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Nutrition and Antioxidant Potential of Three Cauliflower (*Brassica oleracea* L. Var. Botrytis) Cultivars Cultivated in Southern Part of Bangladesh

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	This study aimed to evaluate the biochemical and nutritional profiles of three cauliflower						
Research Article	cultivars-Valentena, Carotena, and Snow White-focusing on chlorophyll, carotenoids,						
	anthocyanins, vitamin C, flavonoids, phenolics, and antioxidant activity. Uniform curds were						
Received : 14.08.2024	harvested at 60 days post-sowing from Baratia, Dumuria, Khulna, and analyzed at Khulna						
Accepted : 03.10.2024	Agricultural University. Valentena exhibited the highest chlorophyll content (40.06±0.39 µg/100g						
	FW chlorophyll a, 28.98±3.35 µg/100g FW chlorophyll b), superior lycopene (8.71±0.38 µg/100g						
Vanuarda	FW) levels. Carotena showed the highest total carotenoid content ($60.52\pm1.76 \ \mu g/100g FW$) and β -						
Chlorophyll content	carotene (26.99 \pm 0.44 µg/100g FW), while Snow White had the lowest values across most						
Carotenoids	parameters. Valentena also led in anthocyanins (101.56±3.9 mg/L FW) and total flavonoids						
Antioxidant activity	$(79.56\pm10.36 \text{ mg}/100 \text{g FW})$, with Carotena having the highest vitamin C content ($60.05\pm2.93 \mu\text{g/g}$						
Anthocyanins	FW). DPPH assays indicated that Valentena showed the most effective antioxidant ($IC_{50} =$						
Nutritional profiles	43.65±3.56 mg/mL FW), followed by Carotena and Snow White. Hierarchical clustering and						
	pricipal component analysis (PCA) revealed distinct biochemical profiles: Valentena and Carotena						
	shared similarities in carotenoids and antioxidant activity, whereas Snow White differed						
	significantly. Linear discriminant analysis identified lycopene, chlorophyll b, and β-carotene as						
	major differentiators, nignighting the diverse nutritional and antioxidant properties of these						
	caunitower varieties. The findings highlight the potential of Carolena and Valentena for health-						
	conscious consumers seeking nument-men, antioxidant benefits in functional meals.						
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Introduction

People from all walks of life have shown increasing interest in colorful fruits and vegetables for their diseasepreventing properties (Minich, 2019). With a significant rise in chronic diseases due to various factors (Stuckler, 2008), doctors are emphasizing the consumption of foods rich in pigments and antioxidants (Saiwal et al., 2019; Arumugam et al., 2021). The science behind this recommendation is that antioxidant-rich foods contain biologically active substances that protect cells from oxidative stress and other damage (Kar et al., 2021). As the color intensity of fruits and vegetables increases, their antioxidant content and capacity also rise. Vibrant hues, particularly in dark-colored foods like red, purple, and orange/yellow, indicate higher levels of beneficial pigments and antioxidants (Cömert et al., 2020; Sharma et al., 2021). Therefore, researchers are working to develop colored variants of traditional varieties with the aim of increasing their nutritional value, especially their bioactive components.

The Brassicaceae family is renowned for its healthpromoting phytochemicals, which empower its species to reduce chronic non-communicable diseases (Ahmed & Ali, 2013; Radünz et al., 2024). Brassica vegetables, in particular, boast a high nutritional composition, including vitamins (carotenoids, tocopherol, ascorbic acid, folic acid), carbohydrates, amino acids, minerals (Cu, Zn, P, Mg, etc.), and various phytochemicals such as indole phytoalexins, glucosinolates, and phenolics (Jahangir et al., 2009; Biondi et al., 2021; Soengas et al., 2021). These phytochemical properties enable Brassica vegetables to reduce age-related chronic illnesses, such as cardiovascular health issues (hypertension, stroke), degenerative diseases, obesity, type-2 diabetes, osteoporosis, and various cancers (Favela-González et al., 2020). Cauliflower, a member of the Brassicaceae family, is highly regarded for its nutritional value and health-promoting properties. This versatile vegetable is a staple in many diets worldwide, prized not only for its culinary applications but also for its rich content of essential nutrients and bioactive compounds (Picchi et al., 2020). Like other Brassica vegetables, its leaves and curd are excellent sources of antioxidants like phenolic compounds, carotenoids, glucosinolates and vitamins (Picchi et al., 2020); as well as dietary fibers, and other phytochemicals (Bux Baloch et al., 2015). Cauliflower is also a rich source of glucosinolate, a wellknown anticarcinogenic compound. These bioactive compounds contribute to reducing the risk of chronic diseases, including various cancers and coronary heart disease (Picchi et al., 2020).

