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REVIEW



Anthocyanins in Brassicaceae: composition, stability, bioavailability, and potential health benefits

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ABSTRACT

Brassicaceae family includes various economic plants for edible or ornamental purposes. Brassicaceae vegetables are considered to be a major part of human diet as sources rich in phytochemicals. Among them, anthocyanins provide red to blue colors in Brassicaceae plants and as well have nutritional value and pharmaceutical potential. This review aims to summarize the chemical composition, stability, bioaccessibility, bioavailability, potential health benefits, and applications of anthocyanins from Brassicaceae. Additionally, the potential for incorporation of Brassicaceae anthocyanins in food systems as food additives and functional ingredients was demonstrated.

KEYWORDS

Acylated anthocyanins; bioactivity; Brassica; cyanidin 3-diglucoside-5-glucoside; functional food

Introduction

The Brassicaceae (Cruciferae) with a total of nearly 338 genera and 3709 species distributes worldwide. Many are economically important crop and ornamental species such as *Brassica oleracea* L., *Brassica rapa* L., *Raphanus sativus* L., *Arabidopsis thaliana*, and *Matthiola incana* (Warwick, Francis, and Al-Shehbaz 2006). Brassicaceae vegetables are highly distributed in Asia, North America, and European countries with various genera, species, and cultivars, such as *B. oleracea* (kale, cabbage, broccoli, kohlrabi, Brussels sprouts, and cauliflower), *B. rapa* (Chinese cabbage, bok choy, and pak choi), *Brassica juncea* (mustard), and *R. sativus* (radish) (Cartea et al. 2010; Dominguez-Perles et al. 2014). Additionally, *Brassica napus* crops are mainly used like oilseed, which rapeseed ranks currently the third source of vegetable oil (Thiyam et al. 2004; Wanasundara 2011). The roots of the horseradish (*Armoracia rusticana*) and Japanese wasabi (*Eutrema japonica*) are processed for condiments to provide pungent flavor in human diet (Agneta, Möllers, and Rivelli 2013). *A. thaliana* is the plant model widely utilized in biological research. There are also a limited number of plants that are cultivated as ornamentals, e.g., dame's rocket or violet (*Hesperis matronalis*), wallflower (*Erysimum cheiri*), and candytuft (several species of *Iberis*).

Anthocyanins are one of the most widespread natural pigments responsible for the red, purple, and blue colors of Brassicaceae plants. They are considered as important dietary bioactivity compounds with potential health benefits. The aim of this article is to provide an overview for better understanding the composition, stability, bioaccessibility,

bioavailability, health benefits of anthocyanins in Brassicaceae.

Anthocyanins in Brassicaceae

Anthocyanins are a kind of naturally occurring water soluble pigments responsible for the blue, purple, pink, red, and black colors of fruit, vegetables, flowers, and cereal grains. Chemically, anthocyanins belong to the class of flavonoid compounds possessing the structure of a C6-C3-C6 carbon skeleton. They are glycosides of hydroxyl and/or methoxy derivatives of 2-phenylbenzopyrylium or flavylium salts (Kong et al. 2003). Over twenty naturally occurring anthocyanidins has been identified and only six of them are commonly found in fruits and vegetables, including pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin (Castañeda-Ovando et al. 2009). Brassicaceae anthocyanins have been identified in many *Brassica* and *Raphanus* vegetables as well as the flowers in *Moricandia*, *Heliophila*, *Matthiola*, and *Iberis* species.

The levels and profiles of anthocyanins in Brassicaceae are summarized in Tables 1 and 2, respectively. The concentrations of anthocyanins reported in Brassicaceae vary greatly among varieties (Table 1). Anthocyanins concentrations in red cabbage cultivar Langedijker Polona reached up to 629 mg/100 g dry weight (DW), whereas total anthocyanins were 0.2285 mg/100 g fresh weight (FW) in broccoli sprouts (Moreno et al. 2010; Wiczowski, Topolska, and Honke 2014). Edible sprouts, especially broccoli, kale, mustard, and radish sprout, are good sources of anthocyanins, while anthocyanins are not found in the corresponding adult

Table 1. Anthocyanin concentrations in Brassicaceae.

Scientific name	Common name	Cultivar	Tissue	Anthocyanins	Reference
<i>Brassica oleracea</i> L. var. <i>italica</i>	Broccoli	Marathon	Sprout	0.2912 mg/100 g FW ^{a,c}	Moreno et al. (2010)
		Nubia	Sprout	0.2804 mg/100 g FW ^{a,c}	Moreno et al. (2010)
		Intersemillas	Sprout	0.2285 mg/100 g FW ^{a,c}	Moreno et al. (2010)
		Viola	Sprout	0.6360 mg/100 g FW ^{a,c}	Moreno et al. (2010)
		Plenck	Sprout	12.66 mg/100 g DW ^{b,c}	de la Fuente et al. (2019)
<i>Brassica oleracea</i> L. var. <i>botrytis</i>	Violet cauliflower	Graffiti	Head of cauliflower	73.9 mg/100 g FW ^{b,c}	Volden, Bengtsson, et al. (2009)
		Violetto di Catania	Head of cauliflower	7.72 mg/100 g FW ^{b,c}	Scalzo et al. (2008)
		Natalino	Head of cauliflower	1.81 mg/100 g FW ^{b,c}	Scalzo et al. (2008)
		Sammartinaro	Head of cauliflower	4.01 mg/100 g FW ^{b,c}	Scalzo et al. (2008)
		Early purple Vienna	Peel	78.58 mg/100 g DW ^{a,c}	Park et al. (2012)
<i>Brassica oleracea</i> var. <i>gongylodes</i>	Purple kohlrabi	Azur-Star	Peel	3 mg/100 g DW ^{a,c}	Rahim et al. (2018)
		Kolibri	Peel	302 mg/100 g DW ^{a,d}	Zhang et al. (2015)
		Red Dove	Leaves	173 mg/100 g FW ^{b,c}	Zhang et al. (2012)
<i>Brassica Oleracea</i> var. <i>acephala</i> f. <i>tricolor</i>	Purple kale	n.s.	Leaves	1.39 mg/100 g DW ^{b,c}	de la Fuente et al. (2019)
<i>Brassica oleracea</i> var. <i>sabellica</i> L.	Green curly kale	n.s.	Sprout	1.39 mg/100 g DW ^{b,c}	de la Fuente et al. (2019)
<i>Brassica oleracea</i> L. var. <i>capitata</i> L. f. <i>rubra</i>	Red cabbage	Langedijker Dauer 2	Leaves	214 and 424 mg/100 g DW ^{a,c,i}	Wiczowski, Topolska, and Honke (2014)
		Kissendrup	Leaves	181 and 373 mg/100 g DW ^{a,c,i}	Wiczowski, Topolska, and Honke (2014)
		Koda	Leaves	154 and 332 mg/100 g DW ^{a,c,i}	Wiczowski, Topolska, and Honke (2014)
		Kalibos	Leaves	113 and 317 mg/100 g DW ^{a,c,i}	Wiczowski, Topolska, and Honke (2014)
		Langedijker Polona	Leaves	226 and 629 mg/100g DW ^{a,c,i}	Wiczowski, Topolska, and Honke (2014)
		Violetto di Catania	Leaves	75.6 mg/100 g DW ^{b,c}	Scalzo et al. (2008)
		Langedijker	Leaves	232 mg /100 g DW ^{a,c}	Wiczowski, Szawara-Nowak, and Topolska (2013)
<i>Brassica juncea</i> (L.) Czern.	Red mustard	n.s.	Sprout	36.40 mg/100 g DW ^{b,c}	de la Fuente et al. (2019)
<i>Brassica juncea</i> var. <i>tumida</i> Tsen et Lee	Purple mustard	Zi Ying	Sprout	192.9 mg/100 g FW ^{b,c}	Xie et al. (2014)
<i>Brassica rapa</i> var. <i>chinensis</i>	Purple bok choy	Zi He	Sprout	313 mg/100 g DW ^{a,d}	Zhang et al. (2014)
<i>Brassica rapa</i> L. ssp. <i>pekinensis</i>	Chinese cabbage	11591	Leaves	39.43–1.78 mg/100 g FW ^{a,c,e}	He, Zhang, and Zhang (2016)
<i>Raphanus sativus</i> L.	Radish	n.s.	Sprout	5.57 mg/100 g DW ^{b,c}	de la Fuente et al. (2019)
		Man Tang Hong	Flesh	472 mg/100 g DW ^{a,g}	Park et al. (2011)
		Hong Feng No.1	Peel	448 mg/100 g DW ^{a,g}	Park et al. (2011)
		Yan Zhi# 2	Root	160.74 mg/100 g FW ^{b,h}	Jing et al. (2012)
		Tou Xin Hong	Root	144.56 mg/100 g FW ^{b,h}	Jing et al. (2012)
		Xin Ling Mei	Root	63.77 mg/100 g FW ^{b,h}	Jing et al. (2012)
		China rose	Sprout	15.8 mg/100 g FW ^{a,f}	Baenas et al. (2015)
<i>Raphanus sativus</i> var. <i>sativus</i>	China rose radish	China rose	Sprout	180 mg/100 g FW ^{a,f}	Baenas et al. (2015)
<i>Raphanus sativus</i> cv. Rambo	Rambo radish	Rambo	Sprout	14.88–32.13 mg/100 g FW ^{b,c,i}	Guarise et al. (2019)
<i>Sisymbrium officinale</i> (L.) Scop.	Hedge mustard	Milan	Leaves	26.38–38.79 mg/100 g FW ^{b,c,i}	Guarise et al. (2019)
		Bergamo	Leaves		

FW, fresh weight; DW, dry weight; n.s., none specified.

