsive to pH levels. The AE-extracted red cabbage displayed the highest UV and visible light barrier and total phenolic content (TPC). Then, the AE extract was used to formulate the green-based gelatin active films, alongside the 99%E extract, which had similar alcohol content and TPC values. The films averaged 20-21 μm in thickness. Moreover, incorporating both extracts into the gelatin films increased moisture content and water solubility, though the change in water vapor permeability was not significant compared to the neat gelatin film. The gelatin film incorporating AE-extracted red cabbage (GAE) showed higher water solubility than that incorporating the 99%E extract (GE). Both green-based gelatin active films showed differences in appearance color in the third week. The GAE film exhibited greater color stability, attributed to anthocyanin's distinctive chemical structures favoring acidic environments. This gelatin film incorporating red cabbage by AE-extracted holds promise as an eco-friendly active food packaging material.

***Keywords*****:** *anthocyanin; phenolic compounds; antioxidant activity; red cabbage; gelatin active films; active packaging*

## 1. Introduction

Recent advancements in food packaging have given rise to innovative smart packaging technologies aimed at preserving food quality and extending shelf life, known as active packaging, and effectively monitoring changes within food products, known as intelligent packaging (Thirupathi Vasuki et al., 2023). Food products have intricate demands, necessitating packaging solutions that address their physical, chemical, and physicochemical needs. From safety and marketing standpoint, consumers find active packaging and edible films/coatings derived from phenolic compounds, which possess antimicrobial and antioxidant properties, to be more appealing. Phenolic compounds, natural bioactive molecules found in various foods including fruits, vegetables, herbs, oils, spices, tea, chocolate, and wine, offer a promising avenue for enhancing food products' oxidative stability and antimicrobial characteristics

to prolong the shelf life of perishable food (Singh et al., 2022; Wan Yahaya et al., 2019; Orsuwan, & Sothornvit, 2018).

Red cabbage (*Brassica oleracea* var. capital f.), renowned for its potent antioxidant properties, has gained attention in the food and health sectors and innovative packaging solutions (Liang et al., 2019; Musso et al., 2019). The level of phenolics, especially those of the anthocyanins, as well as residue scavenging, were much higher in red cabbage in compared to white cabbage (Leja et al., 2010). Red cabbage contains various beneficial compounds like phenolics, vitamins, and provitamins, such as folic acids and organosulfur compounds, contributing to its antioxidant activity. Anthocyanins in red cabbage, serve as a natural alternative to synthetic colorants, antioxidants, and antimicrobials due to their diverse structures, offering a range of colors based on pH levels determining the indicator properties (Mortensen, 2006; Musso et al., 2019; Zhang et al., 2020; Horbowiczi et al., 2008; Rawdkuen et al., 2020). However, anthocyanins are vulnerable to degradation in aqueous solutions due to chemical, heat, light, and enzyme effects, causing instability in color and biological activity. Musso et al. (2019) explored strategies to enhance their stability, including using biopolymers and compounds such as flavonols and pyruvic acid to create proanthocyanins or by co-pigmentation with other phenolic compounds. Solvent extraction is the most efficient method for deriving active compounds from red cabbage. Utilizing solvents like methanol, acidified ethanol, hexane, and dichloromethane-ethanol mixtures has been pivotal in phytochemical extraction (Metivier et al., 1980; Musso et al., 2019). The effectiveness and yield of extracted substances depend on several factors such as extraction technique, solvent type and concentration, pH, extraction duration, and temperature. According to Musso, Salgado, & Mauri (2019), ethanolic extraction surpasses aqueous extraction in anthocyanin content, antioxidant activity, and total phenolic content. Fuleki, and Francis (1968) and Pajareon (2021) highlighted the pH of the solvent as a critical factor; highly acidic solvents, such as the blend of 1.5 N hydrochloric acid with ethanol at a 15:85 ratio or 0.5-2% citric acid solution, showed superior efficiency in cranberry and rice anthocyanin extraction, offering greater color stability in the flavylium form of anthocyanin. The flavylium cation form of anthocyanin is red and stable in an acid medium.

However, there is little information regarding the color stabilization of different forms of anthocyanin in the gelatin matrix. Therefore, this study aims to compare the changes in physical properties of the gelatin film incorporated with the different forms of red cabbage anthocyanin extract at room temperature.

## 2. Objectives

This study explores the phytochemical composition and properties of red cabbage extract using various available solvents with different pH levels and alcohol concentrations, including purified water (PW) with a pH of 7, 70%, and 99% ethanol (70%E and 99%E) with a pH of 6.5, and acidified ethanol (AE) with a pH of 2. The extract with the highest total phenolic content (TPC) is utilized to create biodegradable active films with gelatin, for evaluating film properties and color stability. The research also investigates the impact of these red cabbage extracts on gelatin-based films, analyzing traits such as moisture content, water solubility, water vapor permeability, and color stability. This analysis aims to determine the effect of storage time at room temperature on the color stability of these green-based gelatin active films to develop active food packaging in the future.

