

Article

Quantification of Phenolic Compounds and Carotenoids in a New Cauliflower Variety: Implications for Antioxidant Potential and Nutritional Breeding

Neil Patrick Uy ¹ , Hak-Dong Lee ^{1,2}, Ju-ri Choi ³ and Sanghyun Lee ^{1,2,*} 

¹ Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea; uyneilpatrick@gmail.com (N.P.U.); gkrehder@naver.com (H.-D.L.)

² Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

³ Functional Vegetable Team, Farm Hannong Co., Ltd., Anseong 17503, Republic of Korea; cjrk97@farmhannong.com

* Correspondence: slee@cau.ac.kr; Tel.: +82-31-670-4688

Abstract: Cauliflower (*Brassica oleracea*) is a highly nutritious cruciferous vegetable that has garnered increasing attention owing to its potential health benefits. This study aimed to quantify the phytochemical constituents of the new cauliflower variety “BetaCaulie” using advanced analytical methods to elucidate their potential contribution to human nutrition and health. Sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), and sinapic acid (4) were abundant in the yellow variety, where their concentration reached a total content of 11.86 mg/g. Among the carotenoids, β -carotene (7) had a concentration of 210.77 mg/g. In the DPPH and ABTS⁺ assays, the control variety exhibited superior antioxidant activity despite having less diverse polyphenols, as evidenced by lower IC₅₀ values of 20.67 and 24.34 mg/mL, respectively. This may be due to the high neochlorogenic acid (2) (14.89 mg/g) content of the control variety. These findings highlight the complexity of plant antioxidant mechanisms, in which different compounds interact uniquely, resulting in an overall antioxidant potential. This dichotomy highlights the potential for targeted breeding strategies that optimize polyphenol or carotenoid levels, depending on the desired health benefits or nutritional goals.

Keywords: glucosinolate; β -carotene; high-performance liquid chromatography; antioxidant



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1. Introduction

Cauliflower (*Brassica oleracea*) is a highly nutritious cruciferous vegetable that has gained increasing interest because of its potential health benefits [1]. Cauliflower is a valuable source of bioactive compounds, such as polyphenols and carotenoids, known for their significant contributions to human health. Its antioxidant [2,3], anti-inflammatory [4], and anti-carcinogenic [5] properties make it particularly beneficial. These compounds, known for their capacity to neutralize free radicals and mitigate oxidative stress, are key contributors to the prevention of chronic diseases such as cardiovascular disorders, certain cancers, and neurodegenerative conditions [6,7].

Cauliflower has a variety of phenolic chemicals, including phenolic acids (e.g., caffeic acid, ferulic acid, and sinapic acid) and flavonoids (quercetin, rhamnetin, and cyanidin), each of which has unique biological activity, including antioxidant, anti-inflammatory, and anticancer properties [8,9]. Cauliflower contains high levels of carotenoids, including α -carotene, cryptoxanthin, and β -carotene, which have been linked to improved vision and skin health [10].

Interestingly, different cauliflower types have varying phytochemical (phenolic, anthocyanin, and carotenoid) profiles, which generally correspond with their head color [11]. For example, yellow and orange cauliflower have more carotenoids compared to purple cauliflower [12]. On the other hand, purple cauliflowers have more anthocyanins compared

to yellow, orange, and regular white cauliflower. These variations show the possibility of breeding and biotechnology tactics targeted at increasing the phytochemical content of crops, such as cauliflower, which can improve their nutritional and medicinal value [13].

Improving crop phytochemical content through breeding or genetic manipulation has the potential to provide significant health advantages [14]. Various approaches, such as developing cauliflower varieties with higher polyphenol and carotenoid content, are being investigated as prospective strategies for combating nutrient deficiencies and improving the disease-preventive characteristics of this commonly consumed crop [15].

As research progresses, it becomes increasingly vital to investigate not just the biochemical profiles of cauliflower varieties but also the underlying mechanisms via which these bioactive substances exert their effects. Understanding these pathways may pave the way for the development of cauliflower as a functional food with added health advantages.

Polyphenols, a diverse group of secondary metabolites, are well known for their antioxidant properties, which contribute to inflammation reduction and protection against cellular damage [16,17]. Polyphenols in cauliflower include flavonoids and phenolic acids, which are associated with numerous health-promoting effects. Similarly, carotenoids are vital for human health due to their role as precursors to vitamin A, their antioxidant capacity, and their involvement in eye health and immune function [18].

Although the nutritional value of cauliflower is widely acknowledged, the precise quantification of its polyphenol and carotenoid content remains a critical area of research [19]. Accurate quantification of these compounds is essential for understanding their bioavailability and potential health benefits [20].

This study aimed to quantify the polyphenol and carotenoid constituents in two cauliflower varieties using advanced analytical methods to shed light on their contribution to potential human nutrition and health and the potential for nutritional breeding.

2. Materials and Methods

2.1. Plants Materials

Two *B. oleracea* varieties were used in this study: a control variety (*B. oleracea* var. *botrytis*) and a yellow commercialized variety known as “BetaCaulie” (Farm Hannong Co., Ltd., Anseong, Korea). BetaCaulie has a deep-orange head, unlike other common varieties (Figure 1). The cultivation area of BetaCaulie was in Anseong, Korea. The main sowing season is March to April, and it could be harvested 65–70 days after transplanting. The average head weight is approximately 750 g. The initial weights of the control and yellow varieties were 45.0 g and 44.9 g. After lyophilization, the samples had a final weight of 23.3 g and 22.4 g, respectively.



Figure 1. Samples of yellow (A) and control (B) cauliflower.