^aHPLC method.^bpH differential method.^cQuantification based on cyanidin 3-glucoside as standard.^dQuantification based on cyanidin 3,5-diglucoside as standard^eThe concentration of anthocyanin was different by internal heading leaves to external heading leaves.^fQuantification based on cyanidin 3-glucoside-β-glucopyranoside as standard^gQuantification based on cyanidin 3-glucoside chloride and pelargonidin 3-glucoside chloride as standard^hQuantification based on perlargonidin 3-glucoside equivalents as standard.ⁱThe concentration of anthocyanin was different by growing season.

vegetables (de la Fuente et al. 2019). Even within the same species, the content of anthocyanins in Brassicaceae may vary with cultivar (Moreno et al. 2010), growing season (Guarise et al. 2019; Wiczowski, Topolska, and Honke 2014), growth condition (Baenas et al. 2015), and plant organ (Park et al. 2012; Rahim et al. 2018). The anthocyanins content in red cabbage varieties Langedijker Polona obtained in 2009 (629 mg/100 g DW) was three times higher than that observed in 2008 (226 mg/100 g DW). Baenas et al. (2015) demonstrated that glucose (277 mM) and sucrose (176 mM) treatment during germination effectively enhanced the production of anthocyanins in China rose radish sprouts

by 57 and 20%. Anthocyanins concentrations in violet cauliflower (*B. oleracea* L. var. *botrytis*) reported by different researchers ranges from 1.81 to 73.9 mg/100 g FW (Scalzo et al. 2008; Volden, Bengtsson, et al. 2009). These differences may be due to cultivar and growing condition.

The anthocyanins profile in Brassicaceae differs greatly among species (Table 2). The major anthocyanins identified in *Brassica* vegetables are cyanidin 3-diglucoside-5-glucoside derivatives acylated with ferulic, *p*-coumaric, malonic, caffeic, oxalic, *p*-hydroxybenzoic, or/and sinapic acids. For instance, cyanidin 3-(*p*-coumaroyl)diglucoside-5-glucoside was the most abundant anthocyanin in violet cauliflower (Scalzo et al. 2008).

Table 2. Anthocyanin composition in Brassicaceae.

Scientific name	Common name	Anthocyanin composition	Core	Acyl group	Reference
<i>Brassica oleracea</i> L. var. <i>italica</i>	Broccoli sprout	Cy 3-diglc-5-glc, Cy 3,5-diglc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Sin)triglc-5-glc, Cy 3-(pCoun)(Sin)diglc-5-glc, Cy 3-(Sin)(Fer)diglc-5-glc, Cy 3-(Sin)(Sin)diglc-5-glc, Cy 3-(pCoun)(Sin)diglc-5-(Mal)glc, Cy 3-(Sin)(Fer)diglc-5-(Mal)glc, and Cy 3-(Sin)(Sin)diglc-5-(Mal)glc	Cy 3,5-diglc, Cy 3-diglc-5-glc, Cy 3-triglc-5-glc	pCoun, Sin, Fer, Mal	Moreno et al. (2010)
<i>Brassica oleracea</i> L. var. <i>botrytis</i>	Cauliflower	Cy 3-glc, Cy 3-glc-5-glc, Cy 3-diglc-5-glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(pCoun)diglc-5-(Sin)glc, Cy 3-(Fer)diglc-5-(Sin)glc, Cy 3-(Sin)diglc-5-(Sin)glc	Cy 3-glc, Cy 3-glc-5-glc, Cy 3-diglc-5-glc	pCoun, Sin, Fer	Scalzo et al. (2008)
<i>Brassica oleracea</i> var. <i>gongylodes</i> L.	Purple Kohlrabi	Cy 3-rut-5-glc, Cy 3-(Caf)(Sin)glc-5-glc, Cy 3-(Caf-Fer-Sin)glc-5-glc, Cy 3-(Caf-pCoun-Sin)diglc-5-glc, Cy 3-diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Caf)(pCoun)diglc-5-glc, Cy 3-(Glu-Fer)diglc-5-glc, Cy 3-(pCoun)(Sin)triglc-5-glc, Cy 3-(Fer)(Sin)triglc-5-glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Glu-Sin)diglc-5-glc, Cy 3-(pCoun)(Sin)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-glc, Cy 3-(Sin)(Sin)diglc-5-glc	Cy 3-glc-5-glc, Cy 3-diglc-5-glc, Cy 3-rut-5-glc, Cy 3-triglc-5-glc	Glu, Sin, Caf, Fer, pCoun	Park et al. (2012); Zhang et al. (2015)
<i>Brassica oleracea</i> var. <i>acephala</i>	Red kale sprout	Cy 3-diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Caf)(pCoun)diglc-5-glc, Cy 3-(Sin)glc-5-glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(pCoun)(Sin)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-glc, Cy 3-(Sin)(Sin)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-glc	Cy 3-diglc-5-glc, Cy 3-glc-5-glc	Sin, Caf, pCoun, Fer	Jeon et al. (2018)
<i>Brassica oleracea</i> L. var. <i>capitata</i> L. f. <i>rubra</i>	Red cabbage	Cy 3-diglc-5-glc, Cy 3,5-diglc, Cy 3-(pHy)diglc-5-(Oxa)glc, Cy 3-diglc-5-xyl, Cy 3-(Caf)(pCoun)diglc-5-glc, Cy 3-(Caf)(Fer)diglc-5-glc, Cy 3-(Caf)(Sin)diglc-5-glc, Cy 3-(pCoun)triglc-5-glc, Cy 3-(pCoun)diglc-5-(Suc)glc, Cy 3-(Sin)(pCoun)triglc-5-glc, Cy 3-(Sin)(Fer)triglc-5-glc, Cy 3-(pCoun)glc-5-glc, Cy 3-(Glu-Sin)(pCoun)diglc-5-glc, Cy 3-(Glu-Sin)(Fer)diglc-5-glc, Cy 3-(Sin)(pCoun)diglc-5-glc, Cy 3-(Glu-Sin)(Sin)diglc-5-glc, Cy 3-(Sin)(Fer)diglc-5-glc, Cy 3-(Fer)diglc-5-(Sin)glc, Cy 3-(Sin)diglc-5-(Sin)glc, Cy 3,5-diglc, Cy 3-(Fer)triglc-5-glc, Cy 3-(Sin)triglc-5-glc, Cy 3-(Fer)(Fer)triglc-5-glc, Cy 3-(Caf)diglc-5-glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Fer)glc-5-glc, Cy 3-(Sin)glc-5-glc, Cy 3-(Fer)(Fer)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-glc, Cy 3-(Sin)(Sin)diglc-5-glc	Cy 3-diglc-5-glc, Cy 3,5-diglc, Cy 3-diglc-5-xyl, Cy 3-triglc-5-glc, Cy 3,5-diglc	Sin, Caf, pCoun, Fer, pHy, Oxa, Glu	Arapitsas, Sjoberg, and Turner (2008); Wiczowski, Szawara-Nowak, and Topolska (2013)