## 3. Materials and methods

### 3.1 Raw materials

The red cabbage was obtained from the local market of Kamphaengsaen, Nakhon Pathom province, Thailand.

### 3.2 Chemicals and Reagents

Gelatin was purchased from McGarrett (Thailand). Glycerol and sodium hydroxide (NaOH) were obtained from Kemaus (Australia). Hydrochloric acid (HCl) and 99% ethanol were obtained from QRëC (New Zealand). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (USA). Folin–Ciocalteu's phenol reagent was purchased from Sigma Aldrich (Merck 1KGaA, Darmstadt, Germany). Water was purified in a Milli-Q water purification system (Millipore, Burlington, MA, USA).

### 3.3 Red cabbage extraction

The red cabbage was extracted using the solvent extraction technique slightly modified by

Prietto et al. (2017). First, the fresh leaves of the red cabbage were washed and cut into small pieces of about 1 cm. Then, ten grams of red cabbage were added into the beakers containing 50 mL of purified water (PW) and gently stirred with magnetic stirrer for one hour at room temperature (25 ± 5 ºC). After that, the sample was filtered through Whatman No.1 filter paper. The red cabbage extract (RCE) was transferred into a brown bottle with a tight lid and stored in the refrigerator for further characterization and experimentation. Next, the extraction solvents were changed from PW to 70% ethanol (70%E), 99% ethanol (99%E), and the blending between 99% ethanol: 1.5 M HCl at a ratio 85:15 or acidified ethanol (AE), and the experiments continued following the extraction steps.

### 3.4 Characterization of red cabbage extract (RCEs) properties

*3.4.1 Color measurement*

The color of RCEs was measured using a color spectrophotometer (MiniScan EZ 4500L BYK-Gardner GmbH, Geretsried, Germany). RCE CIELAB color parameters (L*, a*, and b*) were averaged from three RCEs sample readings.

*3.4.2 Indicator color change*

Color changes of RCEs were recorded using a digital camera and measured by the UV-visible spectra of the RCEs in the different pH solutions (pH 2-12) at a ratio of 1:10 was recorded at 200-800 nm with UV-visible spectroscopy.

*3.4.3 Total anthocyanins*

The total anthocyanin (TAC)of the RCE was analyzed using the pH-differential method modified by Siti Azima et al. (2014). The RCE was diluted in the pH 1 and 4.5 solutions at a ratio of 1:10. The UV-visible spectroscopy was applied to measure the absorbance of the RCE in different pH solutions at the maximum absorption wavelength ($A_{max}$) and 700 nm ($A_{700}$). Then, the differentiation between the absorbance at pH 1 and 4.5 and the total anthocyanin content was calculated as (1) and (2) equations, respectively.

$$A = (A_{max} - A_{700})_{pH1} - (A_{max} - A_{700})_{pH4.5} \quad (1)$$

$$\text{Total Anthocyanin (mg cyanidin 3-glucoside/100g FW)} = \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times 1)} \quad (2)$$

Where A is a differentiation between the absorbance at pH 1 and 4.5.

MW is a molecular weight of cyanidin 3-glucoside 484.83 g/mol.

DF is a Dilution factor.

ε is a molecular absorptivity of cyanidin 3-glucoside 26,900 L/mol/cm$^{-1}$.

*3.4.4 Total phenolic content*

The total phenolic content of the red cabbage extracts was analyzed using a method modified from Macedo et al. (2021). An aliquot of 0.9 mL of the ethanolic extract (0.05 mL of the RCE sample and 0.85 mL of ethyl alcohol) was added to 1.5 mL of 10% Folin–Ciocalteu reagent. After keeping the mixture in the dark cabinet for 8 min, 1.6 mL of 7.5% sodium carbonate was added to the reaction mixture. The mixture was maintained at room temperature (25 ± 2 ºC) for 30 minutes. Absorbance at 740 nm was measured and converted to the phenolic content using a calibration curve based on gallic acid. The TPC was expressed in mg gallic acid equivalents (GAE)/100 g of fresh weight (FW) sample.

*3.4.5 DPPH radical scavenging effect*

The antioxidant activity of the RCEs samples was evaluated using a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay modified from Abdelwahed et al. (2007). Briefly, 0.02 mL of RCE and 0.38 mL of ethyl alcohol were mixed in the 10 mL glass tube. Then, 0.4 mL of the prepared sample was mixed with 3.6 mL of 0.1 mM of a DPPH solution (2,2-diphenyl-1-picrylhydrazyl). The mixture was incubated in a dark place for 30 min. Each sample's free radical scavenging activity was quantified by observing the decolorization of DPPH at 517 nm by a UV-visible spectrophotometer (V-770 UV/VIS/NIR Jasco Corporation, Tokyo, Japan). The percentage of DPPH free radical scavenging activity (RSC) was determined using the following equation:

$$\text{DPPH radical scavenging activity (\% RSC)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (3)$$

$A_{blank}$ and $A_{sample}$ were the absorbance values at 517 nm for the ethanolic solution of blank (0.4 mL ethanol and 3.6 mL of 0.1 mM DPPH) and the sample extract, respectively.