2.2. Chemicals and Apparatus

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) was performed using an LC system with a Thermo Vanquish ultra-high-performance liquid chromatography (UHPLC) apparatus. Phytochemical quantification was conducted using an HPLC

system (Agilent Technology 1290 Infinity II, Santa Clara, CA, USA), which consisted of a pump, auto-sampler, and UV detector. HPLC solvents, water, and acetonitrile (ACN) were supplied by Honeywell (Burdick and Jackson, Muskegon, MI, USA), and trifluoroacetic acid (TFA) was obtained from J. T. Baker (Phillipsburg, PA, USA). Standard compounds such as sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), sinapic acid (4), lutein (5), zeaxanthin (6), and β -carotene (7) were generously provided by the Natural Product Institute of Science and Technology (www.nist.re.kr; accessed on 7 July 2024), Anseong, Korea (Figure 2).

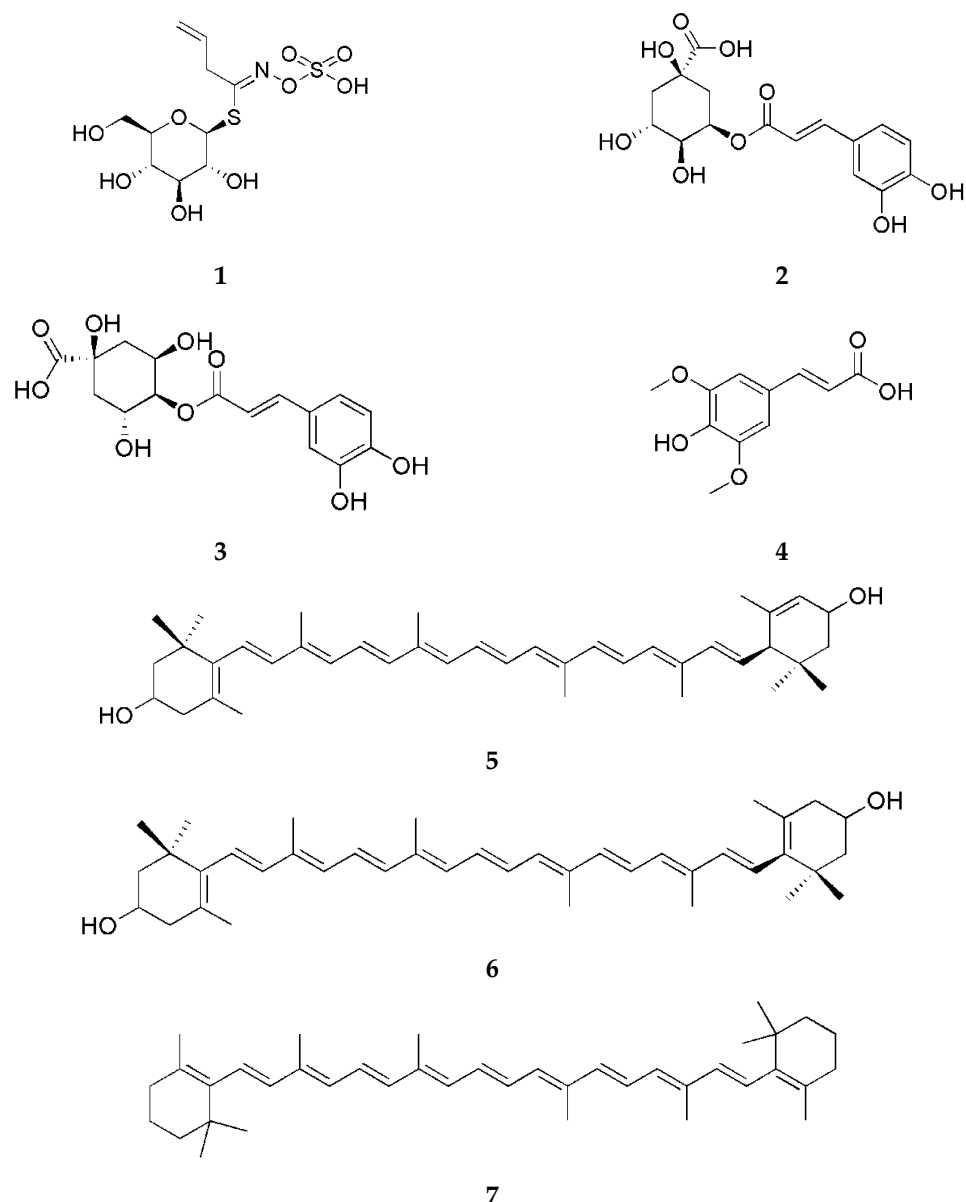


Figure 2. Chemical structures of sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), sinapic acid (4), lutein (5), zeaxanthin (6), and β -carotene (7).

2.3. Phytochemical Extraction

For polyphenol analysis, freeze-dried yellow and control *B. oleracea* samples were finely ground to obtain homogenous powders. One gram of each sample was combined with 10 mL of absolute ethanol (EtOH) and extracted with an ultrasonic shaker for 3 h at 60 °C. The extracts were filtered and subsequently concentrated in vacuo using a rotary evaporator. The concentrated extracts were set aside for further use.