<i>Brassica oleracea</i> cv. <i>Red Danish</i>	Red cabbage sprout	Cy 3-diglc-5-glc, Cy 3-(Mal)diglc-5-glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(pCoun)(pCoun)diglc-5-glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Fer)(Fer)diglc-5-glc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Sin)(Sin)diglc-5-glc	Cy 3-diglc-5-glc	Sin, pCoun, Fer, Mal	Hrazdina, Iredale, and Mattick (1977)
<i>Brassica juncea</i> var. <i>tumida</i> Tsen et Lee	Tumorous stem mustard	Cy 3-(Fer)diglc-5-glc, Cy 3-(Caf)(Sin)diglc-5-glc, Cy 3-(pHy)(Fer)(Caf)diglc-5-(Mal)glc, Cy 3-(Fer)diglc-5-(Mal)glc, Cy 3-(Caf)diglc-5-(Mal)glc, Cy 3-(Fer)diglc-5-(Mal)glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(Caf)(Fer)diglc-5-glc, Cy 3-(Caf)(Sin)diglc-5-(Mal)glc, Cy 3-(Fer)(Fer)diglc-5-glc, Cy 3-(pCoun)diglc-5-(Mal)glc, Cy 3-(Fer)(Caf)diglc-5-(Mal)glc, Cy 3-(pCoun)(Fer)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-(Mal)glc, Cy 3-(pCoun)(Sin)diglc-5-(Mal)glc, Cy 3-(Fer)(Sin)diglc-5-(Mal)glc, Cy 3-(pCoun)(Fer)diglc-5-(Mal)glc, Cy 3-(Fer)(Fer)diglc-5-(Mal)glc, Cy 3-(pCoun)triglc-5-(Mal)glc, Cy 3-(pCoun)(pCoun)diglc-5-(Mal)glc	Cy 3-diglc-5-glc, Cy 3-diglc-5-diglc	Sin, pCoun, Fer, Mal, Caf, pHy	Xie et al. (2014)
<i>Brassica rapa</i> var. <i>chinensis</i>	Bok choy sprout	Cy 3-(Sin)diglc-5-glc, Cy 3,5-diglc, Cy 3-diglc-5-(Mal)glc, Cy 3-(pHy)diglc-5-(Mal)glc, Cy 3-(Sin)diglc-5-(Mal)glc, Pt 3,5-diglc, Cy 3-(Mal-Glu-pHy)ara-5-(Mal)glc, Cy 3-(Fer-Sin)diglc-5-(Mal)glc, Cy 3-(pHy-Mal)glc-5-(Mal)glc, Cy 3-(pHy-pHy-Mal)glc-5-(Mal)glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Sin)diglc-5-(Mal)glc, Cy 3-(Caf-Sin)rut-5-glc, Cy 3-(pHy-Fer-Mal)glc-5-(Mal)glc, Cy 3-(Mal-Glu-pHy-pCoun)ara-5-(Mal)glc	Cy 3,5-diglc, Cy 3-diglc-5-glc, Pt 3,5-diglc, Cy 3-rut-5-glc, Cy 3-ara-5-glc	Sin, pCoun, Fer, Mal, Caf, pHy	Zhang et al. (2014)
<i>Brassica rapa</i> L. ssp. <i>pekinensis</i>	Chinese cabbage	Dp 3-glu, Pn 3-diglc-5-glc, Dp 3-diglc-5-glc, Cy 3-diglc-5-glc, Cy 3-diglc-5-(Mal)glc, Dp 3,5-diglc, Cy 3-(Caf)diglc-5-glc, Cy 3-(Oxa-pHy)diglc-5-glc, Cy 3,5-diglc, Dp 3-(Fer)glc-5-glc-7-glc, Dp 3-(Sin)glc-5-glc-7-glc, Pt 3-(Mal)glc-5-glc, Cy 3-(Mal)glc-5-(Sin)glc-7-glc, Pt 3,5-diglc, Cy 3-(Sin)diglc-5-glc, Cy 3-(Mal)glc-5-(pHy)rut-7-(Mal)glc, Cy 3-(Fer)diglc-5-glc, Cy 3-(Mal)glc-5-(pHy)rut-7-(Ace)glc, Cy 3-(Sin-Fer)diglc-5-(Mal)glc, Cy 3-(pCoun)diglc-5-glc, Cy 3-(pCoun)diglc-5-(Mal)glc, Cy 3-(Fer)diglc-5-(Mal)glc, Cy 3-(Sin-Fer)diglc-5-glc, Cy 3-(Sin-pCoun)diglc-5-glc, Cy 3-(Sin-Fer)diglc-5-diglc, Cy 3-(Sin-Fer)diglc-5-(Mal)glc, Cy 3-(Sin-pCoun)diglc-5-(Mal)glc, Cy 3-(Mal)glc-5-(Caf-Fer)rut, Pt 3-(Mal)glc	Dp 3-glu, Dp 3-diglc-5-glc, Dp 3,5-diglc, Dp 3,5,7-tiglc, Pt 3,5-diglc, Pt 3-glc Pn 3-diglc-5-glc, Cy 3-diglc-5-glc, Cy 3-glc, Cy 3,5-diglc, Cy 3,5,7-tiglc, Cy 3-glc-5-rut-7-diglc, Cy 3,5-diglc, Cy 3-glc-5-rut	Sin, pCoun, Fer, Mal, Caf, pHy, Oxa	He, Zhang, and Zhang (2016)
<i>Raphanus sativus</i> L. var. <i>raphanistroides</i> Makino	Radish flowers	Cy 3-(Caf)(pCoun)diglc-5-(Mal)glc, Cy 3-(Caf)(Fer)diglc-5-(Mal)glc, Cy 3-(pCoun)diglc-5-(Mal)glc, Cy 3-(Caf)diglc-5-(Mal)glc, Cy 3-(pCoun)diglc-5-(Mal)glc, Cy 3-(Fer)diglc-5-(Mal)glc	Cy 3-diglc-5-glc	Sin, pCoun, Fer, Mal, Caf	Tatsuzawa (2016)
<i>Raphanus sativus</i> var. <i>niger</i>	Red radish	Cy 3-(Caf)(Fer)diglc-5-glc, Pg 3-(Caf)(Caf)diglc-5-glc, Pg 3-(Caf)(Fer)diglc-5-glc, Pg 3-(pCoun)diglc-5-glc, Pg 3-(pCoun)(Caf)diglc-5-glc, Pg 3-(Fer)diglc-5-glc, Pg 3-(Fer)(Caf)diglc-5-glc, Pg 3-(pCoun)(Fer)diglc-5-glc, Pg 3-(Fer)(Fer)diglc-5-glc, Pg 3-diglc-5-glc, Pg 3-diglc-5-(Mal)glc, Cy 3-(Glu)rha, Pg 3-(Caf)diglc-5-glc, Pg 3-(Caf)diglc-5-(Mal)glc, Pg 3-(pCoun)glc-5-glc, Pg 3-(pCoun)(Caf)diglc-5-(Mal)glc, Pg 3-(pCoun)diglc-5-(Mal)glc, Pg 3-(Fer)diglc-5-(Mal)glc, Pg 3-(Fer)(Caf)diglc-5-(Mal)glc, Pg 3-(pCoun)(Fer)diglc-5-(Mal)glc	Pg 3-diglc-5-glc, Cy 3-diglc-5-glc, Cy 3-rha, Pg 3,5-diglc	Mal, pCoun, Fer, Caf	Park et al. (2011); Wang et al. (2010)
<i>Raphanus sativus</i> cv Sango	Radish sprout	Cy 3-(Sin)(Fer)diglc-5-glc, Cy 3-(Fer)(Sin)diglc-5-(Mal)glc, Cy 3-(Fer)(Fer)diglc-5-(Mal)glc, Cy 3-(Fer)(Fer)diglc-5-(Mal)glc, Cy 3-(Sin)diglc-5-(Mal)glc, Cy 3-(Sin)(Sin)diglc-5-glc, Cy 3-(Fer)diglc-5-(Mal)glc, Cy 3-(Fer)(Fer)diglc-5-(Mal)glc	Cy 3-diglc-5-glc, Cy 3-diglc-5-diglc	Fer, Sin, Mal	Matera et al. (2015)
<i>Arabidopsis thaliana</i>	n.s.	Cy 3-(Xyl)glc-5-glc, Cy 3-(Xyl)(pCoun)glc-5-glc, Cy 3-(Xyl)(pCoun)glc-5-(Mal)glc, Cy 3-(Xyl)(pCoun)diglc-5-glc, Cy 3-(Sin-Xyl)(pCoun)glc-5-(Mal)glc, Cy 3-(Sin-Xyl)(pCoun)diglc-5-(Mal)glc, Cy 3-(Sin-Xyl)(pCoun)diglc-5-glc, Cy 3-(Xyl)(pCoun)glc-5-(Mal)glc	Cy 3-diglc-5-glc, Cy 3,5-diglc	Mal, pCoun, Sin, Xyl	Bloor and Abrahams (2002); Nakabayashi et al. (2009)