**3.5 Gelatin-based film preparation**

The gelatin-based film was formed by a solution-casting method modified by Musso et al. (2019). Gelatin powder 5 g was slowly poured into the 100 mL beaker containing 50 mL of 60 °C warm water. Then, the gelatin film solution and 25% (w/w) glycerol were continuously mixed under the magnetic stirrer for 20 min. After the film solution cooled to nearly room temperature (25 ± 5 °C), 50 mL of RCEs with high TPC were added to the film solution. The 20 mL of the film solution was cast onto a plastic plate with a diameter of 9 cm and then dried in a hot-air oven at 40 °C for three hours. Films were kept in the control temperature (25 ± 5 °C) and humidity (50 ± 5%RH) cabinet for at least 48 hours before being used for characterization. The film thickness was measured using a manual micrometer (No. 7326, Mitutoyo Manufacturing, Kawasaki, Japan) to the nearest 0.0001 in (0.00254 mm). The average thickness of each film was calculated from five random measurements.

**3.6 Film characterization**

*3.6.1 Film response to pH change*

The gelatin film incorporated with the selected RCEs was tested with the different pH solutions from 2-12. A film size of 3x3 cm was placed on the plastic plate, and then the pH solution of 1-2 drops was dropped on the film surface, and the color change resulted in 5 minutes. The color changes of the gelatin film indicator were recorded using the digital camera.

*3.6.2 Moisture content, Water vapor permeability, and water solubility*

The moisture content (MC) of the films was determined using a drying oven method (Rhim, & Wang, 2013). Film samples were cut into a square of 3 cm × 3 cm and subsequently dried at 105 °C for 24 h using a drying oven. The MC was calculated from the weight loss and expressed as percent MC.

The film samples' water solubility (WS) was determined as the percentage of dissolved dry matter after immersion in water. For this, three randomly selected specimens of each type of film (3 cm × 3 cm) were first dried at 60 °C for 24 h to determine the initial dry matter ($W_1$). Each film was immersed in 30 mL of distilled water in a 50 mL beaker with gentle stirring for 24 h. The film samples were removed after 24 h and dried in a drying oven at 105 °C for 24 h to determine the undissolved final dry weight ($W_2$). The WS of the sample was calculated as follows:

$$\text{WS}\ (\%) = \frac{W_1 - W_2}{W_1} \times 100 \qquad (4)$$

The films' water vapor permeability (WVP) was determined by a gravimetric modified cup method modified from the ASTM standard method E-96 (1995). The selected film, without pinholes and defects, was cut and sealed to a cup base (cup radius = 25.4 mm) previously filled with 7 mL of distilled water. The sealed cup was placed in a pre-equilibrated cabinet at 27 ± 2 °C and 50 ± 5% RH, with a fan. The 50 ± 5% RH condition was maintained using a desiccant (silica gel) in the chamber, confirmed by a temperature-RH data logger (model DM-760). The chamber was located inside a controlled temperature room at 27 ± 2 °C. Steady-state moisture transfer was obtained once the film reached equilibrium with the cup and chamber conditions. The total mass of the sealed cup was recorded after achieving a steady state at two-hour intervals; changes in the weight were recorded to the nearest 0.0001 g and graphically plotted as a function of time. The slope of each line was calculated by linear regression ($r^2 > 0.99$), and the water vapor transmission rate (WVTR) was calculated from the slope of the straight line divided by the cup base area. The WVP of the films was calculated by multiplying the steady state WVTR by the film thickness (d) and dividing by the water vapor partial pressure difference across the films ($P_{A1} - P_{A2}$). An average of 5 values of film thickness was used in the equation to determine WVP for each film replicate.

$$\text{WVP}\ (\text{g.mm/kPa.day.m}^2) = \frac{(\text{WVTR} \times \text{d})}{(P_{A1} - P_{A2})} \qquad (5)$$

*3.6.3 Film color and its stability check*

The color of the samples was measured using a color spectrophotometer (MiniScan EZ 4500L BYK-Gardner GmbH, Geretsried, Germany) on the white standard. CIELAB color parameters (L*, a*, and b*) were averaged from three RCE sample readings.

The film samples were exposed to ambient conditions in a clear glass desiccator under open fluorescent light for eight hours. Subsequently, the color of the samples was measured weekly until it approximated that of the neat gelatin film.

### 3.7 Statistical analysis

Measurements for each property of the Red Cabbage Extracts (RCEs) and the gelatin-based films containing the selected RCEs were conducted in triplicate, with individually prepared samples as the replicated experimental units. Mean values, along with standard deviations, were reported. One-way analysis of variance (ANOVA) was employed, and the significance of each mean property value was determined ($p < 0.05$) using Duncan's multiple range test within the SPSS statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA).

