Comparative Analysis of Antioxidant Activity, ROS, and Relative Water Content Between Red and Green Cabbage

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Abstract

Cabbage, the second largest leafy vegetable, is highly valued for its nutritional richness and

versatility. As health consciousness increases, the worldwide demand for cabbage continues

to grow steadily. Cabbages come in various forms, varying in size, color, texture, and

nutritional properties. An experiment was conducted to distinguish significant differences in

relative water content (RWC of leaf and RWC of head), relative oxygen species (MDA and

H_2O_2), and antioxidant properties (POD, APX, and CAT) between red and green cabbage

varieties. Cabbage samples were grown under fertilizer and control conditions to observe the

impact of fertilizers on the acquisition of these properties. The results indicated that fertilizer

application positively influenced the acquisition of relative water content, relative oxygen

species, and antioxidant properties in both cabbage varieties. The results emphasized that red

cabbage excelled in antioxidants and ROS levels, containing higher amounts compared to

green cabbage. Conversely, green cabbage showed greater relative water content in both

cultivation conditions. These findings suggest that consumers seeking higher antioxidant and

ROS levels in their diet may benefit from incorporating more red cabbage into their meals.

Further research into the mechanisms behind differences in red and green cabbage could

inform breeding programs, enhancing nutritional traits for agricultural and dietary purposes.

Keywords: Antioxidant, Comparison, Green cabbage, Reactive oxygen species, Red

cabbage, Relative water content

Introduction

Cabbage (*Brassica oleracea* var. *capitata*, 2n = 18), one of the most important cruciferous

vegetables, is commonly grown worldwide (Singh et al. 2006) for its significance due to its

high nutritional properties and wider adaptability. Due to improved livelihood and health

consciousness, the requirement for vegetables and vegetable products is increasing day by

day (Braga, Coletro, and Freitas 2019). Cabbage can be consumed either raw or processed in

different ways, e.g., boiled, fermented, or used in salads, so a steady supply is required year-

round. In 2020, world production of cabbages (combined with other brassicas) was 71 million

tons (FAOSTAT 2022).

A wide range of vitamins, minerals, fiber, and carbohydrates can be obtained from the Cole

crops which also contain high amounts of vitamin C, soluble fiber, and multiple potential

anti-cancer nutrients (Chun et al. 2004). Among them, the antioxidant property is the most

important dietary point of view. Phytochemicals such as vitamin C, flavonoids, carotenoids,

and other phenolics are the constituents that contribute to this property. Due to its antioxidant (Vega-Galvez et al. 2023; Liang et al. 2019) anti-inflammatory (Kasarello et al. 2022), and antibacterial (Arrais et al. 2022) properties, cabbage has widespread use in traditional medicine (Gruszecki et al. 2022), in the alleviation of symptoms associated with gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as in the treatment of minor cuts and wounds and mastitis (Melim et al. 2022; Abramowitz et al. 2022; Tsania, Willy, and Sawitri 2023). Fresh cabbage juice, prepared either separately or mixed with other vegetables such as carrots and celery, is often included in many commercial weight-loss diets (Šamec et al. 2011).

The main varieties of cabbage cultivars are red, white (including spring greens and green), and savoy cabbages (Pérez and Cartea 2008). The different cultivated types of cabbage show great variation in terms of size, shape, color of leaves, and the texture of the head (Moreb et al. 2020; Singh et al. 2006). The mineral, nutritional, and antioxidant content varies throughout cabbage cultivars (Yue et al. 2024). Consequently, to maintain a balanced diet, understanding the distinctions between the major varieties of cabbage is crucial (Turner, Luo, and Buchanan 2020). Green cabbage (*Brassica oleracea* var. *capitata*) is the most commonly consumed type of cabbage. Previous research indicates that Red cabbage (*B. oleracea* var. *capitata* f. *rubra*) exhibits a superior nutritional profile, characterized by elevated levels of nutrients and bioactive compounds, thereby establishing its precedence over Green cabbage. Red cabbage boasts a wealth of nutrients, including amino acids and organic acids, making it a notable source of dietary fibre for individuals (Zayed et al. 2023). Recent investigations have further illuminated its polyphenolic content, particularly anthocyanins, renowned for their potent antioxidant properties and adeptness in scavenging free radicals (Ghareaghajlou, Hallaj-Nezhadi, and Ghasempour 2021). Epidemiological inquiries have also implicated red cabbage consumption in mitigating the risk of various ailments, encompassing cancer, cardiovascular disease, metabolic disorders, and Alzheimer's disease (Manchali, Chidambara Murthy, and Patil 2012). Collectively, these findings underscore the favorability of red cabbage in dietary contexts, owing to its low-calorie profile, ample fiber content, and nutritional richness.