(continued)

Table 2. Continued.

Scientific name	Common name	Anthocyanin composition	Core	Acyl group	Reference
<i>Heliophila coronopifolia</i> L.	n.s.	Dp 3-(Xyl)(pCoum)glc-5-(Mal)glc, Dp 3-(Xyl)(Caf)glc-5-(Mal)glc, Dp 3-(Xyl)(pCoum)glc-5-glc, Dp 3-(Xyl)(Fer)glc-5-glc, Dp 3-(Xyl)(pCoum)glc-5-(Mal)-glc, Dp 3-(Xyl)(Fer)glc-5-(Mal)glc, Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Xyl)(Fer)glc-5-(Mal)glc	Cy 3,5-diglc	Mal, pCoum, Fer, Caf, Xyl	Saito et al. (2011)
<i>Moricandia arvensis</i> (L.) DC.	n.s.	Cy 3-(Glu-Caf-Glu-Caf)(Caf)diglc-5-(Mal)glc, Cy 3-(Glu-Caf-Glu-Caf)(Sin)diglc-5-(Mal)glc	Cy 3-diglc-5-glc	Clu, Caf, Mal, Sin	Tatsuzawa et al. (2013); Tatsuzawa et al. (2015)
<i>Moricandia ramburii</i> Webb.	n.s.	Pn 3-(Fer)(pCoum)diglc-5-(Mal)glc, Pn 3-(Fer)(pCoum)diglc-5-(Mal)glc, Pn 3-(Sin)(pCoum)diglc-5-(Mal)glc, Cy 3-(Fer)(pCoum)diglc-5-(Mal)glc	Pn 3-diglc-5-glc, Cy 3-diglc-5-glc	Fer, Sin, Mal, pCoum	Tatsuzawa et al. (2012)
<i>Malcolmia maritima</i> (L.) R. Br.	n.s.	Cy 3-[(Sin)(Glu-Xyl)](pCoum)glc-5-(Mal)glc, Cy 3-[(Sin)(Glu-Xyl)](pCoum)glc-5-(Mal)glc, Cy 3-[(Sin)(Glu-Xyl)](pCoum)glc-5-glc, Cy 3-(Glu-Xyl)glc-5-glc	Cy 3,5-diglc	Sin, Glu, Xyl, pCoum, Mal	Tatsuzawa et al. (2008)
<i>Matthiola incana</i>	n.s.	Cy 3-(Fer)(Sin-Xyl)glc-5-(Mal)glc, Cy 3-(pCoum)(Sin-Xyl)glc-5-(Mal)glc, Cy 3-(Caf)(Sin-Xyl)glc-5-(Mal)glc, Cy 3-(Fer)(Sin-Xyl)glc-5-glc	Cy 3,5-diglc	Sin, Fer, Xyl, pCoum, Mal	Saito et al. (1995)
<i>Matthiola longipetala</i> subsp. <i>bicornis</i> (Sm) P. W. Ball.	n.s.	Cy 3-(Fer-Xyl)(Fer)glc-5-(Mal)glc, Cy 3-(Sin-Xyl)(Fer)glc-5-(Mal)glc, Cy 3-(Xyl)(Fer)glc-5-(Mal)glc	Cy 3,5-diglc	Fer, Xyl, Mal, Sin	Tatsuzawa et al. (2014)
<i>Ionopodium acaule</i> (Desf.) Rchb.	n.s.	Cy 3-(Xyl)(Fer-Glu-pCoum)glc-5-(Mal)glc, Cy 3-(Fer-Xyl)(Fer-Glu-pCoum)glc-5-(Mal)glc, Cy 3-[(Fer)(Glu-Xyl)](Fer-Glu-pCoum)glc-5-(Mal)glc	Cy 3,5-diglc	Glu, Fer, Xyl, pCoum, Mal	Tatsuzawa et al. (2014)
<i>Cheiranthus cheiri</i> L.	n.s.	Cy 3-(Xyl)(pCoum)glc-5-glc	Cy 3,5-diglc	Xyl, pCoum	Tatsuzawa et al. (2006)
<i>Lunaria annua</i> L.	n.s.	Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Xyl)(Fer)glc-5-(Mal)glc	Cy 3,5-diglc	Fer, Xyl, pCoum, Mal	Tatsuzawa et al. (2006)
<i>Lobularia maritima</i> (L.) Desv.	n.s.	Cy 3-(Xyl)(pCoum)diglc-5-glc, Cy 3-(Caf-Xyl)(pCoum)diglc-5-glc, Cy 3-(Caf-Xyl)(pCoum)glc-5-glc	Cy 3,5-diglc, Cy 3-diglc-5-glc	Caf, Fer, Xyl, pCoum	Tatsuzawa et al. (2006)
<i>Arabis blepharophylla</i> Hook. & Arn.	n.s.	Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Xyl)(Sin)glc-5-(Mal)glc, Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Xyl)(pCoum)glc-5-glc, Cy 3-(Xyl)(Sin)glc-5-glc	Cy 3,5-diglc	Sin, Xyl, pCoum, Mal	Ito, Kato, and Tatsuzawa (2013)
<i>Hesperis matronalis</i> L.	n.s.	Cy 3-(Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Sin-Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Caf-Xyl)(pCoum)glc-5-(Mal)glc, Cy 3-(Fer-Xyl)(pCoum)glc-5-(Mal)glc	Cy 3,5-diglc	Sin, Caf, Xyl, pCoum, Mal, Fer	Tatsuzawa et al. (2012)
<i>Iberis umbellata</i> L.	n.s.	Cy 3-(pCoum)diglc-5-(Mal)glc, Cy 3-(Fer)(pCoum)diglc-5-(Mal)glc, Cy 3-(Sin)(pCoum)diglc-5-(Mal)glc, Cy 3-(Glu)(pCoum)diglc-5-(Mal)glc, Cy 3-(Fer)(Glu-pCoum)diglc-5-(Mal)glc, Cy 3-(Sin)(Glu-pCoum)diglc-5-(Mal)glc, Cy 3-(Fer-Glu-pCoum)diglc-5-(Mal)glc, Cy 3-(Sin)(Fer-Glu-pCoum)diglc-5-(Mal)glc, Cy 3-(Glu-Fer-Glu-pCoum)diglc-5-(Mal)glc, Cy 3-(Fer)(Glu-Fer-Glu-pCoum)diglc-5-(Mal)glc, Pg 3-(pCoum)diglc-5-(Mal)glc, Pg 3-(Sin)(Glu-pCoum)diglc-5-(Mal)glc, Pg 3-(Fer)(Glu-pCoum)diglc-5-(Mal)glc, Pg 3-(Sin)(Fer-Glu-pCoum)diglc-5-(Mal)glc, Pg 3-(Fer)(Fer-Glu-pCoum)diglc-5-(Mal)glc, Pg 3-(Fer)(Glu-Fer-Glu-pCoum)diglc-5-(Mal)glc	Cy 3-diglc-5-glc, Pg 3-diglc-5-glc	Glu, Fer, Sin, pCoum, Mal	Saito et al. (2008); Tatsuzawa (2019)
<i>Orychophragorus violaceus</i>	Violaceus	Cy 3-(Glu-Caf-Glu-Caf-Xyl)(Glu-pCoum)glc-5-glc, Cy 3-(Glu-Caf-Glu-Caf-Xyl)(Glu-Fer)glc-5-glc, Cy 3-(Glu-Caf-glc-Caf-Xyl)(Glu-pCoum)glc-5-(Mal)glc	Cy 3,5-diglc	Glu, Fer, Xyl, pCoum, Mal, Caf	Honda et al. (2005)
<i>Erysimum × cheiri</i> (L.) Crantz "Aurora"	n.s.	Pg 3-(Xyl)(pCoum)glc-5-glc, Pg 3-(Xyl)(Fer)glc-5-glc, Pg 3-(pCoum)(Glu-Xyl)(pCoum)glc-5-glc, Pg 3-(pCoum-Xyl)(pCoum)glc-5-glc	Cy 3,5-diglc	Glu, Fer, Xyl, pCoum	Tatsuzawa (2019)

Cy, cyanidin; Pg, pelargonidin; Pn, peonidin; Dp, delphinidin; Pt, petunidin; glc, glucoside; diglc, diglucoside; triglc, triglucoside; rut, rutinoside; ara, arabinoside; rha, rhamnoside; xyl, xyloside; Ace, acetyl; Caf, caffeoyl; Fer, feruloyl; Mal, malonyl; n.s., none specified; Oxa, oxalic acid acyl; p-Coum, p-coumaroyl; pHy, p-hydroxybenzoyl; Sin, sinapoyl; Xyl, xyloyl; Glu, glucosyl; Suc, succinoyl.

The major anthocyanins identified in broccoli sprouts and red cabbage were cyanidin 3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside and cyanidin 3-(sinapyl)diglucoside-5-glucoside, respectively (Moreno et al. 2010; Wiczowski, Szawara-Nowak, and Topolska 2013). Cyanidin 3-triglucoside-5-glucoside was well represented in kohlrabi, while it was absent in red kale (Zhang et al. 2015). The *p*-coumaroyl and feruloyl esterified forms of cyanidin 3-diglucoside-5-glucoside were predominant in mustard, and the sinapoyl ester was predominant in bok choy (Xu et al. 2019).

The anthocyanins in genus *Raphanus* has characteristic of acylated cyanidin and pelargonidin. The major anthocyanins are anthocyanidin 3-diglucoside-5-glucoside acylated with malonic, caffeic, ferulic, and/or *p*-coumaric acids to form anthocyanidin 3-(acyl)diglucoside-5-glucoside, anthocyanidin 3-(acyl1)(acyl2)diglucoside-5-glucoside, and anthocyanidin 3-(acyl1)(acyl2)diglucoside-5-(malonyl)glucoside (Park et al. 2011; Wang et al. 2010). Red radish contributed to the highest level of pelargonidin in common foods (Wu et al. 2006).

A range of different anthocyanins have been identified in the flowers of Brassicaceae. Saito et al. (2011) identified six acylated delphinidin glycosides, one acylated kaempferol glycoside, and two acylated cyanidin glycosides in the blue

flowers of cape stock (*Heliophila coronopifolia*). Typical anthocyanins in purple-violet flowers of *Matthiola longipetala* subsp. *bicornis* (Sm) P. W. Ball. was cyanidin 3-(feruloyl-xylopyranosyl)(feruloyl)glucoside-5-(malonyl)glucoside, cyanidin 3-(sinapoyl-xylopyranosyl)(feruloyl)glucoside-5-(malonyl)glucoside, cyanidin 3-(xylopyranosyl)(feruloyl)glucoside-5-(malonyl)glucoside.

Overall, acylated cyanidin 3,5-diglucoside and cyanidin 3-diglucoside-5-glucoside tend to be the major anthocyanins (Figure 1), though the anthocyanin composition (e.g., the amount of acylated anthocyanins) greatly varied among different species. The acyl groups found attached to the anthocyanins of Brassicaceae are aromatic acid (*p*-coumaric, caffeic, ferulic, sinapic, and *p*-hydroxybenzoic acid), aliphatic acid (acetic, oxalic, succinic, and malonic acid), glucoside, and xylose (Figure 1).

Stability of Brassicaceae anthocyanins

Anthocyanins are relatively unstable compounds. The stability of anthocyanins was affected by itself structure, pH, light, temperature, the presence of copigments, metal ions, ascorbic acid, and antioxidant (Patras et al. 2010). The

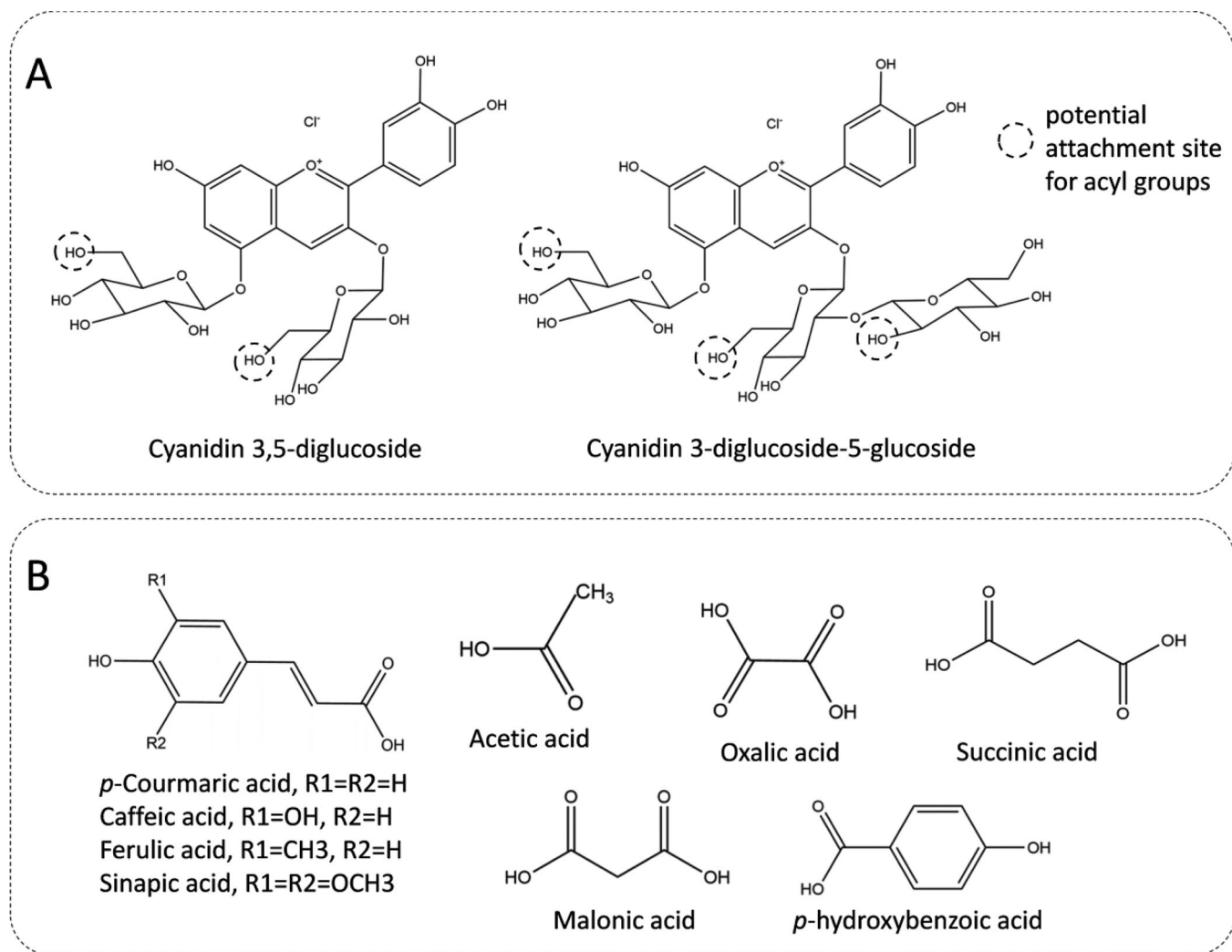


Figure 1. Chemical structures of major anthocyanins core of Brassicaceae (A) and common organic acids that acylated the sugar moiety of anthocyanins (B).