Anthocyanins and carotenoids are pigments that are important to plant biology and human health (Jayakumar et al., 2023). In plants, these pigments are necessary for photosynthesis and UV protection (Markwell & Namuth, 2003; Roleda et al., 2004). Carotenoids, which are present in orange and yellow cauliflower varieties, are rich sources of antioxidants and are precursors to vitamin A, which is important for immunological response, skin health, and vision (Selly Msungu et al., 2022; Çoka & Akınoğlu, 2023). In addition to giving purple pigmentation, anthocyanins have potent antioxidant properties that shield cells from oxidative damage and lower inflammation (Frestasya & Pangsibidang, 2024). According to studies conducted by SellyMsungu et al. (2022) and Jayakumar et al. (2023), dietary consumption of plants' pigments is linked to a decreased risk of chronic illnesses like cardiovascular disease and some types of cancer. Once more, plants' color, flavor, and ability for withstanding both biotic and abiotic stresses are all influenced by phenolic compounds, a diverse group of secondary metabolites (Kelebek & Selli, 2012; Khanday et al., 2024; Salehi & Safaie, 2024). According to Phuyal et al. (2020), phenols are known for their antioxidant properties, which reduce oxidative stress and neutralize dangerous free radicals to improve human health. Increased phenol intake has been linked to a lower incidence of long-term conditions such cancer, diabetes, and cardiovascular disease (Rodriguez-Mateos et al., 2024). Furthermore, phenols possess anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, making them essential for preserving health and averting illness (Phuyal et al., 2020; Alsaadi, 2024). Fruits, vegetables, and drinks like tea and wine are rich sources of flavonoids, a subclass of phenolic chemicals. These substances are well-known for their vivid hues and for helping to tint plants (Davies et al., 2022). In terms of health benefits, flavonoids are powerful antioxidants and play a significant role in reducing the risk of chronic diseases(Alsaadi, 2024). They help improve cardiovascular health by enhancing endothelial function, reducing blood pressure, and decreasing LDL cholesterol levels (Hashizume & Tandia, 2024). Additionally, flavonoids possess anti-inflammatory and anti-cancer properties, support immune function, and may improve cognitive function and reduce the risk of neurodegenerative diseases (Spagnuolo et al., 2018).

Cauliflower is an important crop in areas where agriculture is the primary source of income, such as southern Bangladesh. Numerous cauliflower varieties are grown in the area due to its good climate and soil, each of which has a distinct nutritional profile and antioxidant capacity. Colorful cauliflower cultivars have recently been produced by researchers to improve nutrient and antioxidant consumption. Nevertheless, nothing is known about these new cultivars' pigment richness, nutritional makeup, and antioxidant potential (total phenolics and flavonoids) in this area. In this study, three cauliflower cultivars-two colorful variants (orange/yellow and purple) and the conventional white cultivar-grown in southern Bangladesh are evaluated for their nutritional makeup and antioxidant potential. We anticipate that our study can bring light on the distinctive nutritional profiles and antioxidant qualities of these colorful new cultivars and offer insightful information about their scientific advantages.

Materials and Methods

Sample Collection

On November 23, three cauliflower cultivars (Snow White, Carotena, and Valentena) curd of uniform age (60 days after sowing/20 days after flowering) and size were collected from a cauliflower cultivation field in Baratia, Dumuria, Khulna and placed in ziplock bags. The collected sample was then taken to the Crop Botany Department Laboratory at Khulna Agricultural University in Khulna for further chemical analysis. From each cultivar, four individual plants were selected, and one curd was harvested from each plant, ensuring that each curd represented one of the four replications (Figure 1).



Figure 1. Three cauliflower (*Brassica oleracea* L. Var. Botrytis) cultivars collected from Baratia, Dumuria, Khulna for pigment, antioxidant and nutrition analysis.

