Acta Pharm. Sci. Vol 62:(4), 2024
Original article
DOI: 10.23893/1307-2080.APS6259

# Isolation and quantification of anthocyanins from red cabbage (*Brassica oleracea* L.) and its potential uses as antioxidant in natural food

Muhammad Khalid SAEED, Naseem ZAHRA*, Asma SAEED, Laiba BABAR, Manal MALIK, Momna SHEHBAZ, Muhammad Hassan RAZA

Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

**ABSTRACT**

Red cabbage (*Brassica oleracea* L. *var. capitata*) is a widely cultivated vegetable known for its diverse varieties and health benefits. This article focuses on the extraction and analysis of anthocyanins, phenolic compounds, and antioxidant activities from red cabbage powder and paste. The physicochemical properties of red cabbage, including its nutritional composition, were evaluated. The anthocyanin content was found to be highest in red cabbage paste in water, followed by red cabbage powder in water, while the lowest level was observed in red cabbage powder in methanol. The total anthocyanin content in red cabbage was determined to be 78.47 mg/100g. The total phenolic content was highest in red cabbage paste in methanol, followed by red cabbage paste in water. The antioxidant activity was assessed using the DPPH assay, showing promising results for red cabbage extracts. The findings demonstrate the potential of red cabbage as a natural source of anthocyanins and phenolic compounds with antioxidant properties, which can be utilized in various applications, including the food industry.

**Keywords:** *Brassica oleracea* L., DPPH, antioxidants, anthocyanin

*Corresponding author: Naseem ZAHRA
E-mail: drnaseemzahra@gmail.com
ORCIDs:
Muhammad Khalid SAEED: 0000-0003-0613-0896
Naseem ZAHRA: 0000-0002-3993-5079
Asma SAEED: 0009-0008-6370-0692
Laiba BABAR: 0009-0009-0294-0999
Manal MALIK: 0009-0003-9213-6035
Momna SHEHBAZ: 0009-0008-3916-1107
Muhammad Hassan RAZA: 0000-0002-7685-1368
(Received 15 Nov 2023, Accepted 9 Feb 2024)

## INTRODUCTION

One of the most significant vegetables farmed worldwide is cabbage (*Brassica oleracea* L. *var. capitata*). It is a member of the Cruciferae family, along with Brussels sprouts, broccoli, cauliflower, and kale[1]. In terms of the size, shape, color, and texture of the leaves as well as the size, shape, color, and texture of the head, the different cultivated varieties of cabbage exhibit enormous variability. The various cultivars of cabbage are divided into three categories: white, red, and savoy cabbage[2]. People across the world use brassica vegetables in the human diet[3]. Numerous epidemiological and clinical researches have shown that cabbage is good for your health[4,5]. Red cabbage is a blooming plant that is herbaceous, biennial, and dicotyledonous. Its leaves are typically served as coleslaw, salad, and beverage[6]. Red cabbage stands out for its flavour, colour, and texture. It was first grown and harvested in Europe, but it is now grown and harvested all over the world. This vegetable contains a lot of micronutrients and phytochemicals, like glucosinolates, vitamin C and K, beta-carotene, minerals, fibre, and total polyphenols[7]. Red cabbage is becoming increasingly popular across the world, and it is consumed raw as well as after technical and home treatment[8].

The most important pigments found in vascular plants are anthocyanins (anthos, which means flower and kianos, which means blue). Because they are non-toxic and simple to absorb into aqueous mediums, anthocyanins are attractive as natural water-soluble colourants[9]. Aglycone, acylated and glycosidic anthocyanins are all absorbed by people. Anthocyanins are ingested by humans at levels that may be physiologically relevant as components of plant food items. A rise in interest in these polyphenols was caused by this discovery as well as the pro-health qualities of anthocyanins (anticancer, cardioprotective, eyesight-improving, antidiabetic)[10]. Anthocyanins have been shown to have several potential health benefits, including a reduced risk of chronic illnesses like cancer and coronary heart disease[11], obesity and type 2 diabetes prevention[13], vision and acuteness enhancement[12], and anti-allergic and antibacterial activities[14]. Red cabbage has a distinct anthocyanin pattern. It includes a substantial number of anthocyanins, the primary structure of which is cyanidin glycosides usually. However, most anthocyanin compounds in red cabbage are acylated[15]. When compared to anthocyanins from other natural sources, red cabbage anthocyanins represent the colour throughout a wide range of pH values, which is extremely appealing to the food industry as natural food colourants. It is critical to pick a suitable approach with high anthocyanin recovery from red cabbage, with organic solvent extraction being the most often used extraction method[16]. Red cabbage's anthocyanins can be used in neutral foods as well as acidic ones because they range in colour from red at low pH to blue and green at high pH. They therefore have the poten-

tial to offer a natural substitute for artificial colourants[17].