relationship between molecular structure (e.g., methylation, hydroxylation, glycosylation, and acylation) and stability has been widely concerned. For instance, increased methylation or decreased hydroxylation enhanced the stability of anthocyanins (Hrazdina, Borzell, and Robinson 1970). Recent researches have shown that Brassicaceae anthocyanins with complex patterns of acylation exhibit remarkable stability to thermal treatment, light exposure, pH change, and storage.

Temperature is recognized to have a crucial impact on anthocyanins integrity. Although magnitude and duration of heating has a strong influence on anthocyanin stability, Jing et al. (2012) observed that the half-lives of red radish anthocyanins at 90 and 100 °C were 14.5 and 8.7 h in an acidic solution (pH 2.5), respectively, which were much longer than that of purple corn (2.0 or 1.8 h), purple carrot (4.6 or 3.0 h), and red sweet potato (4.6 or 2.8 h) at 98 °C in an acidic (pH 1 or 3) media, respectively. Similarly, Dyrby, Westergaard, and Stapelfeldt (2001) reported that anthocyanins in red cabbage were more stable than those in blackcurrant, grape skin, and elderberry during heating at 25–80 °C. Thermal degradation of anthocyanins was also observed during processing. The total anthocyanins content of violet cauliflower showed a significant loss of 80.2% by cooking process and a marginally loss of 4.6% by microwave treatment, comparing

with that of raw sample (Scalzo et al. 2008). This study also noted that the recovery rate were 9.97% to 35.41% after blanching, which were different for three cultivars.

Additionally, several other factors were believed to affect the stability of anthocyanins, including light, pH, and metal ions. Prietto et al. (2017) developed pH-sensitive film based on corn starch plasticized with anthocyanin extract. They reported that the film with red cabbage anthocyanins exhibited a higher stability than that with black bean anthocyanins when exposed to light. The photostability also depended on the pH of environment. Matsufuji et al. (2007) found that the retention of red radish anthocyanins was more than 60% at pH 3 and 5 after 20 days light exposure, while anthocyanin content reduced to 40% within 1 day at pH 7. Studies have shown degradation of anthocyanins during storage. For example, the anthocyanins half-life were 22 and 10 weeks for red radish and red-fleshed potatoes during storage at room temperature (25 °C), respectively, suggesting that red radish anthocyanins were resistant to storage (Rodriguez-Saona, Giusti, and Wrolstad 1999). Another study similarly found that twelve months freezer storage did not significantly affects the content of anthocyanins in purple cauliflower (Volden, Bengtsson, et al. 2009). It has been known that metal ions may participate in stabilizing co-

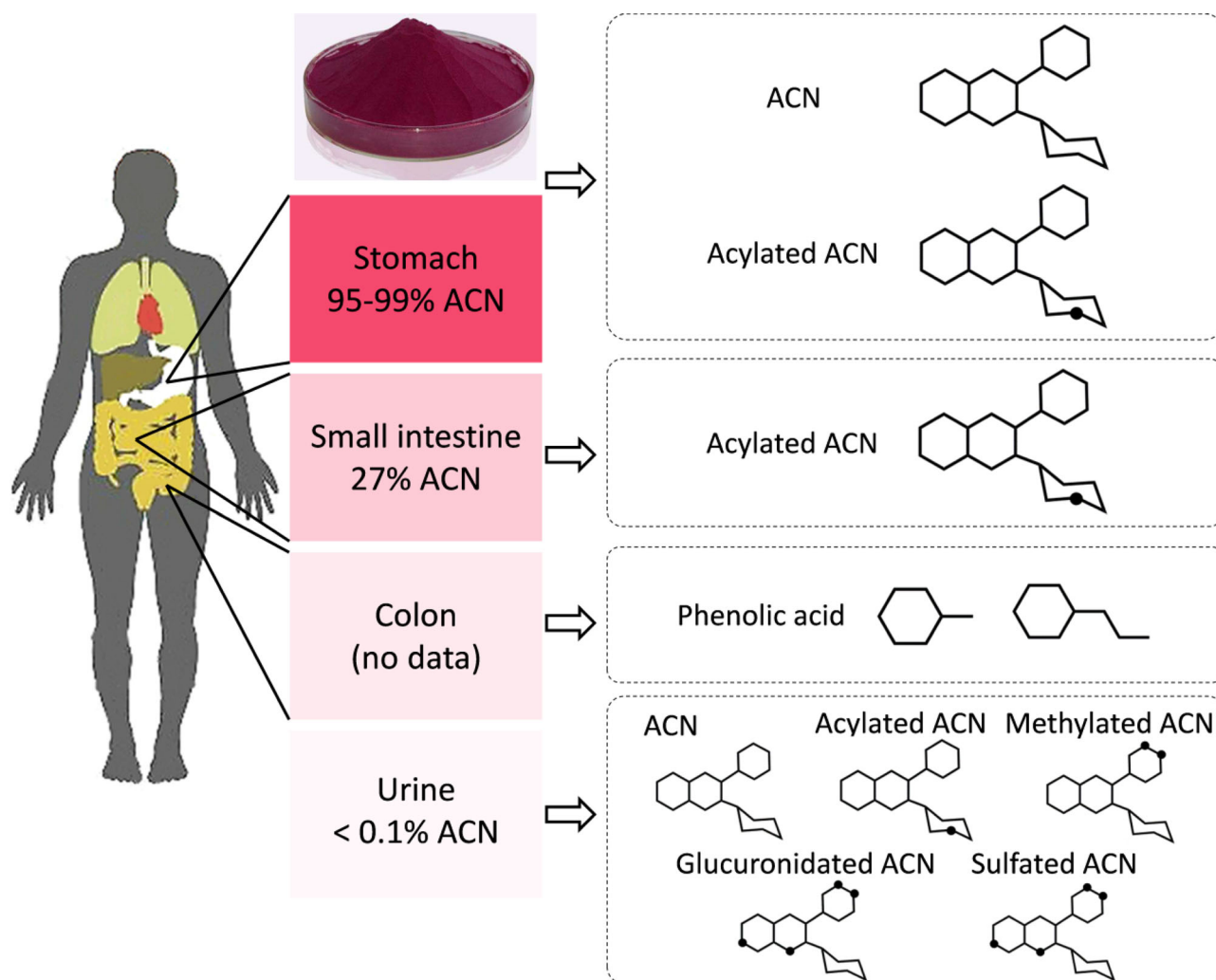


Figure 2. Schematic representation of the metabolism of Brassicaceae anthocyanins. ACN: anthocyanin-glucoside.

pigmentation complexes (Trouillas et al. 2016). In contrast, Ratanapoompinyo et al. (2017) found that the addition of metal ions (Al^{3+} , Ca^{2+} , Fe^{3+} , or Sn^{2+} with a molar ratio at 1:1) accelerated the degradation of red cabbage anthocyanins under thermal treatment, spring drying, and storage.

The stability of anthocyanins is also affected by the number and type of acyl group. Numerous studies have indicated that diacylated anthocyanins were more stable than monoacylated anthocyanins (Matsufuji et al. 2007). Additionally, the type of acyl group would also appear to play a role in the stability of anthocyanins. Wiczowski, Szawara-Nowak, and Topolska (2015) reported that anthocyanins acylated with sinapic acid exhibited the highest loss among seven anthocyanins compounds in red cabbage under stewed. Similar trends were reported for anthocyanins under pancreatic digestion (McDougall et al. 2007). Matsufuji et al. (2007) demonstrated that anthocyanins acylated with *p*-coumaric or ferulic acids were more stable than with caffeic acids at pH 7. Cyanidin 3-diglucoside-5-glucoside acylated with two sinapic acids were less stable than acylated with two ferulic acid (Wiczowski, Szawara-Nowak, and Topolska 2015). Interestingly, the antioxidant activity of hydroxycinnamic acids increased in the

sequence *p*-coumaric < ferulic < caffeic < sinapic acid (Natella et al. 1999), suggesting low comparative stability might due to higher antioxidant activity (McDougall et al. 2005; McDougall et al. 2007).