Determination of Pigment

The concentrations of photosynthetic pigments were measured using a modified spectrophotometric technique based on Lichtenthaler (1987) method. Exactly 0.5 grams of cauliflowers' leaf and curd (composite sample) were finely chopped and placed in a small vial containing 10 mL of 80% ethanol. The vials were kept in the dark for 10 days to allow pigment extraction. A spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) was used to measure absorbance at wavelengths of 480, 453, 495, 505, 645, and 663 nm. Subsequently, the concentrations of chlorophyll *a*, chlorophyll *b*, carotenoids, lycopene, β -carotene, and lutein were determined using specific equations (Sumi et al., 2024).

Chlorophyll $a = (\lambda 663 \times 0.999 - \lambda 645 \times 0.0989) \times V/W$

Chlorophyll $b = (\lambda 645 \times 1.77 - \lambda 663 \times 0.328) \times V/W$

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Total Carotenoids = { $(\lambda 663 \times 0.114 - \lambda 645 \times 0.638) + \lambda 480$ } × V/W

$$\begin{split} Lycopene &= (\lambda 663 \times 0.0458 + \lambda 645 \times 0.204 + \lambda 505 \times \\ & 0.372 - \lambda 453 \times 0.0806) \times V/W \end{split}$$

$$\label{eq:beta-carotene} \begin{split} \beta\text{-carotene} &= (\lambda 663 \times 0.216 - \lambda 645 \times 1.22 - \lambda 505 \times \\ &0.304 + \lambda 453 \times 0.452) \times V/W \end{split}$$

Lutein = $(\lambda 480 \times 11.51 - \lambda 495 \times 20.61) \times V/W$

In this context, $\lambda 663$ represents the absorbance at a wavelength of 663 nm, $\lambda 645$ denotes the absorbance at 645 nm, $\lambda 505$ signifies the absorbance at 505 nm, $\lambda 495$ indicates the absorbance at 495 nm, $\lambda 480$ corresponds to the absorbance at 480 nm, and $\lambda 453$ pertains to the absorbance at 453 nm.

Vitamin C Determination

The 2,6-dichlorophenol indophenol dye solution, which changes color from blue to red when ascorbic acid is present, is the basis for the titrimetric determination of vitamin C. With ascorbic acid, this reaction is both quantitative and selective within the concentration range of 10-35µg/mL. The indophenol dye solution and metaphosphoric acid were among the reagents utilized. A known weight curd tissue sample was extracted using 3% meta-phosphoric acid, and the sample was then diluted to a predetermined volume. When an aliquot of this solution was titrated with the indophenol dye solution and a persistent pink color developed, the reaction had reached its endpoint. After the dve solution was adjusted using a standard ascorbic acid solution, the dye factor was determined. In the end, the vitamin C content was calculated using the formula below. This process was modified in light of Xiao et al. (2012).

Vitamin C (
$$\mu$$
g/g FW) = $\frac{e \times d \times b}{c \times a \times 100}$

Where, a represents the weight of the sample, b denotes the volume made with metaphosphoric acid, c indicates the volume of the aliquot taken for estimation, d stands for the dye factor, and e signifies the average burette reading for the sample.

Determination of Anthocyanin

Using a mortar and pestle, the 3 g fresh curd sample were homogenized in 30 mL of 99.9% ice-cooled methanol. After that, the mixture was sealed in a glass bottle and left for half an hour in the dark. Two distinct 1.5 mL Eppendorf tubes were filled with the supernatant extracts. Following a 5-minute centrifugation at 15,000 rpm, the tubes were refrigerated at -10°C to measure the anthocyanin, phenol, flavonoid, and DPPH radical scavenging capacity. Using a modified technique that combined the pН differential method with spectrophotometry, the total amount of monomeric anthocyanins was ascertained (Gordillo et al., 2018). The foundation of this technique is the way the anthocyanin's structure changes in response to pH in two buffer solutions (pH 1.0 and pH 4.5). The monomeric anthocyanins concentration is directly proportional to the difference in absorbance between the pH 1.0 and pH 4.5 solutions, as per this approach. At 510 nm and 700 nm, the absorbance of the samples buffered in two distinct pHs is measured. The most prevalent anthocyanin, cyanidin-3-glucoside, is measured using the following equation to determine the total amount of monomeric anthocyanins (TAMA), which is expressed as mg/L (Khoo et al., 2017; Galvão et al., 2020).