Red cabbage contains more than 150 flavonoids, among which anthocyanins are abundant. Among the Brassica species, red cabbage is the vegetable with the best antiradical defence system. Food oxidation can be inhibited or controlled by antioxidant substances. The main antioxidants in red cabbage are phenolic compounds. Their chemical activity is proportional to the quantity of hydroxyl groups they contain. They are natural antioxidants with numerous roles, including free radical scavengers and potential pro-oxidant producers[18]. During a reaction, the activation energy of the radial molecule is very less it is composed of a chain reaction of kinetic property. The free radicals are prevented from causing any harm with the help of antioxidants. Since the antioxidants are composed of phenolic hydroxyl groups, these groups react with the present free-radical molecule, thus producing a stable product i.e., imiquimod radical. Another way to stop the free radical from creating harm is to reduce itself and donate an electron to the radical[19,20].

The purpose of the current study is to isolate and quantify the anthocyanins from red cabbage along with their antioxidant activity.

## METHODOLOGY

### Plant material

Three fresh red cabbages were acquired at a nearby grocery, cash and carry Punjab society in Lahore, Pakistan. The red cabbages were washed and cleaned under running water to eliminate any signs of dust and contaminants[21]. Following that, raw red cabbage (1770g) was weighed and sliced into small pieces. The chopped cabbage was then placed in a polythene bag and refrigerated for later use.

### Chemical and reagents

All additional chemicals used in the current experiment were analytical graded, except for the following: sodium carbonate, the Folin-Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), ethanol, and methanol, which were all acquired from sigma Chemical Co.

### Extraction procedure

Two procedures were used to extract the red cabbage powder:

In the first method, fresh red cabbage was sliced and weighed before being placed in a tray and dried in a dry oven at 50 degrees Celsius for 56 hours. After drying, the dried cabbage was converted into a fine powder.

The second method used to create red cabbage powder was solvent extraction.

In this procedure, 200g of chopped cabbage was placed in a flask along with a 1:1 combination of 250 mL ethanol and 250 mL water (v/v) and covered with foil and plastic wrap for 1 week. After a week, the red cabbage colour was released into the solution, which was then dried and pulverized in a dry oven. A red cabbage paste was obtained at the end[22].

**Physiochemical analysis**

AOAC techniques were used to measure physicochemical properties such as moisture, ash, fat fibre, and protein content of fresh red cabbage and red cabbage powder, and then the measured parameters were compared.

**Isolation and quantification of anthocyanins**

The colours (purple, blue, and red) in many plants are caused by anthocyanins, which are water-soluble flavonoid pigments that come in a spectrum of hues ranging from red to blue depending on the pH of the vacuole water. Anthocyanins are becoming more and more important as antioxidants in addition to serving as food colouring. Several therapeutic benefits, including as Vaso protective and anti-inflammatory properties, have been associated with anthocyanins[23].

The total monomeric anthocyanin was calculated using the pH differential method. Using pH 1 (0.025 M potassium chloride) and pH 4.5 (0.4 M sodium acetate) buffers, the extract was diluted 100 times. 15 minutes were given for the solutions to equilibrate in the dark. On cuvettes with a 1 cm path length, absorbance was measured at 530 and 700 nm using a Shimadzu UV/visible spectrophotometer[24].

The following formula is used to calculate the anthocyanin content:

$$Abs = (A_{530} - A_{700}) - (B_{530} - B_{700})$$

$$\frac{Abs \times Molar\ Facror \times 1000 \times (dilution\ factor)}{29600 \times 1}$$

**Determination of total polyphenols content**

A modified Folin-Ciocalteu technique was used to measure the number of total polyphenols in the sample[25]. 250 L of Folin-Ciocalteu reagent and approximately 50 L of each sample's diluted extract were blended with minor adjustments. 1.5 mL of sodium carbonate solution (7%) was added after 5 minutes, and the mixture was shaken occasionally for the next 30 minutes. The absorbance of the resulting solution was measured at 760 nm. Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) were used to assess the amount of phenol in the sample[26].