## 4. Results and Discussion

### 4.1 Characterization of the red cabbage extract properties

#### *4.1.1 Extraction and characterization of RCEs' physical properties*

The phytonutrients from red cabbage were obtained using various solvents—purified water, PW, ethanol 70%, 70%E, ethanol 99%, 99%E, and acidified ethanol, AE—resulting in distinct pH levels of the Red Cabbage Extracts (RCEs): 7.08, 6.46, 6.84, and 2.18, respectively. The visual appearance of RCEs revealed different colors: aqueous extraction with PW displayed a deep purple hue (Figure 1A), while alcoholic extraction with 70%E and 99%E exhibited a similar magenta tone (Figures 1B and 1C). Conversely, RCEs obtained by AE displayed a deeper red with an orange tint (Figure 1D), in line with findings by Mortensen (2006). These color variations are linked to differing anthocyanin structures, hydroxyl group orientations, glycosidic patterns, and acylations. The number of hydroxyl groups plays a pivotal role in the purple coloration of anthocyanins at neutral pH levels. Anthocyanins demonstrate varying structures based on pH levels, transitioning from quinoidal structures (purple) to anionic quinoidal base (blue) or carbinol pseudobase (colorless) at low pH (pH 2-6), then transforming into chalcone structures (yellow) at pH >7 (Roy, & Rhim, 2021).

The color analysis of the RCEs is presented in Table 1. The RCE derived from PW exhibited a lower brightness value (L*) than other extracts. This aqueous extraction (PW) produced the lowest yellow tone (b*), indicating a slightly blueish hue. However, the redness value (a*) of PW-extracted RCE fell between AE and 99%E. Moreover, the RCE from 99%E displayed the highest L* value but the lowest a*. Notably, the AE-derived RCEs demonstrated the highest a* and b* values, with an L* value higher than PW but lower than 70%E and 99%E, respectively. These color metrics align with the observed apparent color variations among all RCEs, as previously described.

The UV-visible spectra of the RCEs are illustrated in Figure 2. Figure 2A shows that the RCE derived from PW exhibited absorbance in both regions of the UV (200-400 nm), covering the UVA (315-400 nm), UVB (280-315 nm), and UVC (200-280 nm) regions. In addition, a small absorbance in the visible region (500-700 nm) was also observed in the PW-extracted RCE. Both 70%E (Figure 2B) and 99%E (Figure 2C) RCEs exhibited comparable absorbance levels within the 200-380 nm range. Conversely, the AE-extracted RCE (Figure 2D), characterized by higher acidity, displayed elevated absorbance in the UVA range (300-400 nm) and notable absorption in the red region of visible light (550-700 nm). It was found that AE-extracted RCE had the highest absorbance in the visible region, followed by PW and ethanol-extracted RCEs, respectively. This suggests potential UV light barrier properties of all RCEs in different ways, making these extracts suitable for active packaging applications for UV-sensitive food items. Ultraviolet rays are destructive and cause deterioration, yellowing, and discoloration of foods. The main food substances affected by light, including vitamins, chlorophyll, myoglobin, and common colorant in foods (fats and oils), are considered photosensitizers. Photooxidation plays a role in changing the external and internal characteristics of raw and packed food in a short period by changing the sensory and nutritional characteristics. Red cabbage extracts' barrier qualities against UV and visible light can be employed in composite materials or eco-friendly food packaging, preserving the quality of packaged foods, and shielding them from photochemical damage caused by UV and visible rays (Ezati et al., 2023).

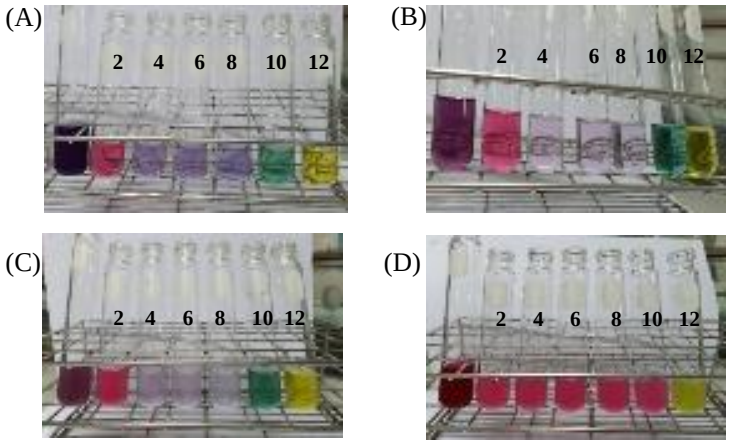


**Figure 1** Color of the red cabbage extract (left) and the color changed testing of the red cabbage extracted by; purified water; PW (A), 70% ethanol; 70%E (B), 99% ethanol; 99%E (C) and acidified ethanol; AE (D) in the different pH solutions (pH 2-12).