TAMA (mg/L) =
$$\frac{(A510 - A700) - (B510 - B700) \cdot MW * DF * 100}{\varepsilon \cdot l}$$

Here, at 510 nm, A510 and B510 represent the absorbance for pH 1 and pH 4.5 buffers, respectively. At 700 nm, A700 and B700 represent the absorbance for pH 1 and pH 4.5 buffers, respectively. The variables that represent the molar mass (MW) of cyanidin-3-glucoside (449.2 g/mol), dilution factor (DF), molar extinction coefficient (ϵ) (26,900 L/mol/cm), conversion factor (1000) from g to mg, and wave path (in centimeters) in the cuvette are all represented.

Determination of Total Phenolic Content

To quantify the total phenolic compound content, a technique adapted from (Albano & Miguel, 2011) was used. For this determination, gallic acid served as the standard. Various concentrations of gallic acid or plant extracts were added to test tubes, followed by 16μ L Folin–Ciocalteu reagent and 3mL of 10% sodium carbonate solution. The mixture was left in the dark at room temperature for 30 minutes. The absorbance of each sample was then measured at 760nm wavelength to indicate the overall phenolic content. These absorbance values were plotted against their corresponding concentrations, generating a linear relationship that formed a standard curve used to determine the total phenolic content in the test samples (Sumi et al., 2024).

Determination of Total Flavonoid Content

The flavonoid contents of the leaf and stem extracts were calculated using catechin as a reference. More specifically, $300 \,\mu\text{L}$ of each AlCl₃ and NaNO₂ solution was reacted with 1 mL of previously obtained plant extracts. After the mixture was allowed to sit at room temperature for five minutes, two milliliters of NaOH solution and ten milliliters of distilled water were added. The mixture was then vortexed and allowed to sit for 30 minutes. As per Baba & Malik, (2015), the compound's total flavonoid concentration (TFC) was determined by measuring the absorbance at 510 nm. The TFC was expressed as follows: one gram of fresh extract was equal to a microgram of catechin.

DPPH Radical Scavenging Capacity Assay

Using 2-diphenyl-1-picrylhydrazyl radical (DPPH), the ability of medicinal plant extracts to scavenge free radicals was evaluated using the procedure outlined by (Brand-Williams et al., 1995). Stable free radical DPPH, which has a distinct violet hue, turns yellow as the sample's antioxidants scavenge the free radicals. The transition from violet to yellow in color corresponds to the level of radical scavenging action. One milligram of DPPH in methanol was mixed with different quantities of plant extracts that were made using methanol and previously extracted material. The materials were completely mixed by vortexing, and then they were incubated for 30 minutes at room temperature (24–30°C). A DR 6000 UV spectrophotometer was used to measure the reduction in absorbance at 517 nm. Using the following formula, the proportion of scavenging activity was determined:

Scavenging Activity (%) =
$$\frac{(Ac - As) \times 100}{Ac}$$

Where, As is the sample absorbance and Ac is the control absorbance (without extract). The IC_{50} value, representing the concentration of antioxidant required to scavenge 50% of the free radicals, was determined by plotting the percentage of radical scavenging activity against the extract concentration.

Statistical Analysis

Minitab 17.3 was used to do the statistical analysis. After determining whether differences were significant using one-way ANOVA, Tukey's HSD test ($p \le 0.05$) was run. Using euclidean distances, a hierarchical clustering analysis was performed and a heatmap was made using the "pheatmap" package in R 4.3.2. Principal component analysis (PCA) was carried out using the "GGally" and

"factoextra" programs. The correlation study was performed using the "Corrplot" software (Yasmin et al., 2024). The "MASS" program was utilized for Linear Discriminant Analysis (LDA).

Result and Discussion

Chlorophyll Content of Three Cauliflower Cultivars

The chlorophyll content varied significantly among the Valentena, Carotena, and Snow White cauliflower varieties. Valentena exhibited the highest chlorophyll levels, demonstrating superior photosynthetic potential with a chlorophyll *a* (Chl a) content of $40.06\pm0.39 \ \mu g/100g$ FW (100%) and a chlorophyll *b* (Chl b) content of 28.98±3.35 $\mu g/100g$ FW (100%), leading to a total chlorophyll (TChl) value of $69.04\pm3.64 \ \mu g/100g$ FW (100%) (Table 1). Carotena and Snow White had considerably lower chlorophyll levels, although their chlorophyll content was not significantly different. Carotena showed moderate values with Chl a at 77.46%, Chl b at 36.78%, and a TChl of 60.38%, while Snow White recorded the lowest with Chl a at 71.85%, Chl b at 24.35%, and a TChl of 51.91% (Table 1).