**DPPH assay**

The hue of the organic nitrogen radical, DPPH free radical, is dark purple. When DPPH reagent is introduced in a solution that includes an antioxidant, this then changes from purple hue to yellow. The DPPH test is also regarded as the simplest colorimetric technique for such uses. It is a stable radical in solution, and when present in methanol, it looks purple in colour. The DPPH test works on the idea that when the DPPH ion absorbs a hydrogen atom from the antioxidant, the absorbance decreases and the colour changes from purple to yellow[27].

The approach recommended by Brand-Williams was modified to determine the overall free radical-scavenging activity of the samples under analysis. In this experiment, 25–100 L of each red cabbage extract was mixed with 2.95 mL of a methanolic solution containing 0.4 mmol/L of DPPH. The absorbance at 517 nm was measured after standing at room temperature in the dark for 30 minutes[28,29].

Using the equation, the percentage of DPPH scavenging (RSA%) was calculated:

$$\% \text{ Scavenging of DPPH} = [(A0 - A1)/A0] \times 100$$

Where A0 = absorbance of the control and A1 = absorbance of the test extracts[30].

**Statistical analysis**

The average and standard deviation (SD) of the three replicates were used to express the experimental results. Using the SAS v. 9.1.3 programme, the results of each analysis were compared using an ANOVA and Duncan's multiple range test.

**RESULTS and DISCUSSION**

Colour is a sensory property of food that makes it more appealing, enticing, and tasty to customers. Colour has a strong impact on flavour perception and is frequently recognized before fragrance[31]. In addition to the food industry, the textile, and cosmetics sectors both heavily rely on colour. These sectors typically employ artificial colours, which represent a health risk, and as the globe changes, everyone is gravitating toward natural and organic goods. Keeping that in mind, the present research involves the extraction of natural colour from red cabbage. In this study, red cabbage powder was extracted (Figure 1) and tested for anthocyanin, phenolic, and antioxidant activities.

**Figure 1.** Red cabbage flakes, extract and powder

## Physiochemical analysis

Red cabbage powder was assessed for its physiochemical properties that include its nutritional value shown in Table 1. The nutritional composition of red cabbage which includes moisture, ash, carbs, protein, fats, and fibre was assessed. The findings revealed that 100 g of edible red cabbage contains the following nutritional value: calories (31 kcal), water (90 g), protein (2.0 g), and fat (0.2 g)[32]. The nutritional value of red cabbage powder was also assessed, and it was then compared to the nutritional value of fresh cabbage. red cabbage powder has the following nutritional value: red cabbage powder contains 8.3% moisture, and this value was like Drozdowska's study who also reported 8.33% of moisture in dehydrated red cabbage powder[33].

**Table 1.** Nutritional composition of red cabbage

| Parameters | Value (Fresh red cabbage) | Value (Red cabbage powder) |
|---|---|---|
| Moisture (%) | 89.70 | 8.30 |
| Ash (%) | 0.90 | 6.37 |
| Fats (%) | 0.12 | 3.21 |
| Fiber (%) | 2.20 | 6.50 |
| Protein (%) | 1.52 | 9.45 |
| Carbohydrates (%) | 5.56 | 66.17 |

## Anthocyanin content in red cabbage powder and paste

Red cabbage paste and powder were tested for total anthocyanin content using a UV spectrophotometer, and the results are shown in Table 2. The result of anthocyanin content in red cabbage shows that the highest anthocyanin level was detected in red cabbage paste in water (27.55mg/100g) and then in red cabbage powder water (26.14mg/100g) and (14.93mg/100g) anthocyanin was present in red cabbage paste in methanol. The minimum level of anthocyanin was seen in red cabbage powder in water (9.85mg/100g). Total anthocyanin determined in red cabbage powder and paste was 78.47mg/100g. The total anthocyanins contents decreased in the following order: Red cabbage paste (Water) > Red cabbage powder (Water) > Red cabbage paste (Methanol)>Red cabbage powder (Methanol)[34].