The enhancement of the stability of Brassicaceae anthocyanins might due to the intermolecular copigmentation (Malien-Aubert, Dangles, and Amiot 2001; Trouillas et al. 2016). Anthocyanins exist four molecular species in aqueous in equilibrium: flavylium cation, quinonoidal base, carbinol pseudobase, and chalcone pseudobase forms. Formation of chalcone might be the first degradation step of anthocyanins (Markaris, Livingston, and Fellers 1957). The anthocyanins glycosyls acylated by hydroxycinnamic acids, especially those attached to the C3 site, are flexible and can rotate freely (Zhao et al. 2017). Therefore, planar aromatic acyl groups are thought to form π - π interactions with benzopyrylium ring due to hydrophobic forces in aqueous (Rodriguez-Saona, Giusti, and Wrolstad 1999). Aromatic acyl group of diacylated anthocyanins can stack on both side of pyrylium nucleus by π - π interactions, forming sandwich-type complex to preclude the nucleophilic attack of water, while only one side of the pyrylium ring can be protected by one acyl group

in the monoacylated anthocyanins (Figueiredo et al. 1999; Zhao et al. 2017). The inter-copigmentation inhibits the chalcone formation and prevents the C ring cleavage.

Bioaccessibility, bioavailability, and metabolism of Brassicaceae anthocyanins

Anthocyanins are considered to be poorly bioavailable (Fernandes et al. 2014; Tian et al. 2019). One of the limitations of bioavailability might be chemical degradation in the gastrointestinal tract. Notably, acylated anthocyanins were more stable than non-acylated anthocyanins as discussed above, suggesting a high bioaccessibility. McDougall et al. (2007) reported that red cabbage anthocyanins were stable in the acidic gastric condition, while the recovery after pancreatic digestion was ~27% of anthocyanins-rich extract (Figure 2). Similarly, Podsędek et al. (2014) demonstrated that the recovery rates of anthocyanins-rich extract after gastric and intestinal digestion were 99.9% and 13.2%, respectively. In large intestine, gastrointestinal digests incubated with human fecal microflora caused a further decline in anthocyanins content (Fleschhut et al. 2006; Podsędek et al. 2014). Anthocyanins absorption is also affected by food matrix (Yang et al. 2011). For example, the recovery of red cabbage anthocyanin-rich extract after pepsin and pancreatin-bile digestion was 13.23%, while those of raw red cabbage were 67.71% (Podsędek et al. 2014).

The bioavailability is the proportion of nutrient reaching systemic circulation, which is a major issue regarding anthocyanins biological activity. Two previous studies examined the bioavailability of red cabbage anthocyanins, which found 14 and 30 cyanidin derivatives in the plasma and urine of volunteers (Charron et al. 2007; Wiczowski, Szawara-Nowak, and Romaszko 2016). Urinary recoveries of nonacylated, monoacylated and diacylated anthocyanins from red cabbage were 0.28%, 0.07% and 0.03%, respectively, suggesting that the recovery of acylated anthocyanins was lower than that of nonacylated anthocyanins (Wiczowski, Szawara-Nowak, and Romaszko 2016). Similarly, Charron et al. (2007) reported that the recovery of nonacylated anthocyanins was 4-fold higher than that of acylated anthocyanins and no differences in total recovery of monoacylated and diacylated anthocyanins. However, individual cyanidin-3-diglucoside-5-glucoside acylated with *p*-coumaric, ferulic, or sinapic acid was detected in urine, while the counterpart additionally acylated with sinapic acid was not (Charron et al. 2007). It is plausible that the hydrophobicity of acyl group could be significant in affecting bioavailability. Apart from the native anthocyanins, glucuronidated, sulfated, and methylated derivatives were also detected in plasma and urine (Figure 2).

Large amounts of unabsorbed anthocyanins reached the large intestine where they were extensively metabolized by the intestine microbiota (Faria et al. 2014). Bacterial metabolism involved the cleavage of glycosidic linkages and the breakdown of heterocyclic C-ring. Protocatechuic acid was recognized as the major metabolite of cyanidin 3-O-glucoside (Aura et al. 2005). Fleschhut et al. (2006) have reported the catabolism of red radish anthocyanins. The acyl group

could be cleaved by human fecal microflora and main intermediate degradation products were pelargonidin 3-sophoroside-5-glucoside and pelargonidin 3-sophoroside. After cleavage of the sugar moiety, the aglycones could be further metabolized by the bacteria or spontaneous cleavage to 4-hydroxybenzoic, *p*-coumaric, ferulic, and caffeic acid, which can be absorbed into the circulatory system (Figure 2) (Fleschhut et al. 2006). Brassicaceae anthocyanins were metabolized forming simple anthocyanins, which was much more bioavailable. Additionally, the degradation products can also be absorbed, and when these events are taken into account, the Brassicaceae anthocyanins might be more bioavailable than previously perceived (Kay et al. 2017; Tian et al. 2019).

Health benefits of Brassicaceae anthocyanins and mechanisms of action

Anthocyanins might possess wide range of biological activities, including antioxidant property, anti-tumor activity, cardio- and hepatoprotection, glucose control in diabetics, immunomodulation, and effects on aging (Al-Dosari 2014; Cruz et al. 2016; Lee et al. 2002; Rojo et al. 2012; Shiyani, Herlina, and Rizkika Sari 2018; Taverniti et al. 2014; Yousuf et al. 2016). Consumption of anthocyanin-containing foods may also be beneficial to human health. Epidemiologic evidence indicated that high dietary intakes of anthocyanins-rich foods may lower the risk of cardiovascular diseases, blood pressure, and type 2 diabetes mellitus (Cassidy et al. 2016; Guo et al. 2016; Zhu et al. 2017). The estimated daily intake of anthocyanins was 12.5 mg/day/person in the United States, while Brassicaceae anthocyanins contributed 7.68% of daily intake (Wu et al. 2006). Many in vivo and in vitro studies related to the biological activities of Brassicaceae anthocyanins have been done.

Relief of oxidative stress

Oxidative stress is an imbalance of antioxidants and free radicals, which causes various chronic diseases (Lee et al. 2011). Various chemical methods have been used to estimate the antioxidant activity of Brassicaceae anthocyanins, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), and trolox equivalent antioxidant capacity (TEAC). In numerous studies, Brassicaceae anthocyanins have been shown to possess high antioxidant capability (Jing et al. 2014; Matsufuji et al. 2007; Matsufuji et al. 2003; Pliszka et al. 2009; Volden, Borge, et al. 2009; Wang et al. 2010; Wiczowski, Topolska, and Honke 2014). Antioxidant activity is supposed to positively correlated with the anthocyanins content and also strongly varied with cultivars (Wiczowski, Topolska, and Honke 2014). Anthocyanins extracted from “Langedijker Polona” exhibited the highest ORAC value among red cabbage cultivars “Langedijker Dauer 2,” “Kissendrup,” “Koda,” and “Kalibos” (Wiczowski, Topolska, and Honke 2014).

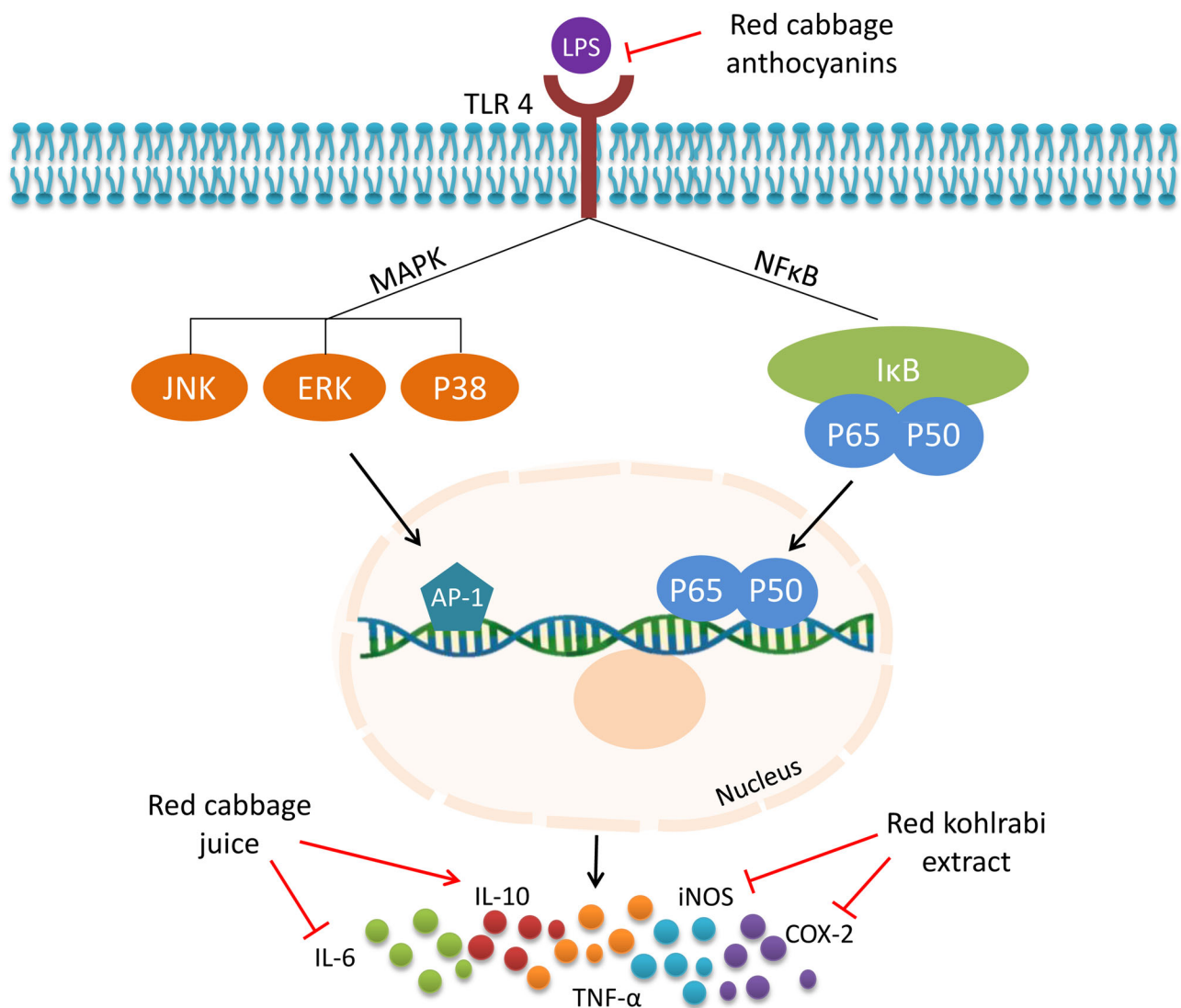


Figure 3. Schematic model showing the mechanisms of Brassicaceae anthocyanins in inflammatory signaling pathway. Red cabbage anthocyanins are a potent competitive inhibitor of LPS at Toll-like receptors (TLR) 4. Red cabbage juice attenuated lipopolysaccharide (LPS)-induced inflammatory response through inhibition of interleukin (IL)-6 and promotion of IL-10. Red kohlrabi extract inhibit NO production by suppressing inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 protein production in LPS-induced RAW 264.7 cells.

