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Determination of anthocyanin content of two forms of *Brassica oleracea*

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Abstract

The present study was carried out to determine the total anthocyanin content of two cultivars of *Brassica oleraceae* belongs to the family Cruciferae. Total phenolics and total flavonoid content were also determined. pH differential method was used to determine total anthocyanins present and aluminium chloride and Folin-Ciocalteu reagents were used for the determination of flavonoids and phenolics, respectively. Comparatively, anthocyanin flavonoid and phenolic contents were varied between white and red cabbage. Red cabbage showed high amount of these phytochemicals when compared to white cabbage. Thus depending on results we say that red cabbage variety may serve as a potential source of nutraceuticals and functional food development.

Keywords: Red cabbage, White cabbage, anthocyanin, flavonoid, phenolics, Spectrophotemeter.

1. Introduction

Anthocyanins are water-soluble vacuolar pigments that, depending on their pH, may appear red, purple, or blue. They belong to a parent class of molecules called flavonoids synthesized via the phenylpropanoid pathway. They occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. They are derived from anthocyanidins by adding sugars [1]. They are odorless and moderately astringent. They have a long history as part of the human diet. They are probably the most important group of visible plant pigments besides chlorophyll and are mainly the glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The flower part of the plant *Brassica oleracea* is selected for the present study. Based on the colour of the head there are two forms viz *Brassica oleracea* var. *capitata* f. *alba* (white cabbage) – white or greenish head, *F. rubra* (red cabbage) – red cabbage. The plant changes color according to the pH value of the soil, due to the pigment anthocyanin [2]. Acidic soils – grow reddish, alkaline soil-greenish colored cabbage. The present study was aimed to determine total anthocyanin, flavonoid and phenolic contents present in these cultivars of *Brassica oleracea*.

2. Materials and methods

2.1 Collection of Plant Material

Brassica oleracea var. *capitata* f. *alba* (white cabbage) and *Brassica oleracea* var. *capitata* f. *rubra* (red cabbage) were purchased from the local market in Warangal and authenticated by Dr. V S Raju, Department of Botany, Kakatiya University, Warangal.

2.2 Extraction of Anthocyanins

Methanolic extraction is the classical method of extracting anthocyanins from plant materials. This

procedure involves maceration or soaking of the plant material in methanol containing a small concentration (0.01%) of mineral acid (e.g., HCl). Methanol extraction is a rapid, easy, and efficient method for anthocyanin extraction. However, a crude aqueous extract with several contaminants is obtained, and methanol evaporation can result in hydrolysis of labile acyl linkages, which is aggravated by the presence of HCl [3].

2.3 Determination of total anthocyanin content

The total amount of anthocyanin content was determined by using pH differential method. A spectrophotometer and 1 cm path length cuvette was used for spectral measurements at 210 and 750 nm [4].

The absorbance of the samples (A) was calculated as follows:

$$A = (A_{520} - A_{700})\ pH\ 1.0 - (A_{520} - A_{700})\ pH\ 4.5$$

Anthocyanin pigment content (mg / liter) =

$$(A \times MW \times DF \times 1000) / e \times 1)$$

Where,

Molecular weight of anthocyanin (cyanidine-3-glucoside equivalent) = 449, Extinction coefficient (**ε**) = 29,600, DF= dilution factor

2.4 Estimation of Total Flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination. One milliliter of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with ultraviolet (UV) visible spectrophotometer. The content was determined from extrapolation of calibration curve which was prepared by using quercetin solution (2-10µg/ml) in methanol. The concentration of flavonoid was expressed in terms of mg/ml [5].

2.5 Determination of Total Phenolics

The amount of total phenolic content of the extracts was determined by Folin-Cio calteau reagent as oxidizing agent, gallic acid as standard. Exactly 0.5 ml of the extract was transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by addition of distilled water. 1 ml of Folin-Ciocalteau reagent was added and incubated at room temperature for 3 min. 3 ml of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as the standard (20-100µg/ml) for a calibration curve. The phenolic compound content was expressed in terms of mg/ml [6,7].

2.6 Statistical analysis

The experiments were performed in triplicate. The results were expressed as mean ± SD. Also, linear regressions between the content of total anthocyanins with the results of the antioxidant assays were assessed.

3. Results and discussion

3.1 Total anthocyanin content

The total quantity of anthocyanins in red and white cabbage was found to be 58.41 mg/kg and 0.3mg/kg, respectively with equivalent to cyanindin-3- glucoside.

3.2 Total flavonoid content

Total flavonoid content of RCAE, WCAE (1mg) equivalent to 7.016, 2.05µg respectively of quercetin was detected.

3.3 Total phenolic content

Phenolics are the most wide spread secondary metabolites in plant kingdom. Phenols are very important constituents because of their scavenging ability due to their hydroxyl groups. In the present study total phenolics content of RCAE, WCAE (1mg) equivalent to 69.73, 45.4 µg respectively of gallic acid was detected.

The two cultivars of *Brassica* were analysed spectrometrically for their anthocyanin, flavonoid and phenolic contents. The obtained results revealed differences in the concentrations of total anthocyanins in two cultivars according to the variety. The phenolics, flavonoids and anthocyanins are the main class of natural compounds with significant antioxidant activities which have been identified and quantified in several fruits, vegetables and berries [8,9]. Phenolics, flavonoids and anthocyanins are primary antioxidants which can donate hydrogen or electron, and radical intermediates can be stabilized by these types of compounds [10].

As the red cabbage has highest amount of anthocyanin, flavonoids and phenolics it has the greatest potential as a source of compounds to be applied as natural antioxidants in food. This study showed that the red cabbage variety is rich in anthocyanins might be utilised as functional food and natural remedy against various diseases and disorders related to oxidative stress and free radical effects. Thus, the red cabbage variety rich in anthocyanins may serve as a new potential source of nutraceuticals and functional food development.

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