2.4. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The DPPH assay was employed according to the methods of a previous study [21]. To prepare a 0.2 mM DPPH working solution, the stock DPPH solution was diluted using 95% EtOH. In a 96-well microplate, each well received 200 µL of the working solution along with 10 µL of either the yellow or control cauliflower extract. All experiments were conducted in triplicate to ensure precision. The mixtures were homogenized using a microplate shaker and incubated in the dark for 30 min to prevent photodegradation. Absorbance was then recorded at 514 nm (OD₅₁₄) using a spectrophotometer, with ascorbic acid as the reference positive control. The DPPH radical scavenging activity (%) was determined using the following equation:

$$\text{DPPH radical-scavenging activity (\%)} = (\text{Blank OD}_{514} - \text{Sample OD}_{514}) / \text{Blank OD}_{514} \times 100$$

2.5. ABTS^{•+} (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic Acid)) Assay

Similarly, the ABTS^{•+} assay was employed according to the methods of a previous study [21]. A 7.4 mM ABTS^{•+} stock solution was prepared and diluted with water to create the ABTS^{•+} working solution. In a 96-well microplate, 200 µL of the ABTS^{•+} working solution was combined with 10 µL of the yellow or control extract in designated wells. Each sample was analyzed in triplicate for data reliability. After thorough mixing on a microplate shaker, the mixtures were incubated in the dark for 30 min. Absorbance at 734 nm (OD₇₃₄) was measured using ascorbic acid as the reference standard. The ABTS^{•+} radical scavenging activity (%) was calculated using the following equation:

$$\text{ABTS}^+ \text{ radical-scavenging activity (\%)} = (\text{Blank OD}_{734} - \text{Sample OD}_{734}) / \text{Blank OD}_{734} \times 100$$

2.6. LC-MS/MS Analysis

The LC-MS/MS analysis was carried out using a Thermo Vanquish UHPLC system coupled with a Waters Cortex T3 column (150 mm × 2.1 mm, 1.6 µm particle size). The column temperature was set to 45 °C to ensure optimal separation conditions. The mobile phase consisted of two eluents: water containing 0.1% formic acid (eluent A) and ACN with 0.1% formic acid (eluent B). The gradient program was run as follows: 97% A from 0 to 0.5 min, 85% A at 15 min, 0% A from 50 to 55 min, and 3% B from 55.1 to 60 min. The flow rate was maintained at 0.25 mL/min. Mass spectrometric analysis was conducted using a Triple TOF 5600⁺ system (AB SCIEX, USA) equipped with a heated electrospray ionization (ESI) source. The mass spectrometer operated in both positive and negative ion modes to acquire full-scan MS spectra (*m/z* 100–1500) using a quadrupole system with a resolution of 70,000. In positive ion mode, the spray voltage was set to 3.5 kV. For MS2 fragmentation, the 16 most intense precursor ions were selected and analyzed at a resolution of 17,500. Additional parameters included a capillary temperature of 320 °C, sheath gas at 50 AU, auxiliary gas at 10 AU, and sweep gas at 0 AU.

2.7. HPLC Analysis of Phenolic Compounds and Glucosinolates

To prepare stock solutions of the standard compounds sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), and sinapic acid (4), each compound was dissolved in 1 mL of HPLC-grade methanol (MeOH) to a final concentration of 1 mg/mL. For the extracts, 50 mg of each concentrated sample was diluted with 1 mL of the same solvent to obtain a final concentration of 50 mg/mL. All solutions were filtered with a 0.45 µm PVDF filter. The extracts (yellow and control) were analyzed quantitatively using a reverse-phase HPLC system equipped with an Inertsil ODS-3 column (3.0 cm × 150 mm, 3 µm). The samples were evaluated with a 5 µL injection volume and absorbance at 227 nm. The flow rate was set to 0.4 mL/min, and the column temperature was maintained at 30 °C. The gradient elution was carried out using a mobile phase consisting of 0.1% TFA in water (A) and ACN (B), and a gradient program proceeded as follows: 87% A from 0 to 10 min, 70% A from 20 to 25 min, 30% A until 40 min, 0% A from 41 to 45 min, and 13% B from 50 to 60 min.

2.8. HPLC Analysis of Carotenoids

For carotenoid analysis, 200 mg of each powdered sample was first dissolved in a chloroform/methanol (3:7) solvent system to obtain a concentration of 50 mg/mL and then extracted at room temperature using an ultrasonic shaker and filtered through a 0.22 µm PVDF membrane filter to prepare a test solution. Similarly, the standard solutions were prepared by precisely weighing 2.0 mg of each standard compound lutein (5), zeaxanthin (6), and β-carotene (7) and dissolving it in chloroform/MeOH (3:7) with an ultrasonic shaker and filtering with a 0.22 µm PTFE membrane filter. The extracts (yellow and control) were analyzed quantitatively using a reverse-phase HPLC system equipped with a YMC carotenoid column (4.6 × 250 mm, 3 µm). The samples were evaluated with a 10 µL injection volume and absorbance at 450 nm. The flow rate was set to 1 mL/min, and the column temperature was maintained at 35 °C. The mobile phase used for gradient elution was 0.1% TFA in water (A) and ACN (B). The gradient program was run as follows: 90% A from 0 to 15 min, 60% A at 30 min, 30% A from 37 to 40 min, and 10% B from 44 to 60 min.

2.9. Calibration Curve

Standard stock solutions were serially diluted to obtain concentrations ranging from 500 to 31.25 µg/mL, which were then used to prepare the working solutions for the calibration curve. Calibration functions for the extracts were determined based on the peak areas (Y) and the corresponding concentrations (X, µg/10 µL). The results are expressed as mean values ± SD (n = 5).

2.10. Statistical Analysis

All analyses were performed in triplicate, and the results were expressed as mean ± SD. The data were analyzed using Student's *t*-test and Tukey's post hoc test. All statistical analyses were performed with GraphPad Prism 8.0.2 software (GraphPad Software, Boston, MA, USA). A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. DPPH and ABTS^{•+} Assays

DPPH and ABTS^{•+} assays were used to evaluate the antioxidant capacities of the two *B. oleracea* varieties (Figure 3). Both samples showed concentration-dependent inhibitory activity against the DPPH and ABTS^{•+} reagents, indicating that as the concentration of the sample increases, the inhibitory activity also increases. Both samples generally had higher IC₅₀ values than the positive control ascorbic acid, which had an IC₅₀ value of 0.15 µg/mL in the ABTS^{•+} assay and 0.17 µg/mL in the DPPH assay. Furthermore, the concentration range used for the two *B. oleracea* varieties was 50–3.125 mg/mL, whereas that used for the ascorbic acid was only 200–6.25 µg/mL. However, when comparing the two samples, the control variety had better antioxidant activity than the yellow variety, as depicted in their IC₅₀ values of 20.67 mg/mL and 24.34 mg/mL, respectively, in the ABTS^{•+} assay. Similarly, the control variety showed better antioxidant activity than the yellow variety in the DPPH assay, with IC₅₀ values of 15.40 mg/mL and 30.42 mg/mL, respectively.