**Table 2.** Total anthocyanin content in red cabbage

| Samples | Total anthocyanins content |
|---|---|
| Red cabbage paste (Water) | 27.55 mg/100g |
| Red cabbage paste (Methanol) | 14.93 mg/100g |
| Red cabbage powder (Water) | 26.14 mg/100g |
| Red cabbage powder (Methanol) | 9.85 mg/100g |

Red cabbage anthocyanin-rich extract improved cell viability and apoptosis by reducing $H_2O_2$-induced intracellular oxidative stress in human hepatocellular carcinoma (HepG2) cells[35]. Anthocyanins are water-soluble pigments that could be used to colour a variety of food goods[36]. Currently, these pigments are used to create food colorings that are produced from horticulture crops and

processing wastes[37]. Additionally, anthocyanins have been found in both in vitro and in vivo studies to have a variety of potential health advantages and to be excellent free radical scavengers, making them good antioxidant chemicals[38,39].

### Total phenolic content

The total phenolic content of red cabbage pastes and powder is shown in Table 3. The TPC was determined by Folin–Ciocalteu reagent and the result obtained shows the highest phenolic content was present in red cabbage paste (methanol) at 140µg/GAE. Then the second highest was seen in red cabbage paste (Water) at 112µg/GAE. Red cabbage powder (methanol) had 107µg/GAE of phenolic content. The lowest level of phenolic content was seen in red cabbage powder (water) at 24µg/GAE. Our results align with the study reported by Izzo L. et al. 2020[40].

**Table 3.** Total phenolic content of red cabbage

| Samples | Total phenolic content |
|---|---|
| Red cabbage paste (Methanol) | 140 µg/g GAE |
| Red cabbage paste (Water) | 112 µg/g GAE |
| Red cabbage powder (Methanol) | 107 µg/g GAE |
| Red cabbage powder (Water) | 24 µg/g GAE |

Red cabbage extract contains 21 hydroxycinnamic acid derivatives (HCAs), the majority of which are leftovers of the organic acids p-coumaric, ferulic, and synaptic or their hydrated forms. As it is well known, different herbs and regions of the world have variable amounts of TPC in plant extracts. Numerous factors, including genetics, the impact of the environment's climate and the type of solvent employed during the extraction technique, can be utilized to explain this variation[41].

### Antioxidant activity using DPPH Assay

By applying the previously reported DPPH technique in a free radical scavenging experiment, the antioxidant activity of Aloe vera extract was determined. The technique is based on a reduction in DPPH in the presence of antioxidants and a progressive change in the colour of DPPH from purple to yellow depending on the concentration of antioxidants, which is shown by a reduction in absorbance[42]. According to the results, the highest antioxidant activity is present in red cabbage paste (Methanol) which lies in the range of 57.71-69.21 in the 25-100µl sample which are in line with the study conducted by Ricci[43,44]. Table 4 shows the antioxidant activity of red cabbage.

**Table 4.** Antioxidant activity of red cabbage

| Antioxidant activity (DPPH) | Sample (25µl) | Sample (50µl) |
|---|---|---|
| Red cabbage paste (Methanol) | 57.71 | 69.21 |
| Red cabbage paste (Water) | 50.74 | 60.90 |
| Red cabbage powder (Methanol) | 29.47 | 55.44 |
| Red cabbage powder (water) | 9.10 | 36.35 |

It was found that red cabbage in paste form showed maximum antioxidant activity in methanol than in water. Similarly, red cabbage in powder form showed more antioxidant activity in methanol than in water.

The above study showed that red cabbage is the best source of antioxidants and can be used as a potential antioxidant source in food and cosmetics. The extracted anthocyanins can be used as natural colorants in different food items.

## STATEMENT OF ETHICS

Ethical approval of this study was obtained from the Food and Biotechnology Research Centre, PCSIR Laboratories Complex Lahore.

## CONFLICT OF INTEREST STATEMENT

There were no known conflicts of interest.

## AUTHOR CONTRIBUTIONS

The authors contributed equally.