The relationship between structure of anthocyanins and antioxidant capability has been studied. A study comparing the antioxidant capability of anthocyanins in red cabbage found that the scavenging activity against 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$) was in the order: cyanidin 3-(sinapoyl)(sinapoyl)diglucoside-5-glucoside > cyanidin 3-(feruloyl)(sinapoyl)diglucoside-5-glucoside > cyanidin 3-(feruloyl)(feruloyl)diglucoside-5-glucoside > cyanidin 3-(feruloyl)diglucoside-5-glucoside > cyanidin 3-(*p*-coumaroyl)diglucoside-5-glucoside > cyanidin 3-diglucoside-5-glucoside, suggesting that the antioxidant activity of anthocyanins was increased by acylation (Wiczowski, Szawara-Nowak, and Topolska 2013). Similarly, Matsufuji et al. (2007) found that acyl units in promoting the DPPH radical scavenging activity was in the order of caffeic acid > ferulic acid > *p*-coumaric acid. This study also noted that the binding site of acyl highly affects antioxidant activity, which is confirmed by a prior study performed by Matsufuji et al. (2003).

The protective effects of Brassicaceae anthocyanins on oxidative damage were promising as shown in *in vivo* models. In a rat study utilizing paraquat-induced oxidative stress model, red cabbage anthocyanins attenuated the increase of catalase and NADPH-cytochrome-P450 reductase activity in liver mitochondrial and microsome, respectively (Igarashi, Kimura, and Takenaka 2000). Kolodziejczyk et al. (2011) reported that red cabbage anthocyanins significantly inhibited blood plasma lipid peroxidation caused by peroxynitrite and hydrogen peroxide. Additionally, red cabbage extracts significantly prevented the decrease of glutathione in brain of mice administered with N-methyl-D-aspartate (NMDA), suggesting a prevention of oxidative imbalance in brain (Lee et al. 2002).

Apart from the direct antioxidant effects, red cabbage anthocyanins extract also protected platelet against lipopolysaccharide (LPS)-induced oxidative damage and competition of anthocyanin with LPS for the binding site in Toll-like receptors (TLR) 4 might be the potential mechanism against the oxidative stress (Figure 3) (Saluk et al. 2015).

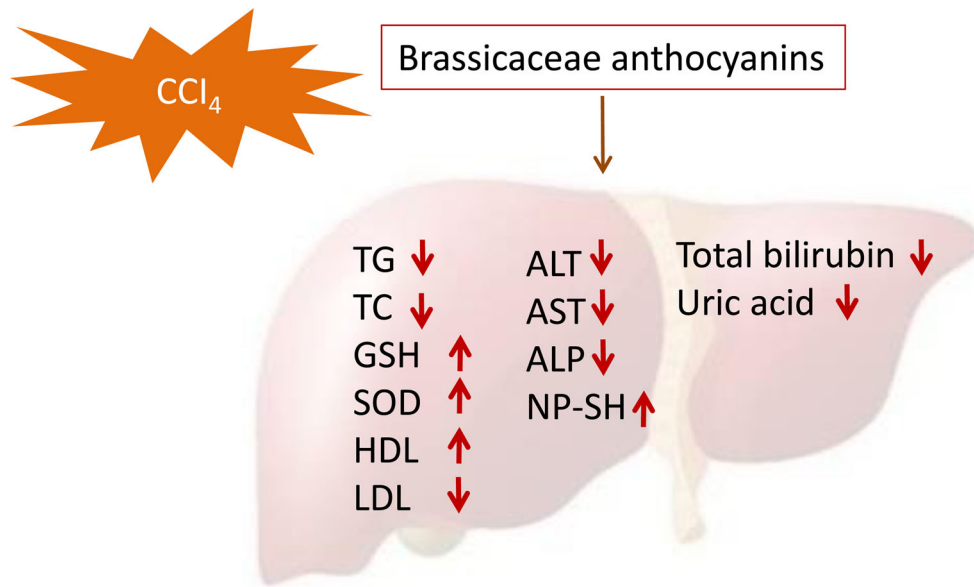


Figure 4. Schematic model showing the role of Brassicaceae anthocyanins in hepatoprotective activity. Brassicaceae anthocyanins decrease the level of total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), low-density lipoprotein (LDL), total bilirubin, and uric acid and increase the level of reduced glutathione (GSH), superoxide dismutase (SOD), high-density lipoprotein (HDL), and nonprotein sulfhydryl (NP-SH).

Prevention of hepatic and renal damage

Long-term oxidative stress leads to an imbalance of organism homeostasis and consequently results in various diseases such as hepatic damage (Figure 4). Aqueous extract of red cabbage has been demonstrated to have inhibitory activity against cholesterol-rich diet induced liver impairment, and also exhibited cytoprotective effect in HepG2 cells (Al-Dosari 2014). Similarly, Sankhari et al. (2012) reported that anthocyanin-rich red cabbage extract ameliorated atherogenic diet-induced hepatic damage by inhibition of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL) and promotion of high-density lipoprotein (HDL), superoxide dismutase (SOD), and reduced glutathione (GSH). In a rat study utilizing N-nitroso-diethylamine (NDEA)-induced hepatocellular carcinoma model, red cabbage extract attenuated changes of biomarkers in serum such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, uric acid, and GSH, therefore reducing the incidence of hepatocellular carcinoma (Morsy 2010). Moreover, pretreatment of turnip juice protected the rat against carbon tetrachloride (CCl_4)-induced hepatotoxicity and alleviated the decrease in nonprotein sulfhydryl (NP-SH), suggesting a hepatoprotective action (Rafatullah et al. 2006). Similarly, anthocyanins fraction of red radish has been shown to reversed the alteration of ALT, AST, ALP, GSH, total bilirubin, and antioxidant enzymes activity induced by CCl_4 in rat and was as effective as hepatoprotective drug silymarin (Dash, Habibuddin, and Baruah 2013).

Direct and indirect research evidences demonstrated the benefits of anthocyanins in kidneys protection. Red cabbage extract attenuated the renal enlargement, renal dysfunction, and oxidative stress of the kidneys in streptozotocin-induced diabetes rat (Kataya and Hamza 2008). Shiyan, Herlina, and Rizkika Sari (2018) also reported that red cabbage

anthocyanins extract repaired the kidney function in a gentamicin-captopril-induced rat model.

Prevention of cardiovascular disease

Dietary anthocyanins provide protection against LDL oxidation and cardiovascular disease (Wallace 2011). In a Triton WR-1339-induced hyperlipidemic rat model, aqueous extract of red cabbage ameliorated the levels of LDL, very low-density lipoprotein (VLDL), cholesterol, and triglycerides alterations in rats (Cruz et al. 2016). Additionally, pigment from red radish decreased the blood pressure and the heart rate of spontaneously hypertensive rats, suggesting an anti-hypertensive effects (Shindo et al. 2007). Brassicaceae anthocyanins may also play a key role in cholesterol metabolism. Studies have demonstrated that the administration of red turnip anthocyanins and red cabbage extract significantly lowered the triacylglycerol and cholesterol levels in rat treated with cholesterol-rich diet (Al-Dosari 2014; Igarashi, Abe, and Satoh 1990).

Apart from the direct cardiovascular disease prevention, the anthocyanins from Brassicaceae have been shown to inhibit platelet activation (Saluk et al. 2012). Platelets are sensitive blood cells and the hyperactivation of platelets leads to overproduction of reactive oxygen species (ROS), which considered to be one of the risk factors of cardiovascular disease (Lee et al. 2012).

Anti-inflammatory activity

Inflammation is a complex biological response involved in multiple signal pathways including nuclear transcription factor kappa-B (NF- κ B) and mitogen-activated protein kinases (MAPKs). Activated transcription factor of NF- κ B and activator protein-1 (AP-1) can translocate into the nucleus and induce the transcription of specific genes, producing pro-

inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α , as well as inflammatory mediators like inducible NO synthase (iNOS) and cyclooxygenase (COX)-2 (Figure 3). Red cabbage juice has been found to inhibit pro-inflammatory cytokine IL-6 and promote anti-inflammatory cytokine IL-10 secretions in LPS-stimulated murine splenocyte cultures (Lin, Li, and Hwang 2008). The study also noted that malvidin 3-glucoside, malvidin 5-glucoside, and malvidin 3,5-diglucoside could be responsible for inhibition of the IL-6 secretion. Additionally, recent study has shown that methanol extracts of red kohlrabi inhibit NO production by suppressing iNOS and COX-2 protein production in LPS-induced RAW 264.7 cells (Jung et al. 2014). Red cabbage extracts also attenuated inflammation in the mouse model of acute and chronic Crohn's disease (Zielińska et al. 2015).