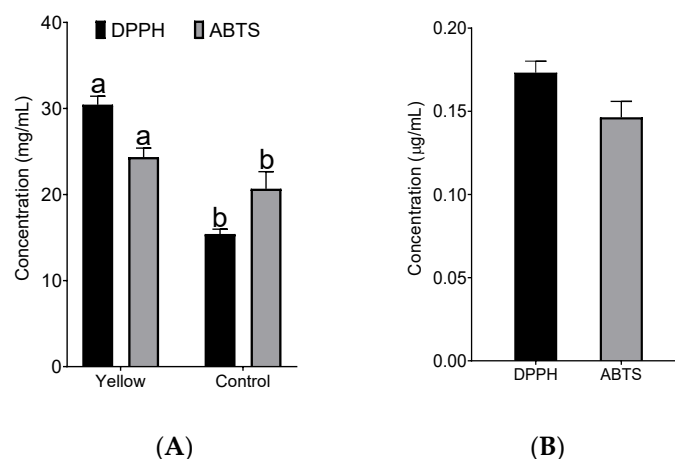


Figure 3. IC₅₀ values of the two *B. oleracea* varieties (A) and ascorbic acid (B) in DPPH and ABTS^{•+} assays. Lowercase letters on columns of the same color indicate a significant difference ($p < 0.005$).

3.2. LC-MS/MS Analysis

LC-MS/MS was employed to characterize the phytochemicals present in the yellow *B. oleracea* variety (Table 1). Although, 16 compounds were detected (Figure 4). Despite detecting 16 compounds, only sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), and sinapic acid (4) can be quantified because the amount of the rest of the compounds in the samples was either too small or the standard compounds were not available.

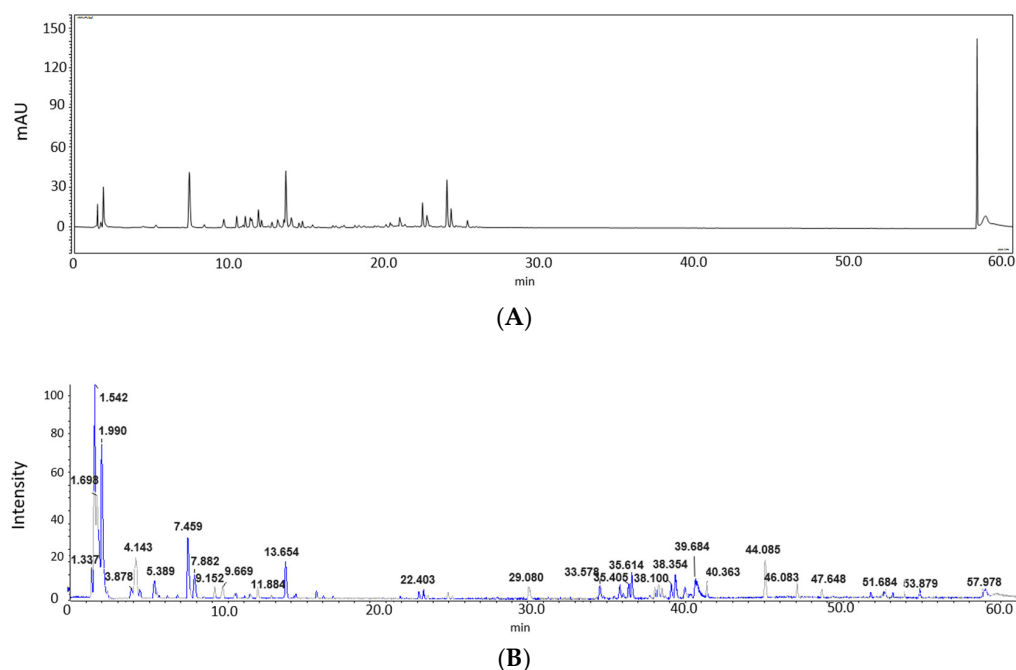


Figure 4. UV chromatogram (A) and base peak chromatogram (B) of the yellow *B. oleracea* variety, analyzed in negative ionization mode.

Table 1. Proposed compounds identified by LC-MS/MS analysis (negative mode).

Retention Time	Molecular Weight	Tentative Identification
1.66	423.0	Glucobrassicin
2.01	359.0	Sinigrin
3.89	464.1	4-Hydroxyglucobrassicin
4.15	407.0	3-Methylthiopropyl-glucosinolate
6.80	421.1	Glucobrassicin

Table 1. Cont.

Retention Time	Molecular Weight	Tentative Identification
7.47	354.1	Neochlorogenic acid *
7.85	448.1	Glucobrassicin
9.65	338.1	5-Coumaroyl quinic acid
11.05	788.2	Quercetin 3,3',4'-triglucoside
11.44	354.1	Cryptochlorogenic acid
11.87	478.1	4-Methoxyglucobrassicin
13.67	224.1	Sinapic acid
15.61	478.1	Neoglucobrassicin
16.65	626.1	Quercetin 3,4'-O-diglucoside
22.40	754.2	1,2-Disinapoyl diglucoside
23.95	754.2	1,2-Disinapoyl glucoside

* Neochlorogenic acid was also detected in the positive data.