## REFERENCES


1. Wei S, Xiao X, Wei L, Li L, Li G, Liu F, et al. Development and comprehensive HS-SPME/GC–MS analysis optimization, comparison, and evaluation of different cabbage cultivars (*Brassica oleracea* L. *var*. *capitata* L.) volatile components. Food Chem, 2020;340:128166. Doi: 10.1016/j.foodchem.2020.128166

2. Godlewska K, Biesiada A, Michalak I, Pacyga P. The effect of botanical extracts obtained through ultrasound-assisted extraction on white head cabbage (*Brassica oleracea* L. *var*. *capitata* L.) seedlings grown under controlled conditions. Sustainability, 2020;12(5):1871. Doi: 10.3390/su12051871

3. Čimo J, Aydın E, Šinka K, Tárník A, Kišš V, Kotuš T. Change in the length of the vegetation period of tomato (*Solanum lycopersicum* L.), white cabbage (*Brassica oleracea* L. *var*. *capitata)* and carrot (*Daucus carota* L.) due to climate change in Slovakia. Agronomy, 2020;10(8):1110. Doi: 10.3390/agronomy10081110

4. Berger MD, Vakula A, Horecki AT, Rakić D, Pavlić B, Malbaša R, et al. Cabbage (*Brassica oleracea* L. *var*. *capitata*) fermentation: Variation of bioactive compounds, sum of ranking differences and cluster analysis. LWT, 2020;133:110083. Doi: 10.1016/j.lwt.2020.110083

5. Dobosy P, Vetési V, Sandil S, Kröpfl K, Óvári M, Záray G. Effect of irrigation water containing iodine on plant physiological processes and elemental concentrations of cabbage (*Brassica oleracea* L. *var*. *capitata* L.) and tomato (*Solanum lycopersicum* L.) cultivated in different soils. Agronomy, 2020;10(5):720. Doi: 10.3390/agronomy10050720

6. Koss Mikołajczyk I, Kusznierewicz B, Wiczkowski W, Płatosz N, Bartoszek A. Phytochemical composition and biological activities of differently pigmented cabbage (*Brassica oleracea var*. *capitata*) and cauliflower (*Brassica oleracea var*. *botrytis*) varieties. J Sci Food Agric, 2019;99(12):5499-5507. Doi: 10.1002/jsfa.9811

7. Batool F, Adeel S, Azeem M, Iqbal N. Natural dye yielding potential and compounds of selected vegetable residues belonging to Brassicaceae: an approach towards sustainability. Pak J Bot, 2022; 54(1):329-336. Doi: https://doi.org/10.30848/PJB2022-1(39)

8. Wei S, Xiao X, Wei L, Li L, Li G, Liu F, et al. Development and comprehensive HS-SPME/GC–MS analysis optimization, comparison, and evaluation of different cabbage cultivars (*Brassica oleracea* L. *var*. *capitata* L.) volatile components. Food Chem, 2021;340:128166. Doi: 10.1016/j.foodchem.2020.128166

9. Sarkar D, Rakshit A. Bio-priming in combination with mineral fertilizer improves nutritional quality and yield of red cabbage under Middle Gangetic Plains, India. Sci Horti, 2021;283:110075. Doi: 10.1016/j.scienta.2021.110075

10. Kamal KY, Khodaeiaminjan M, ElTantawy AA, Moneim DA, Salam AA, Ash Shormillesy, et al. Evaluation of growth and nutritional value of Brassica microgreens grown under red, blue and green LEDs combinations. Physiol Plant, 2020;169(4):625-638. Doi: 10.1111/ppl.13083

11. Zhang Y, Xiao Z, Ager E, Kong L, Tan L. Nutritional quality and health benefits of microgreens, a crop of modern agriculture. J Fut Foods, 2021;1(1):58-66. Doi: 10.1016/j.jfutfo.2021.07.001

12. Abedi-Firoozjah R, Yousefi S, Heydari M, Seyedfatehi F, Jafarzadeh S, Mohammadi R, et al. Application of red cabbage anthocyanins as pH-sensitive pigments in smart food packaging and sensors. Polymers, 2022;14(8):1629. Doi: 10.3390/polym14081629

13. Zanoni F, Primiterra M, Angeli N, Zoccatelli G. Microencapsulation by spray-drying of polyphenols extracted from red chicory and red cabbage: effects on stability and color properties. Food Chem, 2020; 307:125535. Doi: 10.1016/j.foodchem.2019.125535