**Table 1** Color (L*, a* and b*), total anthocyanin (TAC), total phenolic compounds (TPC), and antioxidant activity by DPPH radical scavenging assay (RSC) of the red cabbage extract by different organic solvents such as purified water (PW), 70%ethanol (70%E), 99%ethanol (99%E) and acidified ethanol (AE)

| Extracted solvents | Color | | | TAC | TPC | RSC |
|---|---|---|---|---|---|---|
| | L* | a* | b* | (mg Cyd-3dGlu /100g FW) | (mg GAE/100 g FW) | (%) |
| **PW** | 12.6±0.4$^{d}$ | 24.5±6.6$^{b}$ | -11.9±2.0$^{c}$ | 423.0±3.0$^{b}$ | 141.5±14.0$^{ab}$ | 46.2±1.8$^{b}$ |
| **70%E** | 54.9±3.1$^{b}$ | 26.8±3.1$^{b}$ | -9.3±2.0$^{c}$ | 482.0±22.0$^{a}$ | 113.0±6.5$^{b}$ | 44.0±1.2$^{b}$ |
| **99%E** | 67.3±1.8$^{a}$ | 17.8±1.7$^{c}$ | -0.3±0.2$^{b}$ | 305.0±14.5$^{c}$ | 142.0±20.5$^{ab}$ | 67.5±2.7$^{a}$ |
| **AE** | 29.4±0.2$^{c}$ | 66.9±0.4$^{a}$ | 19.1±0.1$^{a}$ | 349.0±25.0$^{c}$ | 157.5±20.5$^{a}$ | 74.2±4.2$^{a}$ |

Values are given as mean ± standard deviation, and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Turkey's multiple range test.

*4.1.2 Total anthocyanin, total phenolic content, and the antioxidant activity*

The active phenolic phytochemicals in RCEs, such as total anthocyanin (TAC), total phenolic compounds (TPC), and the DPPH radical scavenging effect (RSC), are also shown in Table 1. Notably, the deep purple RCEs extracted via 70%E exhibited the highest TAC at 482.0 ± 22.0 mg Cyd-3dGlu/100 g FW, closely followed by PW at 423.0± 3.0 mg Cyd-3dGlu/L. RCE extracted by 99%E (305.0±14.5 mg Cyd-3dGlu/100 g FW) and AE (349.0±25.0 mg Cyd-3dGlu/100 g) exhibit lower in TAC than using the PW and 70%E as the solvent. Ethanol's efficiency in anthocyanin content extraction, as highlighted by Musso et al. (2019) and Oancea et al. (2012) in cranberry and blueberry studies, was also evident in this observation. Chandrasekhar et al. (2012) and Stoica et al. (2023) found that anthocyanin extraction increased with higher ethanol concentrations in water and 1% HCl acidified water up to50% (v/v) but stable and decreased thereafter. Above 50% (v/v) ethanol, the decrease in anthocyanin might be due to less extraction of hydrophilic anthocyanins as water concentration decreased. This was due to anthocyanins, colored water-soluble pigments belonging to the phenolic group (Khoo et al., 2017). Therefore, the increase of ethanol or decrease of water in this experiment (99%E and AE) resulted in the reduction in the extractable water-soluble anthocyanin as present in TAC in Table 1. The TAC of RCE extracted by AE showed a higher value than that extracted using the similarly based 99%E without the acidity. Stoica et al. (2023) also found that TAC derived from acidified water with 1% glacial acetic acid (320.53±7.12 mg Cyd-3dGlu/100 g) was higher than TAC from pure water (128.57±3.03 mg Cyd-3dGlu/100 g).

The total phenolic content of red cabbage extracts was between 113 and 157.5 mg GAE/100 g FW, depending on the extraction solvent. The PW,

99%E and AE showed no significant difference in TPC values. The orange red RCEs via AE displayed significantly higher TPC and RSC than those derived from 70%E. However, RCE derived from 70% E showed the lowest values in TPC and RSC (113.0±6.5 mg GAE/100 g FW and 44.0±1.2%) (Table 1). These findings are consistent with the results of Fuleki & Francis's (1968) and Stoica et al. (2023) results, indicating that AE, the EtOH-50% AA1% solvents, exhibit the highest RSC and efficacy in TPC extraction. However, it's important to note that the presence of phenolic phytochemicals can vary based on the plant species, cultivation region, and processing (Hernanz et al., 2008).