3.3. Quantification of Phenolic Compounds and Glucosinolate by HPLC

The standard compounds exhibited good retention time and linearity (Table 2 and Figure 5). Quantification of one glucosinolate compound, sinigrin (1), and three phenolic compounds—neochlorogenic acid (2), cryptochlorogenic acid (3), and sinapic acid (4)—revealed significant differences between the yellow and control cauliflower varieties (Table 3).

Table 2. Calibration data for phenolic compounds and carotenoids.

Compound	t _R (min)	Regression Equation	r ²	LOD (mg/mL)	LOQ (mg/mL)
1	2.69	y = 5.0178x + 13.02	0.9994	0.65	1.99
2	9.06	y = 9.7541x + 41.97	0.9999	0.33	1.02
3	10.44	y = 9.7541x + 41.97	0.9993	0.33	1.02
4	12.46	y = 17.826x + 83.105	0.9996	0.18	0.56
5	12.04	y = 19.269x + 2.8766	0.9997	0.17	0.51
6	15.80	y = 44.876x + 1.3339	0.9993	0.07	0.22
7	34.23	y = 80.500x + 9.8383	0.9997	0.04	0.12

t_R: retention time, r²: coefficient of determination, LOD: limit of detection, LOQ: limit of quantification. Sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), sinapic acid (4), lutein (5), zeaxanthin (6), β-carotene (7).

The total polyphenol content was markedly higher in the control (15.78 mg/g) than in the yellow (6.08 mg/g) variety. Among the phenolic compounds, neochlorogenic acid (2) was the most abundant in both varieties, particularly in the control variety, with a maximum concentration of 14.89 mg/g and a total polyphenol concentration of 15.78 mg/g. In contrast, the yellow variety showed a significantly lower neochlorogenic acid (2) content, with a maximum of 5.19 mg/g and a total polyphenol concentration of 11.86 mg/g. Sinigrin (1) was the second-most prevalent compound, primarily concentrated in the yellow variety, where it reached 2.75 mg/g, contributing significantly to the total phenolic content. However, the control variety presented relatively lower sinigrin (1) levels (0.70 mg/g). Cryptochlorogenic acid (3), a compound not detected in the control sample, was found at modest levels in the yellow variety, with a content level of 1.78 mg/g. Conversely, sinapic acid (4) was present in all samples but in much lower quantities than sinigrin (1) or neochlorogenic acid (2), with a content of 2.14 mg/g and 0.19 mg/g in the yellow and control varieties, respectively.

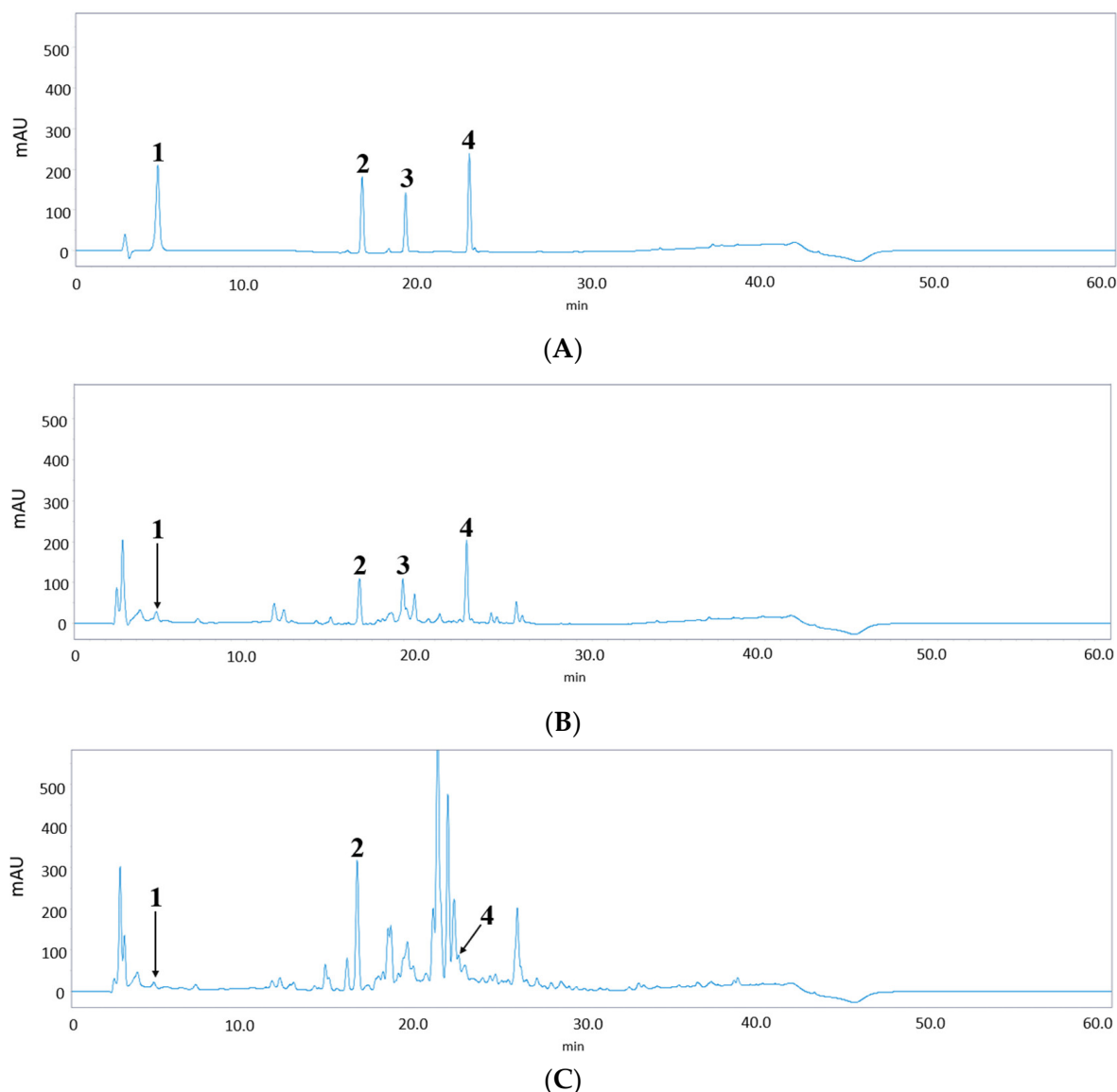


Figure 5. HPLC chromatograms of standard glucosinolate and phenolic compounds (A), yellow variety (B), and control variety (C). Sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), sinapic acid (4).