Anthocyanin of Three Cauliflower Cultivars

Figure 2A summarized the anthocyanin content in the curds of three cauliflower cultivars: Valentena, Carotena, and Snow White.





(A), vitamin C (B), total soluble flavonoids (C), total phenolics (D), total carotenoids (E), and IC50 value for DPPH scavenging (F) across three cauliflower cultivars. Error bars represent the standard error of the mean (SEM, n = 4). Mean values with different letters indicate significant differences at the 5% level.

Table 1	Com	narison (of cl	hlorophy	ll and	carotenoid	content	across	three cai	iliflower	cultivars ((fresh	weight	basis)
r abre r	. com	parison		morophy	in and	carotenoia	content	across	un ce cat		cultivals	, nosn	weight	Jusisj

Cultivars	Chloi	ophyll (µg/ 100g	gFW)	Carotenoids (µg/ 100g FW)			
	Chl a	Chl b	TChl	Lycopene	β-Carotene	Lutein	
Valentena	40.06 ± 0.39^{a}	28.98±3.35ª	69.04 ± 3.64^{a}	$8.71{\pm}0.38^{a}$	7.75±1.71 ^b	$20.04{\pm}4.07^{a}$	
Carotena	31.03 ± 2.08^{b}	10.66 ± 1.04^{b}	41.69±1.36 ^b	5.93 ± 0.47^{b}	26.99±0.44ª	26.20±25.60ª	
Snow White	28.78 ± 2.14^{b}	7.06 ± 1.61^{b}	35.84 ± 2.29^{b}	4.76 ± 0.29^{b}	$1.37{\pm}0.79^{a}$	$10.86{\pm}0.76^{a}$	
* Chl a = chlorophyll a: Chl b = chlorophyll b: TChl = total chlorophyll. No significant difference ($n < 0.05$) between values within each column that							

* Chi a = chlorophyll a; Chi b = chlorophyll b; TChi = total chlorophyll. No significant difference (p<0.05) between values within each column share the same letter(s).

Valentena had a markedly higher average anthocyanin content at 101.56 ± 3.90 mg/L FW (100%), distinguishing it from the other cultivars. In contrast, Carotena and Snow White had significantly lower anthocyanin contents, 7.43% and 3.43%, respectively (Figure 2A). The letters (a, b, c) following the SEM values indicated that Valentena's anthocyanin level was significantly different from both Carotena and Snow White, which did not differ significantly from each other.

Carotenoids of Three Cauliflower Cultivars

Carotena had the greatest average total carotenoid content ($60.52\pm1.76 \mu g/100 g$ FW), much higher than Snow White's and Valentena's combined values of $46.62 \pm 1.56 \mu g/100 g$ FW and $25.58\pm1.36 \mu g/g$ FW (Figure 2E). Once more, Valentena had the greatest lycopene concentration of any cauliflower cultivar, averaging $8.71\pm0.38 mg/100 g$; for reference, this was expressed as 100%. Snow White had 54.65% of lycopene concentration, whereas Carotena had 68.09% (Table 1). Carotena had the greatest average concentration of β -carotene ($26.99\pm0.44 \mu g/100 g$ FW; 100% for comparison). Snow White had 5.07% β -carotene concentration, compared to 28.72% in Valentena. Carotena had the highest level of lutein, averaging $26.20\pm25.60 \mu g/100 g$ FW, or 100%. In contrast, Snow White had 42.27% and Valentena had 77.04% (Table 1).

Vitamin C of Three Cauliflower Cultivars

With an average of $60.05\pm2.93 \ \mu g/g FW$, Carotena curd had the highest vitamin C content among the cultivars, making it very rich in this nutrient, which is renowned for its roles in immune function and antioxidant activity. Valentena was the second highest with an average vitamin C content of $49.49\pm1.69 \ \mu g/g FW$. Snow White had the lowest vitamin C content, averaging $40.25\pm1.81 \ \mu g/g FW$ (Figure 2B). For those looking to increase their vitamin C intake, Carotena may be the most advantageous option, while Snow White and Valentena had moderate to low concentrations of this nutrient.

Total Flavonoid content of Three Cauliflower Cultivars

Figure 2C presented the total flavonoid content in the curd of three cauliflower cultivars: Valentena, Carotena, and Snow White. Valentena had the highest average total flavonoid content at 79.56 ± 10.36 mg/100g FW. Carotena had an average flavonoid content of 55.60 ± 14.12 mg/100g FW, and Snow White had an average of 34.70 ± 6.16 mg/100g FW (Figure 2C). Despite the differences in average flavonoid content, the notation 'a' after the SEM values indicated that there was no statistically significant difference among the cultivars. This means the total flavonoid content was consistent across Valentena, Carotena, and Snow White.