*4.1.3 RCEs Response to pH Changes*

The pH response of the aqueous indicator from pH 2 to 12 is shown in Figure 2. PW, 70%E, and 99%E, having pH levels close to neutrality (7.08, 6.46, and 6.84, respectively), exhibited similar color transitions. These solutions shifted from pink to light magenta, green, and eventually yellow as the pH increased (Figure 2A-2C). On the other hand, AE displayed a unique behavior, appearing pink between pH 2 and 10 and turning yellow at pH 12 (Figure 2D). These color transformations are influenced by the varying forms of anthocyanins in response to pH levels. At acidic pH (pH<3), the prevalent flavylium cation form imparts a red hue. Between pH 4 and 6, a dominant quinoidal blue form is observed. Later, hydration of the flavylium cation leads to reduced color intensity, forming a colorless carbinol pseudo-base (pH 5-6). At pH 7, a purple color emerges, indicating a quinoidal anhydrous base. Ionization at pH 8 shifts to deep blue, and higher pH levels reveal a chalcone form with a light-yellow color (Brouillard, & Delaporte, 1977; Roy, & Rhim, 2020). Since the RCEs by PW, 70%E, and 99%E displayed similar color-changing trends, the UV-visible light spectra of the 70%E-extracted sample across different pH solutions (pH 2-12) were presented as representative data (Figure 3). The samples in all pH solutions exhibited a common peak at 220 nm. At pH 2, the sample peaked at 530 nm in the red region of visible light. Similar small peaks at 280 nm and 330 nm were observed for samples at pH 4, 6, and 8 (Figure 3). Moreover, the sample at pH 10 displayed absorbance peaks at 400 and 600 nm within the visible light range. Additionally, the sample at pH 12 exhibited dominant absorbance in the UV light region (250-300 nm) and a visible yellow color region (300-500 nm). These observations align with changes in anthocyanin structures, which offer diverse colors (Mortensen, 2006; Musso et al., 2019).

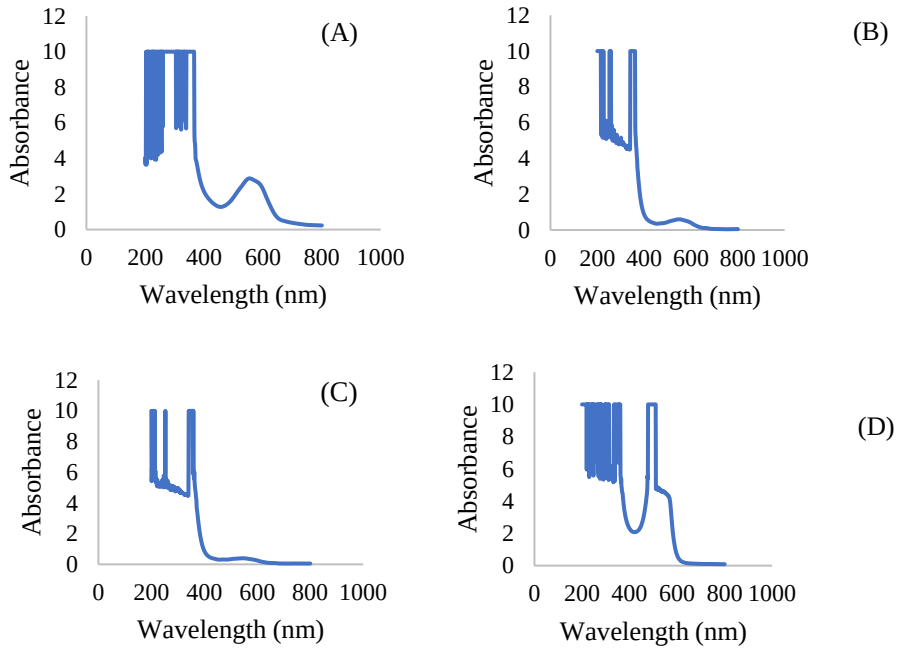


**Figure 2** UV-Visible spectra of the red cabbage extracted by purified water; PW (A), 70% ethanol; 70%E (B), 99% ethanol; 99%E (C) and acidified ethanol; AE (D)