Table 3. Content of phenolic compounds in the *B. oleacea* samples.

Samples	Content (mg/g Extract)				Total
	1	2	3	4	
Yellow	2.75 ± 0.01^a	5.19 ± 0.02^b	1.78 ± 0.83	2.14 ± 0.00^a	11.86
Control	0.70 ± 0.01^b	14.89 ± 0.03^a	ND	0.19 ± 0.00^b	15.78

ND: not detected. Sinigrin (1), neochlorogenic acid (2), cryptochlorogenic acid (3), sinapic acid (4). Lowercase letters indicate significance groupings ($p < 0.005$).

3.4. Quantification of Carotenoids by HPLC

Similar to that of the polyphenols in Section 3.3, the standard carotenoids showed good separation (Figure 6). Quantification of the carotenoids—lutein (5), zeaxanthin (6), and β -carotene (7)—revealed a contrasting trend, with the yellow variety showing significantly higher total carotenoid levels (322.06 mg/g) than those of the control variety (25.87 mg/g) (Table 4). Lutein (5), a carotenoid with strong antioxidant activity, was

present in both the yellow and control samples. Interestingly, the concentrations in the control variety (21.53 mg/g) were significantly lower than those in the yellow sample (107.46 mg/g). Zeaxanthin (6), another carotenoid linked to ocular health, was detected in lower amounts in both varieties, with the control sample showing 4.34 mg/g and the yellow sample showing 3.69 mg/g. β -carotene (7), the precursor to vitamin A and a potent antioxidant, exhibited a substantial difference between the two varieties. It was found in trace amounts in the control sample, while the yellow sample demonstrated a remarkably high concentration (210.77 mg/g).

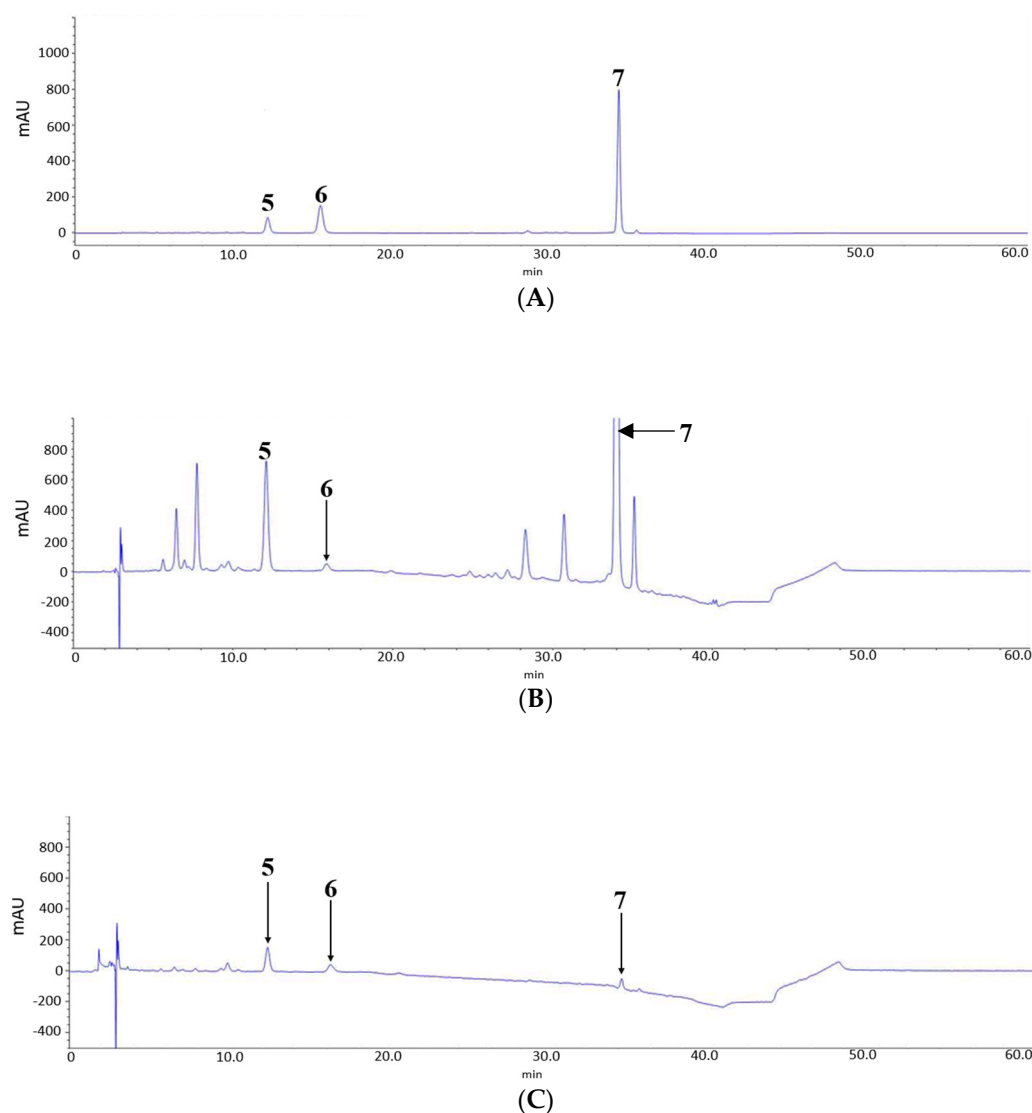


Figure 6. HPLC chromatograms of standard carotenoids (A), yellow variety (B), and control variety (C). Lutein (5), zeaxanthin (6), β -carotene (7).