Total Phenolic Content of Three Cauliflower Cultivars

Valentena curd showed the highest phenolic content with an average of 503.83 ± 15.87 mg/100g FW, indicating a robust concentration of phenolic compounds known for their antioxidant properties. Carotena followed with an average phenolic content of 181.55 ± 19.78 mg/100g FW, which was significantly lower than Valentena. Snow White had the lowest phenolic content, averaging 92.95 ± 5.82 mg/100g FW. The different letters (a, b, c) following the SEM values indicated statistically significant differences among the cultivars.

DPPH radial Scavenging Potential of Three Cauliflower Cultivars

Lower values of DPPH indicate better antioxidant capacity. Among the three cauliflower cultivars, Valentina demonstrated the highest antioxidant potential with an IC₅₀ value of 43.65 \pm 3.56 mg/mL FW (100%). Carotena follows with a value of 139.26%, indicating slightly less effective antioxidant properties than Snow White. Snow White has the lowest antioxidant potential among the three, with a DPPH value of 186.46%. Valentena as the most potent antioxidant, followed by Carotena, with Snow White being the least effective in scavenging DPPH radicals (Figure 2F).

Hierarchical Clustering and Co-Clustering of Variety and Traits

The heatmap with dendrogram in Figure 3 highlighted the similarities and differences among cauliflower cultivars (Valentena, Carotena, Snow White) based on pigments, phytochemicals, nutritional, and antioxidant parameters. Valentena and Carotena showed higher values for certain parameters like TPC and CARO, indicating similarities in antioxidant and carotenoid profiles. Snow White stood out with generally lower values across most parameters, suggesting a distinct biochemical profile. The dendrograms revealed hierarchical clustering of both cultivars and parameters, showing relationships such as the close association between DPPH and TFC, and the grouping of traits like ANT, LCP, CHB, TCHL, TPC, and CHA. Clusters indicated that antioxidant activity and flavonoid content were the highest in Valentena, the lowest in Snow White, and average in Carotena.

Correlation Analysis

The correlation matrix provided showed the pairwise correlations between different parameters: ANT, TFC, TPC, DPPH, VTC, CHA, CHB, TCHL, LCP, BCAR, LUT, and CARO. Each cell in the matrix indicated the correlation coefficient between two parameters, ranging from -1 (perfect negative correlation) to 1 (perfect positive correlation), with 0 indicating no correlation.





Figure 3. Hierarchical co-clustering of three cauliflower traits and cultivars based on standardized pigment, antioxidant, and nutrient values. Colors reflect relative values (-1 to +1), with purple indicating high and white low.

Figure 4: Correlation matrix for three cauliflower cultivars, with pink circles indicating positive correlations, purple circles indicating negative correlations, and circle size reflecting strength (-1 to +1).

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Variable	Principal Components					
variable	PC1	PC2	PC3			
Extracted eigenvalues	8.02	3.98	3.44e-31			
Proportion of varience	0.66	0.33	0.00			
Explained variance (%)	6.69e-01	3.31e-01	2.87e-32			
Cumulative varience (%)	66.87	100	100			
Standard deviation	2.83	1.99	3.80e-16			
Traits		Eigen vectors				
ANT	1.008	-0.732	0.455			
TFC	-0.112	0.095	-0.064			
TPC	-4.629	-0.680	0.056			
DPPH	-0.912	1.452	0.278			
VTC	-0.148	0.375	-0.183			
СНА	0.484	0.175	0.136			
CHB	1.206	-0.293	0.180			
TCHL	0.091	0.197	0.147			
LCP	1.396	-0.246	0.158			
BCAR	1.192	-0.361	-0.244			
LUT	0.288	0.003	-0.540			

This matrix was useful for understanding how these parameters related to each other. For instance, ANT, TPC, CHA, CHB, and TCHL showed strong positive correlations among themselves, while DPPH exhibited strong negative correlations with several parameters. Variable VTC showed moderate positive correlations with BCAR, LUT, and CARO, indicating some degree of positive association. This matrix aided in determining which variables tended to vary together or in opposite directions, revealing potential relationships and dependencies within the dataset.