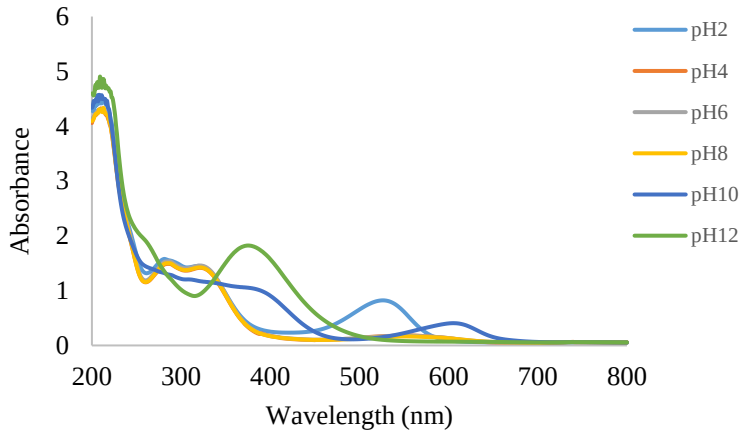


**Figure 3** UV-visible spectra of red cabbage extract with 70 %E

## 4.2 Gelatin-based Film Properties

*4.2.1 Film appearance and Color measurement*

The RCE solvent extract with the highest total phenolic content (TPC) was utilized to create the color-stable gelatin film. The AE-extracted red cabbage displayed the highest total phenolic content (TPC). It was chosen to formulate green-based gelatin active films alongside the 99%E extract, which had similar alcohol content and TPC values. Therefore, gelatin films infused with red cabbage extract (RCE) obtained using AE (GAE) and 99%E (GE) were formed to assess their comparative film properties and color stability. As expected, the control gelatin film (NG) appeared smooth with a clear, pale-yellow color. Interestingly, the films with GAE and GE showed significant visual differences compared to NG ($p<0.05$), even though both RCE-infused films displayed similar color profiles. GE appeared pink with a purple tint, while GAE had a pink hue with an orange tint. Despite balancing the final pH in both film solutions, the color was still influenced by the anthocyanin structures present in the RCE.As expected, the control NG film exhibited higher L* values than the composite films. Interestingly, the L* of the GE did not significantly differ from the GAE film. Regarding color attributes, the GAE film displayed a notably higher redness (a*) than both the GE and the control film. Incorporating both RCEs decreased the L* values of both composite gelatin films. The GAE film showed a slightly more negative b* value than the GE film. This suggests a more bluish hue in the GAE film compared to GE film. Despite similarities in TAC and TPC between GE and GAE, their physical properties, notably color, differed. This discrepancy suggests variations in anthocyanin types and chemical structures in each RCE.

*4.2.2 The physicochemical properties of gelatin films*

Table 2 presents the physicochemical characteristics of the neat gelatin film and the composite gelatin films with RCEs. Comparatively, the moisture content and solubility of GE and GAE were notably higher than those of the NG film. While the GE film exhibited increased water vapor permeability compared to NG and GAE films, the difference was not statistically significant. This indicates that the acidity of the solvent used in the extract in the gelatin film did not significantly impact the film's thickness, moisture content, and water vapor permeability. This observation aligns with research involving blueberry residue and tapioca starch film, which showed similar thickness and moisture content but elevated water vapor permeability. This change was attributed to shifts in intermolecular attraction, transitioning from cohesive forces to weaker adhesion forces between phenolic compounds and starch or plasticizer, as explained by Andretta et al. (2019).

**Table 2** Color (*L**, *a**, and *b**) in the CIE system, thickness, moisture content (MC), solubility (WS), and water vapor permeability (WVP) of gelatin film (NG) and gelatin films incorporated with red cabbage extract with 99%ethanol (GE) and acidified ethanol (GAE)

| Gelatin Films | Color | | | Properties | | | |
|---|---|---|---|---|---|---|---|
| | L* | a* | b* | Thickness (μm) | MC (%) | WS (%) | WVP (g-mm/kPa-h-m$^2$) |
| NG | 19.2 ± 1.2$^a$ | 0.1 ± 0.1$^c$ | 2.5 ± 0.1$^a$ | 18.9±2.4$^a$ | 12.3±0.6$^b$ | 17.5±1.2$^c$ | 15.7±0.6$^a$ |
| GE | 8.8 ± 0.4$^b$ | 0.7 ± 0.2$^b$ | -0.3 ± 0.2$^c$ | 20.6±2.3$^a$ | 13.5±02$^a$ | 20.7±1.1$^b$ | 16.7±0.7$^a$ |
| GAE | 8.7 ± 0.5$^b$ | 1.7 ± 0.2$^a$ | -1.7 ± 0.9$^b$ | 19.6±3.4$^a$ | 13.7±03$^a$ | 23.4±0.9$^a$ | 15.6±0.4$^a$ |

Values are given as mean ± standard deviation, and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Turkey's multiple range test.

*4.2.3 pH response of the film color transition*

The GAE and GE films were tested with different pH solutions (pH 2-12) (Results not shown). As expected, the color of the GAE film did not change when the pH solutions 2-10 was dropped on the film surface for 5 min. However, the GAE film changed from pink to yellow-green when the extremely alkaline pH 12 was applied to the film surface. In addition, the GE film started to change from pink to yellow-green at pH 10. This result was associated with the RCEs' color changes. The different color changes between both composite films might result from the more stable chemical structure of the anthocyanin in the high-acid environment. This experiment showed a similar trend in color changes in the gelatin films incorporated with red cabbage extract (Musso et al., 2019).