14. Sarkar D, Sankar A, Devika OS, Singh S, Parihar M, Datta, R. Optimizing nutrient use efficiency, productivity, energetics, and economics of red cabbage following mineral fertilization and biopriming with compatible rhizosphere microbes. Sci Rep, 2021;11(1):1-14. Doi: 10.1038/s41598-021-95092-6

15. Managa MG, Sultanbawa Y, Sivakumar D. Effects of different drying methods on untargeted phenolic metabolites, and antioxidant activity in Chinese cabbage (*Brassica rapa* L. *subsp. chinensis*) and nightshade (*Solanum retroflexum Dun.*). Molecules, 2020;25(6):1326. Doi: 10.3390/molecules25061326

16. Le TN, Chiu CH, Hsieh PC. Bioactive compounds and bioactivities of *Brassica oleracea* L. *var. italica* sprouts and microgreens: an updated overview from a nutraceutical perspective. Plants, 2020; 9(8):946. Doi: 10.3390/plants9080946

17. Kim JY, Lee SI, Kim JA, Muthusamy M, Jeong MJ. Specific audible sound waves improve flavonoid contents and antioxidative properties of sprouts. Sci Hortic, 2021;276:109746. Doi: 10.1016/j.scienta.2020.109746

18. Drozdowska M, Leszczyńska T, Koronowicz A, Piasna-Słupecka E, Dziadek K. Comparative study of young shoots and the mature red headed cabbage as antioxidant food resources with antiproliferative effect on prostate cancer cells. RSC Adv, 2020;10(70):43021-43034. Doi: 10.1039/D0RA07861A

19. An R, Luo S, Zhou H, Zhang Y, Zhang L, Hu H, et al. Effects of hydrogen-rich water combined with vacuum precooling on the senescence and antioxidant capacity of pakchoi (*Brassica rapa subsp. Chinensis*). Sci Hortic, 2021;289:110469. Doi: 10.1016/j.scienta.2021.110469

20. Salehi B, Quispe C, Butnariu M, Sarac I, Marmouzi I, Martorell M. Phytotherapy and food applications from Brassica genus. Phytother Res, 2021;35(7):3590-3609. Doi: 10.1002/ptr.7048

21. Zahra N, Saeed MK, Shahzad K, Firdous S, Ahmad I, Ashraf M, et al. DPPH assay and reducing power activity of water extract of (*Mentha longifolia*) mint. LGULS, 2022;6(01):38-47. Doi: 10.54692/lgujls.2022.0601198

22. Wen H, Cui H, Tian H, Zhang X, Ma L, Li J. Isolation of neuroprotective anthocyanins from black chokeberry (*Aronia melanocarpa*) against amyloid-β-induced cognitive impairment. Foods, 2020; 10(1):63. Doi: 10.3390/foods10010063

23. Zhao X, Zhang SS, Zhang XK, He F, Duan CQ. An effective method for the semi-preparative isolation of high-purity anthocyanin monomers from grape pomace. Food Chem, 2020;310:125830. Doi: 10.1016/j.foodchem.2019.125830

24. Mansour M, Salah M, Xu X. Effect of microencapsulation using soy protein isolate and gum arabic as wall material on red raspberry anthocyanin stability, characterization, and simulated gastrointestinal conditions. Ultrason Sonochem, 2020;63:104927. Doi: 10.1016/j.ultsonch.2019.104927

25. Chen Q, Zhao J, Liu M, Cai J, Liu J. Determination of total polyphenols content in green tea using FT-NIR spectroscopy and different PLS algorithms. J Pharm Biomed Anal, 2007;46(3):568-573. Doi: 10.1016/j.jpba.2007.10.031

26. Matić P, Sabljić M, Jakobek L. Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. J AOAC Int, 2017; 100(6):1795-1803. Doi: 10.5740/jaoacint.17-0066

27. Menezes BB, Frescura LM, Duarte R, Villetti MA, Rosa MB. A critical examination of the DPPH method: mistakes and inconsistencies in stoichiometry and $IC_{50}$ determination by UV–Vis spectroscopy. Anal Chim Acta, 2021;1157:338398. Doi: 10.1016/j.aca.2021.338398