Principal Component Analysis

The results of the Principal Component Analysis (PCA) revealed essential insights into the structure and variance of the dataset. PCA identified three principal components (PC1, PC2, and PC3) with corresponding eigenvalues of 8.02, 3.98, and close to zero, respectively, indicating that PC1 explained a substantial 66.9% of the total variance,

while PC2 contributed 33.1% (Figure 5). PC3, with a nearly negligible eigenvalue, suggested minimal contribution to the dataset's variance (Table 2). The eigenvectors or traits highlighted the weights and directions of each original variable (ANT, TFC, TPC, etc.) within these components, illustrating their relative importance in shaping the dataset's variability. This analysis underscored the effectiveness of PCA in reducing dimensionality while preserving key information, allowing for clearer interpretation of underlying patterns and relationships among the variables studied.

Coefficients Of Linear Discriminants

Table 3 presents the results of the Linear Discriminant Analysis (LDA) for cauliflower traits, showing the coefficients for two linear discriminants (LD1 and LD2) and their proportions of trace. LD1 explained 63.04% of the variance, while LD2 accounted for 36.96%.



Figure 5. Principal Component Analysis (PCA) visualizes pigment, antioxidant, and nutrient values of three cauliflower cultivars, with arrows from centroids indicating dissimilarities. Vector length reflects variable contribution strength, while angles depict interactions; small angles indicate high positive correlations

Table 3. Linear Discriminant Analysis and Coefficients for Cauliflower Traits in LD1 and LD2

Variable	Linear Discriminants			
variable	LD1	LD2		
Proportion of trace (%)	63.04	36.96		
Studied traits	Coeffi	icients		
ANT	0.204	-0.042		
CARO	0.088	0.144		
CHA	0.083	0.016		
LCP	2.730	-0.057		
BCAR	0.709	0.680		
LUT	0.311	0.482		
TCHL	0.032	0.012		
CHB	1.971	-0.454		
VTC	0.210	0.098		
TFC	0.105	0.174		
TPC	0.060	-0.012		
DPPH	-0.198	-0.026		

The coefficients indicate the contribution of each trait to the discriminants: for example, LCP and BCAR had high coefficients in LD1, suggesting they significantly influence this component, whereas traits like DPPH and TPC had lower coefficients. LD1 and LD2 together highlight which traits are most influential in distinguishing between groups, aiding in the classification and interpretation of cauliflower characteristics.

The research highlights the significant variations in chlorophyll, carotenoid, anthocyanin, and vitamin C content among different cauliflower cultivars, emphasizing their implications for crop quality, nutritional value, and health benefits. Valentena cauliflower exhibited the highest chlorophyll content, particularly Chl a and Chl b in their leaves. Izadpanah et al. (2024) found that purple and orange cauliflower cultivars also had higher chlorophyll levels in their leaves, consistent with our findings. However, their results were higher, likely due to differences cultivation in cultivars, methods, environmental conditions, and analytical techniques. According to Izadpanah et al. (2024) and Sherin et al. chlorophyll-rich cultivars have (2022),better photosynthetic efficiency and growth potential. Also, previous research suggesting chlorophyll-rich cultivars offer increased nutritional benefits due to their robust photosynthetic and antioxidant activities (Lanfer-Marquez et al., 2005; Sherin et al., 2022; Martins et al., 2023). In our study, Carotenoids, such as β -carotene and lutein, were most abundant in Carotena, underscoring its nutritional value and antioxidant properties. According to Izadpanah et al. (2024), orange cauliflower cultivars' dried leaves had significant concentrations of lutein, \beta-carotene, and carotenoids than other studied cultivars. These findings validate our study. Again, they had significantly greater values than we had, probably because we were focused on fresh curds than dried leaves, and because our farming methods and the environment were different. Crupi et al. (2023) and Johra et al. (2020) emphasize the health benefits of β -carotene-rich diets in improving antioxidant defense and eye health. Moreover, the importance of carotenoids in reducing oxidative stress and mitigating chronic diseases has been emphasized in several studies (Koca Bozalan & Karadeniz, 2011; Martí et al., 2016; Muscolo et al., 2024). Anthocyanins, known for their vibrant colors and antioxidant properties, have been extensively studied in various fruits and vegetables, including cauliflower (Scalzo et al., 2008; Mattioli et al., 2020). The potential health advantages of anthocyanin-rich diets, such as reduced inflammation and cardiovascular protection, are well-documented (Mozos et al., 2021; Mohammadi et al., 2024). In our study, Valentena showed significantly higher anthocyanin content compared to Carotena and Snow White, similar to findings in purple cauliflower and red cabbage that correlate higher anthocyanin levels with enhanced antioxidant capacity (Scalzo et al., 2008). Again, Singh et al. (2020) reported a significant variation in anthocyanin content within the F2 population of cauliflower curds, ranging from 3.81 to 48.21 mg per 100 g of fresh weight. They also observed that dark purple curds exhibited higher anthocyanin levels compared to light purple or white curds. These findings closely align with the results of our study. Carotena's high vitamin C content highlights its nutritional superiority, in line with studies by several studies emphasizing vitamin C's role in reducing oxidative stress and improving immune response (Kükürt & Gelen, 2024; Tewari et al.; 2017; Uddin et al., 2021). This finding also aligns with research comparing vitamin C levels in nutrient-dense foods like bell peppers and strawberries (Kishwar et al., 2019; Afnani et al., 2023). Moreover, the ascorbic acid range was consistent with the earlier Vanlalneihi et al. (2020) findings.