Prior studies (Ashfaq 2018; Yue et al. 2024) compared red and green cabbage according to quality indices and mineral elements; however, the antioxidants have not yet been examined. The present study aims to examine the antioxidant, reactive oxygen species, and relative water content of red and green cabbage as well as to determine how fertilizer affects these

variables. This research might help in designing future breeding programs to meet nutritional needs through high-quality cabbage production.

Materials and Methods

Experimental site

The field experiment was conducted at the research field of the Noakhali Science and Technology University, Noakhali-3814, Bangladesh (22.79007, 91.10147). The annual average temperature of the region is 25.6 □ with an average rainfall of 3,302 mm, soil pH of 7.5, and salinity of 4.32 dSm^{-1} (Misu et al. 2023; Osman et al. 2020; Ahmed et al. 2024; Islam et al. 2023).

Plant Materials and Experimental Design

Seedlings of two cabbage genotypes, Ruby King (Red cabbage from Takii and Co. Ltd. Japan) and Dream Ball (Green cabbage from East Bengal Seed Company, Bangladesh), were collected from Nobogram Khamarbari, Noakhali Sadar for the experiment. A Randomized Complete Block Design (RCBD) with three replications was used to conduct the experiment. Two treatments (fertilizers and genotypes) were used for this research and the applied fertilizer dosage rates were presented in the Supplementary Table.

Sample preparation

A small piece of 0.1 g sample was taken from the head of each germplasm and stored in an icebox to prevent microbial growth and slow down enzymatic activity. These prepared samples were then examined to determine the relative water content, and reactive oxygen species (ROS) content and to measure their antioxidant activity. The biochemical analysis of the samples was conducted in the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202.

Determination of Relative Water Content (RWC)

Fresh samples of leaf and head were collected, weighed, and dried in an oven at 70°C for 48 hours. The fresh and dry weight of the samples were used to determine the Relative Water Content using the following formula (González and González-Vilar 2003).

$$RWC = \frac{TW - DW}{FW - DW} \times 100\%$$

Here, TW = Total Weight; DW = Dry Weight and FW = Fresh Weight

Determination of Reactive Oxygen Species

Malondialdehyde (MDA) Activity

A 0.1g sample was ground with 2 ml of 0.1% TCA solution and centrifuged at 11000 rpm for

20 min. The supernatant was transferred to a new tube, mixed with 4ml of 20% TCA

containing 0.5% TBA, and boiled at 70°C for 30 min before quickly cooling on ice. The

resulting supernatant was collected after centrifugation at 5000 rpm for 5 min. Absorbance

was measured at 532nm using a spectrophotometer, with all steps conducted at 4°C except

for absorbance measurement. The following equation is used to calculate MDA concentration

(Bispo et al. 2014).

MDA (μmol/g FW) $= \left(\frac{Absorbance}{Extinction\ coefficient} \right) \times Dilution\ factor$

Here, Extinction coefficient of MDA = 155m/M/cm

Hydrogen Peroxide (H_2O_2) Activity

A 0.1g sample was mixed with 2 ml of 0.1% TCA in a mortar and pestle at 4°C. After

centrifugation at 11000 rpm for 20 min, the supernatant was transferred to a new tube and

combined with phosphate buffer (10 mM) and potassium iodide (1M) in a 0.5 ml: 0.5 ml: 1

ml ratio, left in darkness for 1 hour. The absorbance was measured at 390 nm.