28. Yeo J, Shahidi F. Critical re-evaluation of DPPH assay: presence of pigments affects the results. J Agric Food Chem, 2019; 67(26):7526-7529. Doi: 10.1021/acs.jafc.9b02462

29. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. Food Front, 2020; 1(1):60-69. Doi: 10.1002/fft2.10

30. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules, 2022;27(4):1326. Doi: 10.3390/molecules27041326

31. Plasek B, Lakner Z, Temesi Á. Factors that influence the perceived healthiness of food. Nutrients, 2020;12(6):1881. Doi: 10.3390/nu12061881

32. Kuswandi B, Asih NP, Pratoko DK, Kristiningrum N, Moradi M. Edible pH sensor based on immobilized red cabbage anthocyanins into bacterial cellulose membrane for intelligent food packaging. Packag Technol Sci, 2020;33(8):321-332. Doi: 10.1002/pts.2507

33. Drozdowska M, Leszczyńska T, Koronowicz A, Piasna-Słupecka E, Dziadek K. Comparative study of young shoots and the mature red headed cabbage as antioxidant food resources with antiproliferative effect on prostate cancer cells. RSC Adv, 2020;10(70):43021-43034. Doi: 10.1039/D0RA07861A

34. Ghareaghajlou N, Hallaj-Nezhadi S, Ghasempour Z. Red cabbage anthocyanins: Stability, extraction, biological activities and applications in food systems. Food Chem, 2021;365:130482. Doi: 10.1016/j.foodchem.2021.130482

35. Lee M, Lee M. The effects of C3G and D3G anthocyanin-rich black soybean on energy metabolism in beige-like adipocytes. J Agri Food Chem, 2020;68(43):12011-12018. Doi: 10.1021/acs.jafc.0c04891

36. Mottaghipisheh J, Doustimotlagh AH, Irajie C, Tanideh N, Barzegar A, Iraji A. The promising therapeutic and preventive properties of anthocyanidins/anthocyanins on prostate cancer. Cells, 2022; 11(7):1070. Doi: 10.3390/cells11071070

37. Zhao X, Zhang X, Tie S, Hou S, Wang H, Song Y, et al. Facile synthesis of nano-nanocarriers from chitosan and pectin with improved stability and biocompatibility for anthocyanins delivery: an *in vitro* and *in vivo* study. Food Hydrocoll, 2020; 109:106114. Doi: 10.1016/j.foodhyd.2020.106114

38. Fallah AA, Sarmast E, Jafari T. Effect of dietary anthocyanins on biomarkers of glycemic control and glucose metabolism: a systematic review and meta-analysis of randomized clinical trials. Food Res Int, 2020;137:109379. Doi: 10.1016/j.foodres.2020.109379

39. Mohammadalinejhad S, Kurek MA. Microencapsulation of anthocyanins — critical review of techniques and wall materials. Appl Sci, 2021;11(9):3936. Doi: 10.3390/app11093936

40. Izzo L, Rodríguez-Carrasco Y, Pacifico S, Castaldo L, Narváez A, Ritieni A. Colon bioaccessibility under in vitro gastrointestinal digestion of a red cabbage extract chemically profiled through UHPLC-Q-Orbitrap HRMS. Antioxidants, 2020;9(10):955. Doi: 10.3390/antiox9100955

41. Aksu MI, Turan E, Sat IG, Erdemir E, Oz F, Gürses M. Improvement of quality properties of cemen paste of pastirma by lyophilized red cabbage water extract. J Food Process Preserv, 2020; 44(9):e14714. Doi: 10.1111/jfpp.14714

42. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: a review. Int J Mol Sci, 2021;22(7):3380. Doi: 10.3390/ijms22073380

43. Flieger J, Flieger M. The [DPPH●/DPPH-H]-HPLC-DAD method on tracking the antioxidant activity of pure antioxidants and goutweed (*Aegopodium podagraria* L.) hydroalcoholic extracts. Molecules, 2020;25(24):6005. Doi: 10.3390/molecules25246005

44. Ricci A, Parpinello GP, Teslić N, Kilmartin PA, Versari A. Suitability of the cyclic voltammetry measurements and DPPH• spectrophotometric assay to determine the antioxidant capacity of food-grade oenological tannins. Molecules, 2019;24(16):2925. Doi: 10.3390/molecules 24162925