Valentena's high total flavonoid and phenolic content further underscores its significant antioxidant properties. This aligns with Dos Reis et al. (2015) and recent studies demonstrating the health benefits of flavonoid- and phenolic-rich foods in reducing oxidative stress, inflammation, and chronic disease risk (Sumi et al., 2024; Yang et al., 2021; Liu et al., 2023). Valentena's strong antioxidant capacity, indicated by its low IC₅₀ value in the DPPH assay, supports previous research on the robust relationship between high flavonoid and phenolic levels and antioxidant capacity (Lyu et al., 2023; Sumi et al., 2024). Bhandari et al. (2015) concluded that the Asia purple cultivar has the highest antioxidant activity in its florets due to its considerably higher levels of total phenols, and total flavonoids. The findings validate our results.

Multivariate statistical analyses, such as hierarchical clustering and PCA, were crucial in identifying cultivarspecific traits and biochemical mechanisms influencing nutritional quality (Aleem et al., 2021;Tawonga, 2024). This approach, similar to studies by Granato et al. (2018) and Sharma et al. (2017), enhances understanding of biochemical diversity and assists in identifying cultivars with superior health benefits. One of the crucial methods for dimensionality reduction, machine learning, and pattern recognition is linear discriminant analysis (LDA) (Li et al., 2021). LDA results highlighted key biochemical traits like lycopene, chlorophyll b, β -carotene, anthocyanin, and lutein as significant discriminators, consistent with research on other crops (Bhandari et al., 2016; Fioroni et al., 2023). Since of their antioxidant qualities, these substances are essential to human health since they lower the chance of developing chronic illnesses. Overall, the study reinforces the importance of understanding plant biochemistry to enhance crop quality, nutritional value, and health benefits.

Conclusion

The study focused on diverse cauliflower cultivars, revealing significant biochemical differences that impact their nutritional profiles and health benefits. Valentena cauliflower was found to have the highest level of anthocyanins and chlorophyll, which enhanced its antioxidant capacity and possible health advantages. Superior quantities of lutein and β -carotene were demonstrated by carotena, underscoring its nutritional significance. Carotena's strong vitamin C concentration further emphasizes its significance for antioxidant defense and immunological support. Valentena is a cultivar that is advantageous for consumers who are health-conscious due to its strong antioxidant qualities and high flavonoid and phenolic content. PCA and other multivariate analyses revealed key biochemical traits that differentiate cultivars, guiding future breeding and nutritional enhancement efforts. Future research should explore optimizing cultivation practices for enhancing the beneficial compounds identified in cauliflower cultivars.

Declarations

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Competing Interests

The authors declare that there is no conflict of interest in the article.

Author Contributions

- M.J.S. conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- S.A.S. performed the experiments, authored of the article, and approved the final draft.
- T.K.R. authored or reviewed drafts of the article and approved the final draft.
- K.A. authored or reviewed drafts of the article, funding acquisition and approved the final draft.
- S.R. authored or reviewed drafts of the article, funding acquisition and approved the final draft.
- M.J.D. authored or reviewed drafts of the article, analyzed the data, and approved the final draft.
- M.A. authored or reviewed drafts of the article, and approved the final draft.
- N.U. supervision and curated data, prepared figures and/or tables, funding acquisition, authored or reviewed drafts of the article, and approved the final draft.

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