Cancer chemoprotective properties

Anticancer activity of anthocyanins has been verified by in vitro and in vivo evidence. Red radish extract, rich in acylated pelargonidin derivatives, significantly inhibited hepatocellular carcinoma Bel-7402 cells at lower concentration (Wang et al. 2010). Jing et al. (2008) demonstrated that radish extract exhibited lowest growth inhibition of human colon cancer HT29 cell among purple corn, chokeberry, bilberry, purple carrot, grapes, and elderberry extract, suggesting that non-acylated had greater anticancer activity than acylated anthocyanins. Additionally, anthocyanins from red cabbage significantly reduced colon nodules by 63% in rat treated with 1,2-dimethylhydrazine and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, but the mechanisms were not investigated (Hagiwara et al. 2002).

Other health benefits

Many other health benefits were associated with the consumption of Brassicaceae anthocyanins-rich extract. For instance, pretreatment with the phenolic of red cabbages significantly inhibited amyloid β peptide (A β)-induced cytotoxicity in PC12 cell (Heo and Lee 2006). Posmyk, Janas, and Kontek (2009) revealed the role of red cabbage anthocyanins extract in the protection of lymphocytes against heavy metal toxicity (Cu²⁺). Red cabbage anthocyanins have been shown to prevent irinotecan-induced mucositis by inhibiting intestinal injury and leukopenia (Tong et al. 2017). This study also noted that pretreatment with red cabbage anthocyanins enhanced the transepithelial electrical resistance of Caco-2 cells monolayers.

Mechanisms of action

Experimental studies revealed that Brassicaceae anthocyanins exhibited biological activities through several mechanisms, e.g., maintaining oxidative balance, downregulation of inflammatory mediators (IL-6, IL-10, and TNF- α), suppressing iNOS and COX-2 protein expression, reducing serum and tissue lipids, and reducing liver marker enzymes (AST,

ALT, and ALP) (Cruz et al. 2016; Izzo et al. 2020; Jung et al. 2014; Lee et al. 2002; Lin, Li, and Hwang 2008). Red cabbage anthocyanin was found to be a potent inhibitor of TLR4, which might block subsequent classical pathway activation (Figure 3) (Saluk et al. 2015). Xu et al. (2019) reported that Nrf2/HO-1 signaling pathway participated in the mechanisms of antioxidant activity. Hepatic proteomic analysis showed that cyanidin 3-diglucoside-5-glucoside-rich extract treatment altered the proteins involved in several pathways including glycolysis, tricarboxylic acid cycle, and oxidative phosphorylation, suggesting a decrease in the production and release of ROS (Zhang et al. 2020). However, detailed molecular mechanisms of action of Brassicaceae anthocyanins for these beneficial effects remain to be clearly studied.

Application of Brassicaceae anthocyanins

Color is an important quality criterion in food. Regarding the trends in the food industry, the use of natural colorants has increased in foods as substitutes for their artificial counterparts, which mainly due to the awareness of side-effects of artificial pigments on human health and environment (Francis 1989; Rodriguez-Amaya 2016). Anthocyanins were considered as potential food colorants with distinct red color (ANS 2013). For instance, the visual appearances of red radish, red cabbage, black carrots, red potatoes, and purple sweet potatoes model juices (0.1 M citric acid, pH 3) were close in color to FD&C Red #40, the certified dye with the highest per capita consumption in the USA, while the color attributes imparted by radish to juice were the closest (Giusti and Wrolstad 2003). Several studies have demonstrated the potential use of anthocyanins from grapes, red cabbage, strawberry, black currants, and black ben coats as nature colorants (Aguilera et al. 2016; Mateus and de Freitas 2008; Skrede et al. 1992). However, the application of anthocyanins has been limited due to the disadvantage of low stability during processing and storage.

Red cabbage anthocyanins, typical Brassicaceae anthocyanins, could be used as promising multifunctional food colorants with excellent color stability. First, red cabbage anthocyanins are more resistant to pH changes, which being pink at pH 3, violet at pH 5, and blue at pH 7 (Dyrby, Westergaard, and Stapelfeldt 2001). Second, they are more stable and have shown no reflection in sensory evaluation during the storage, as received high scores of color intensity (Walkowiak-Tomczak and Czapski 2007). Third, they are also can be used in intelligent food packages. Pereira, de Arruda, and Stefani (2015) developed a time-temperature indicator based on chitosan blend films and red cabbage anthocyanins, which exhibited good spectroscopic and physicochemical properties. Besides, red cabbage anthocyanins can also use to develop pH-sensitive films, providing additional value and attractiveness (Prietto et al. 2017).

The application of Brassicaceae anthocyanin pigments with high color intensity is currently limited by the distinct undesirable flavors. The off-flavors were derived from the degradation product of glucosinolates, which is generated by

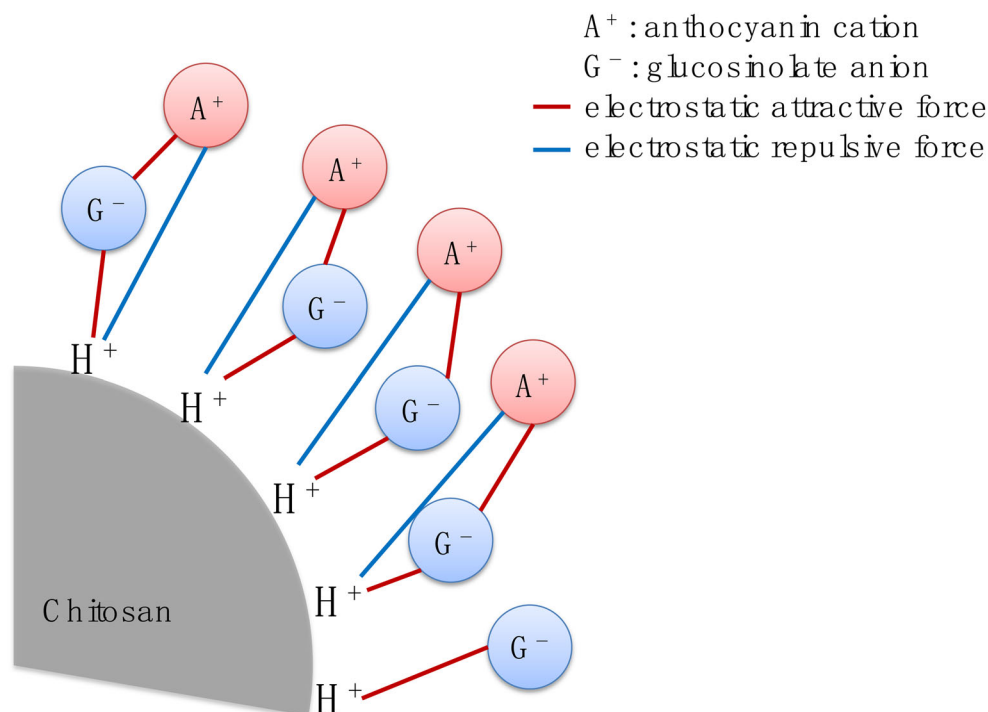


Figure 5. The potential adsorption mechanisms of glucosinolates on chitosan.

endogenous myrosinase or chemical degradation during food processing and storage (Hanschen and Schreiner 2017). Several approaches have been established in literature to eliminate the off-flavors from the Brassicaceae anthocyanin preparations, including resin absorption, flocculation, and membrane processes (Jing et al. 2011; Muller-Maatsch et al. 2019; Sapers 1982). Rodriguez-Saona et al. (2001) reported that direct osmosis concentration processing reduced the undesirable aroma compounds in radish juice, but did not completely solve the problem. In a separate study, Jing et al. (2011) developed an chitosan-treatment procedure with an chitosan concentration of 1.59 g/100 mL at pH 3.92 for 2.74 h, resulting a ~61% glucosinolate reduction and a ~95% anthocyanin retention. The dominant adsorption mechanisms of glucosinolates on chitosan may be electrostatic attractions, including hydrogen bonds and charge neutralization (Gao et al. 2014) (Figure 5).

Conclusions

The Brassicaceae family is a rich source of anthocyanins, which concentrated in the peel, flesh, leaves, sprouts, and flowers. The levels and profiles of anthocyanins differ significantly among Brassicaceae species and varieties. Extensive research has been conducted to investigate the anthocyanin profiles, and most of them are focusing on the identification of anthocyanins. The major anthocyanins identified in Brassicaceae family are cyanidin 3,5-diglucoside and cyanidin 3-diglucoside-5-glucoside derivatives acylated with *p*-

coumaric, malonic, sinapic, caffeic, ferulic, *p*-hydroxybenzoic, acetic, oxalic, succinic acids, glucoside, or/and xylose.

Acylated anthocyanins confer unique chemical and biochemical properties. Brassicaceae anthocyanins with acyl substituents are more stable than others. The improved stabilization attributed to the stacking of the acyl groups with the pyrylium ring of the flavylum cation, thereby prevent the nucleophile attack of water and subsequent formation of chalcone (intramolecular copigmentation). Study on bioaccessibility and bioavailability are increasing our understanding of uptake of Brassicaceae anthocyanins. The remarkable stability of Brassicaceae anthocyanins may be beneficial in preventing certain chemical degradation in the gastrointestinal tract. Additionally, anthocyanins reached blood circulation may be different from the original compounds in food.

The health effects of Brassicaceae anthocyanins have also been conducted. Evidence has been shown that Brassicaceae anthocyanins possess potential antioxidant, anti-cancer, and anti-inflammatory activities, protection against hepatic and renal damage and cardiovascular disease. However, most of the evidence is based on in vitro studies, and more in vivo and possibly clinical studies are necessary to further understand these health benefits, as well as the level of consumption to maximize the benefits and the mechanisms involved.

Despite the excellent color stability and health-promoting benefits, the development and application of Brassicaceae anthocyanins is still in the early stages. Brassicaceae anthocyanins could be used as multifunctional food additives to improve food quality and health function, and thus add value to food products. However, the application of Brassicaceae anthocyanins in food still faces numerous challenges, for

instance, lack of extraction and purification methods and potential negative sensory impacts. These problems need to be addressed by engineers and scientists to benefit both the food industry and improve human health.

Disclosure statement

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