Table 4. Content of carotenoids in the *B. oleracea* samples.

Samples	Content (mg/g Extract)			Total
	5	6	7	
Yellow	107.46 $\pm$ 1.03 ^a	3.69 $\pm$ 0.17 ^b	210.77 $\pm$ 1.47	321.92
Control	21.53 $\pm$ 0.15 ^b	4.34 $\pm$ 0.21 ^a	tr	25.87

tr: trace. Lutein (5), zeaxanthin (6), and β -carotene (7). Lowercase letters indicate significance groupings ($p < 0.005$).

4. Discussion

The LC-MS/MS analysis identified sixteen phytochemicals in the yellow variety, but only four could be quantified, suggesting that other minor compounds in the control variety, or those not detected in the yellow sample, may have contributed to the observed antioxidant activity. The quantification of phenolic compounds and glucosinolates by HPLC in the yellow and control *B. oleracea* varieties revealed significantly higher polyphenol concentrations in the yellow variety than those in the control. This was particularly evident for sinigrin (1), cryptochlorogenic acid (3), and sinapic acid (4), with the yellow sample containing higher levels of these compounds. Conversely, the control variety had a significantly higher neochlorogenic acid (2) content. Given the well-established antioxidant properties of these compounds, it was anticipated that the yellow sample would show superior antioxidant activity in both the DPPH and ABTS^{•+} assays. However, the results from the in vitro antioxidant assays were contrasting. A previous study investigated the scavenging activities of neochlorogenic acid and cryptochlorogenic [22]. Their results showed that neochlorogenic acid had lower IC₅₀ values in both DPPH and ABTS^{•+} assays which might explain why the control variety having significantly more neochlorogenic acid (2) content had higher antioxidant activity than its yellow counterpart. In addition, several studies have revealed that phenolic compounds with considerably lower concentrations than the bulk of phenolic compounds have a significant impact on antioxidant activity due to their synergistic or additive interactions [23,24].

Glucosinolates, a set of compounds with a wide array of structures, are found in commonly consumed *Brassica* crops including radish, mustard, broccoli, and cabbage [25]. Sinigrin (1), a type of glucosinolate, has been studied for its bioactive mechanisms, particularly its anticancer, anti-inflammatory, antibacterial, antifungal, antioxidant, and wound-healing properties [26]. Myrosinase enzymatically breaks down glucosinolates, primarily into isothiocyanates, cyanides, and thiocyanates, which are the major bioactive compounds responsible for pharmacological effects [27]. On the other hand, polyphenols, an extensive family of natural compounds, are the most prevalent natural antioxidants, with structures containing several phenolic units [28]. The natural antioxidant activity of phenolic compounds is attributed to the hydroxyl group in their structure, enabling them to scavenge radicals through hydrogen atom transfer or single-electron transfer processes [29]. Neochlorogenic acid (2), cryptochlorogenic acid (3), and sinapic acid (4) belong to a class of polyphenols known as hydroxycinnamic acids [30]. These compounds are highly valued for their health benefits and use as cosmeceutical components. They are primarily recognized as powerful antioxidants and thus play a role in the prevention of various oxidative stress-related disorders, including cardiovascular and neurological diseases, as well as cancer [31].

As shown in Table 3, the control variety exhibited a higher total phenolic and glucosinolate content (15.78 mg/g extract) than the yellow variety (11.86 mg/g extract). However, the yellow variety contained significant amounts of sinigrin (1), neochlorogenic acid (2), and cryptochlorogenic acid (3), suggesting a more diverse profile of phenolic compounds than that of the control. Despite the diverse polyphenol content of the yellow variety, the DPPH and ABTS^{•+} assays indicated that the control variety had better antioxidant activity, as evidenced by its lower IC₅₀ values compared to the yellow variety. In the ABTS^{•+} assay, the IC₅₀ value of the yellow variety was 20.67 mg/mL, more than that of 15.40 mg/mL for the control. Similarly, in the DPPH assay, the yellow variety had an IC₅₀ value of 30.42 mg/mL, whereas the control sample had an IC₅₀ value of 24.34 mg/mL. This paradox indicates that the antioxidant activity of the samples cannot be exclusively linked to their total phenolic content [32]. Conversely, higher levels of sinigrin (1) and cryptochlorogenic acid (3) in the yellow variety may result in synergistic effects with other compounds, such as carotenoids, enhancing the overall antioxidant potential of the yellow variety [33]. Furthermore, while the control variety demonstrated higher antioxidant activity in both assays, it is crucial to note that these assays only assess certain mechanisms of antioxidant activity (radical scavenging capability). They do not give a complete assessment of a sample's antioxidant

capacity, which may include other pathways such as metal chelation, enzymatic activity modulation, or impacts in biological systems [34]. The yellow variety's increased glucosinolate, phenolic compounds, and carotenoid concentration suggest that it may benefit other antioxidant pathways or have extra functional qualities.

Another possible explanation for this discrepancy is in the neochlorogenic acid (2) content, as the yellow variety had higher levels of sinigrin (1), cryptochlorogenic acid (3), and sinapic acid (4), all of which are known for their antioxidant activities. The control variety contained significantly higher amounts of neochlorogenic acid (2) than the yellow variety. β -carotene (7), a potent antioxidant, may have contributed more significantly to the overall antioxidant activity in the yellow variety [35]. Additionally, phenolic compounds, such as cryptochlorogenic acid (3), have been shown to play an important role in secondary antioxidant mechanisms, including metal chelation and the inhibition of pro-oxidant enzymes, which may not be fully captured by direct radical scavenging assays, such as DPPH and ABTS^{•+} [36,37]. The presence of cryptochlorogenic acid (3) in the yellow variety (1.78 ± 0.83 mg/g extract) but not in the control suggests that this compound could have contributed to the observed antioxidant effects in ways not reflected solely by its concentration [38].