*4.2.4 Film color stability*

The color of GE films by the naked eye is shown in Table 3 and Figure 4, and a noticeable change from pink with a purple tint to pale yellow with a pink tint in the 3$^{rd}$ week was dominantly observed at room temperature. Moreover, the color measurement was expressed in a manner similar to the results in Table 3. Film color stability was investigated; the first week of GE film showed an increase in a* and b* compared to the start film at zero weeks ($p<0.05$). In the 2$^{nd}$ week, this film showed a higher whiteness L* value, the a* and b* were not significantly different from the 1$^{st}$ week. In the 3$^{rd}$ week, the GE film showed significantly higher a* and b* from the 2$^{nd}$. The b* color appeared to change from a negative value (slightly blue) to a positive value (pale yellow). The part in the GAE film expressed higher stability in color than the GE film. However, there was also a noticeable color change in the 3$^{rd}$ week from pink with an orange tint to pale yellow (Table 3). Color measurements of the GAE film showed that the film's colors L*, a*, and b* in the 1$^{st}$ and 2$^{nd}$ weeks did not significantly differ from the starting week. Nevertheless, the L* and a* values were significantly increased in the 3$^{rd}$ week. As time passed, the film's color changed from pink to pale yellow as the neat gelatin film resulted from the sensitivity of anthocyanin to the light. In addition, the destruction of anthocyanin in higher temperatures could be due to the hydrolysis of 3-glycoside structure. Moreover, the hydrolysis of the pyrylium ring resulted in the production of chalcone, which was responsible for the brown color development of anthocyanin in food (Pajareon, 2021). However, the natural color of the anthocyanin might act as a photosensitizer, and it was applied as the UV and visible light barrier for food packaging to prevent the food from deterioration (Orsuwan et al., 2019). As the chemical structure of anthocyanin, anthocyanidins (or aglycons) are basically composed of an aromatic ring bonded to a heterocyclic ring that contains oxygen, and these also bonded by a carbon–carbon bond to a second aromatic ring. Then, it can be considered as a photosensitizer or an agent that absorbs light of a specific wavelength and transforms it into energy. Simultaneously, anthocyanin can serve as an organic UV absorber, protecting against light-induced oxidation that may lead to color bleaching in materials exposed to direct UV and visible light (Ezati et al., 2023). This may cause an increase in L*, a*, and b* values in the third week.

**Table 3** Color change of gelatin films incorporated with red cabbage extract with 99%ethanol (GE) and acidified ethanol (GAE) at 0, 1, 2, and 3 weeks.

| Week | GE | | | GAE | | |
|---|---|---|---|---|---|---|
| | **L*** | **a*** | **b*** | **L*** | **a*** | **b*** |
| **0** | $8.8\pm0.4^{b}$ | $0.7\ 0.2^{c}$ | $-0.3\pm0.2^{c}$ | $8.7\pm0.5^{B}$ | $1.7\pm0.2^{A}$ | $-1.7\pm0.9^{B}$ |
| **1st** | $10.3\pm0.1^{b}$ | $1.4\pm0.1^{b}$ | $0.1\pm0.0^{b}$ | $9.9\pm0.0^{B}$ | $2.2\pm0.1^{A}$ | $-1.0\pm0.0^{B}$ |
| **2nd** | $12.0\pm0.0^{a}$ | $1.7\pm0.0^{b}$ | $1.0\pm0.3^{b}$ | $10.1\pm0.1^{B}$ | $1.4\pm0.1^{A}$ | $0.0\pm0.1^{B}$ |
| **3rd** | $12.7\pm0.2^{a}$ | $2.6\pm0.2^{a}$ | $2.7\pm0.0^{a}$ | $11.1\pm0.0^{A}$ | $2.4\pm0.3^{A}$ | $1.3\pm0.4^{A}$ |

Values are given as mean ± standard deviation, and any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Turkey's multiple range test.

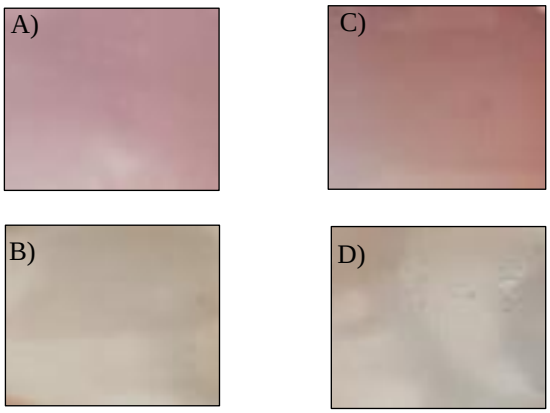


**Figure 4** Color change from the first and third week of gelatin films incorporated with red cabbage extract with 99%ethanol (GE) (A and B) and acidified ethanol (GAE) (C and D)

## 5. Conclusion

The selection of organic solvents for red cabbage extraction significantly impacted the composition and concentration of active substances obtained. Specifically, 70%E proved highly effective in extracting total anthocyanins, which can be considered for application as an indicator. While AE excelled in extracting comprehensive phenolic compounds from red cabbage, making it suitable as the active ingredient in food or biodegradable film/coating due to its UV and visible barrier properties and high value in total phenolic content and radical scavenging activity by DPPH method. These compounds played a crucial role in determining the observed color values. Phenolic-rich extracts from AE and 99%E were used to develop GAE and GE into active gelatin films, respectively. Interestingly, the choice of solvents did not noticeably affect film properties such as thickness, moisture content, and water vapor permeability compared to the neat gelatin film. However, unexpected color transitions from red to green occurred only when the GAE and GE films encountered pH solutions of 12 and 10, differing from anticipated responses. This unpredicted behavior warrants further exploration of the films' reactions to varying pH conditions. Understanding these properties could enhance functionality, paving the way for broader applications in smart packaging for food, such as high-fat meats, olive oil, and red-colored foods, and for medical use in the near future. Furthermore, future investigations should explore the antioxidant and antimicrobial properties of these red cabbage extract-infused gelatin films to assess their potential for innovative packaging applications.

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