The detection of the glucosinolate sinigrin (1) aligns with previous studies indicating the association of sinigrin (1), one type of glucosinolates, with disease resistance and health-promoting properties in cruciferous vegetables [39]. The higher sinigrin (1) concentration in the yellow variety suggests that this variety could offer enhanced health benefits and increased resistance to pathogens. The elevated levels of neochlorogenic acid (2) in the control variety may indicate enhanced antioxidant potential, as this compound is known for its role in oxidative stress regulation [40]. This phytochemical was previously identified as one of the predominant hydroxycinnamoyl acids in *Brassica* plants, like Brussels sprouts and kale [41]. In addition, a previous study investigating the methanolic leaf extracts of *Polygonum cuspidatum* identified neochlorogenic acid (2) as the main compound responsible for the antioxidant activity of the extract [42]. These results point to the same explanation attributed to the findings of the present work. Furthermore, the chromatogram of the control variety showed additional peaks, indicating that other compounds present in this variety might have contributed to its robust antioxidant activity. The absence of cryptochlorogenic acid (3) in the control variety highlights the unique biochemical profile of the yellow variety and suggests that its pigmentation may affect the synthesis or accumulation of this compound. The overall polyphenolic profile of yellow *B. oleracea* demonstrates a significantly higher accumulation of health-related compounds than that of the control variety.

Carotenoids in the two *B. oleracea* varieties were also quantified using HPLC (Table 4). Carotenoids are naturally occurring, brightly colored compounds that play essential roles in photosynthetic organisms, including cyanobacteria, algae, and plants. Found in the chloroplasts of green plant tissues, such as seeds, stems, leaves, and unripe fruits, they work in tandem with chlorophyll to absorb light energy at specific wavelengths [43]. What makes lutein (5), zeaxanthin (6), and β -carotene (7) interesting is the presence of conjugated double bonds in their structures, which allows them to receive electrons from reactive species, neutralize free radicals, isomerize, and aid in oxidation in the presence of light, oxygen, and heat [44].

The HPLC analysis revealed that the yellow variety had a substantially higher carotenoid content, particularly β -carotene (7), than that in the control variety (Table 4). In contrast, the yellow variety, with its much higher β -carotene (7) levels and higher content, exhibited weaker antioxidant activity. This result highlights the complexity of the antioxidant capacity of plant matrices, where different classes of compounds, polyphenols, and carotenoids interact in unique ways [45].

The stark difference in carotenoid content highlights the divergent metabolic pathways between the two varieties, likely influenced by the underlying pigmentation and genetic makeup [46]. The lutein content suggests that while lutein biosynthesis is active in both varieties, accumulation levels may be influenced by other factors, such as light exposure or

pigment-related gene expression [47]. The high lutein content of the yellow variety could make it an excellent contributor to eye health, given lutein's role in preventing age-related macular degeneration [48].

The comparable concentrations of zeaxanthin (6) between the two varieties suggest that its biosynthesis may be less influenced by pigmentation differences than lutein (5) or β -carotene (7). The disparity in the β -carotene (7) content indicates that β -carotene biosynthesis was significantly more active in the yellow variety, which may be related to the absence of yellow pigmentation in the control variety [49]. Similarly, the higher lutein (4) content in the yellow variety may explain why it has a stronger yellow coloration than the control variety. The high β -carotene (7) content in the yellow variety suggests that it could serve as an excellent dietary source of pro-vitamin A, with important implications for addressing vitamin A deficiencies in human diets [50].

Overall, the carotenoid profile underscores a trade-off between polyphenol and carotenoid accumulation in these two varieties of cauliflower, with the yellow variety excelling in polyphenol carotenoid content, particularly β -carotene (7). This dichotomy highlights the potential for targeted breeding strategies that optimize polyphenol or carotenoid levels, depending on the desired health benefits or nutritional goals [51]. The yellow variety may offer greater nutraceutical benefits and could be preferentially selected in breeding programs aimed at enhancing the health-promoting properties of cauliflower, with important implications for addressing vitamin A deficiencies in human diets [52]. The results of this study can be used as a reference for future studies aimed at developing health-promoting cauliflower varieties.

5. Conclusions

The antioxidant assays (DPPH and ABTS^{•+}) revealed that the control variety of *B. oleracea* exhibited superior antioxidant capacity, despite having lower polyphenol content than the yellow variety. This discrepancy can be attributed to the significantly higher neochlorogenic acid content of the control sample. In contrast, the yellow variety, while rich and diverse in polyphenols, showed weaker antioxidant performance, likely due to the limited presence of other influential phytochemicals, as indicated by LC-MS/MS analysis. These findings underscore the complexity of plant antioxidant mechanisms, in which the combined effects of phenolic compounds, glucosinolates, carotenoids, and other minor compounds dictate the overall antioxidant potential. The high antioxidant activity of the control variety may make it a potentially more valuable source of dietary antioxidants, while the yellow variety, with its elevated polyphenol and carotenoid content, may offer other health benefits that warrant further exploration. Future research should focus on understanding the synergistic effects of phenolic compounds, glucosinolates, carotenoids, and other minor compounds in *B. oleracea*, exploring the role of neochlorogenic acid (2) in antioxidant activity and investigating the health benefits of both the control and yellow varieties through in vivo studies. Additionally, long-term studies on the impact of polyphenol- and carotenoid-rich diets could provide insights into their potential in chronic disease prevention.

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