H_2O_2 (nmol/g FW) $= \left(\frac{Absorbance}{Extinction\ coefficient} \right) \times Dilution\ factor$

Here, Extinction coefficient of H_2O_2 = 0.28 μ/M/cm

Determination of antioxidant activity

0.05 grams of each sample were homogenized with 3 mL of 50 mM potassium phosphate

buffer (pH 8.0) and centrifuged at 11000 rpm for 20 minutes. The clear supernatant was then

used to assay POD, APX, and CAT activity.

Peroxidase activity (POD)

Peroxidase activity was assessed following the standard protocol (Nakano and Asada 1981).

A mixture comprising 1.8 mL of 50 mM potassium phosphate buffer (pH 8.0), 0.3 mL of

EDTA solution (2.5 mM), 0.3 mL of hydrogen peroxide (H_2O_2) solution (200 mM), and 0.3

mL of guaiacol solution (100 mM) was prepared in an Eppendorf tube. The enzymatic

reaction commenced upon the addition of 0.1 mL of enzyme extract, and changes in

absorbance were promptly recorded at 470 nm at 30-second intervals over a span of 2

minutes. Peroxidase activity was quantified based on the rate of absorbance increase per minute.

Ascorbate Peroxidase Activity (APX)

Following the protocol (Nakano and Asada 1981), ascorbate peroxidase activity was assessed. Reaction mixtures in Eppendorf tubes contained 1.8 mL of 50 mM potassium phosphate buffer (pH 8.0) supplemented with 0.3 mL each of 2.5 mM EDTA, 200 mM hydrogen peroxide (H_2O_2), and 2.5 mM ascorbic acid. Enzymatic reactions began with the addition of 0.1 mL of enzyme extract. Absorbance changes were tracked at 290 nm using a spectrophotometer at 30-second intervals over 2 minutes. Ascorbate peroxidase activity was determined based on the rate of absorbance increase per minute.

Ascorbate peroxidase (µmol/min/g FW) $= \dfrac{\left[\left(\frac{Absorbance\ difference}{min}\right) \times Dilution\ factor \times 1000\right]}{2.8 \times 1000}$

Here, Extinction Coefficient = 2.8/M/cm of APX

Catalase Activity (CAT)

Catalase activity was evaluated following the standard methodology (Aebi 1984). In brief, a reaction mixture was prepared within an Eppendorf tube, consisting of 2.1 mL of 50 mM potassium phosphate buffer (pH 8.0), 0.3 mL of 2.5 mM EDTA solution, and 0.3 mL of 200 mM hydrogen peroxide (H_2O_2) solution. The enzymatic reaction commenced upon the addition of 0.1 mL of the enzyme extract. Using a spectrophotometer, changes in absorbance at 240 nm were monitored at 30-second intervals for a duration of 2 minutes. Catalase activity was subsequently determined based on the calculated increase in absorbance per minute.

Catalase (mmol/min/g FW) $= \dfrac{\left[\left(\frac{Absorbance\ difference}{min}\right) \times Dilution\ factor \times 1000\right]}{40 \times 1000}$

Here, Extinction Coefficient = 40/M/cm of CAT

Statistical Analysis

A two-way analysis of variance (ANOVA) was conducted to assess the significant differences in mean performances across various parameters. Tukey's (HSD) post-hoc test was employed for multiple comparisons among the groups. Additionally, a correlation matrix was generated using all cabbage variables to explore potential correlations. All statistical analyses were carried out using the R programming environment and visualized using MS Excel 2019.

Results

Antioxidant Activity, ROS, and Relative Water Content Analysis

The comparison of mean values for studied variables between red cabbage and green cabbage

is presented in Figures 1 to 3. In fertilizer-treated plants, The RWC of leaf was higher in

green cabbage (97.197%) than the red cabbage (97.088%) while under controlled conditions

(without fertilizer) the value was lower in red cabbage (95.95%) than in green (96.58%). For

the RWC of the head, a similar pattern was observed in both conditions (Figure 1).

Figure 1. Relative Water Content (percentage) in Red and Green Cabbage. Different letters

indicate statistical differences.

The MDA content was higher in red cabbage (0.242 μM/g FW) compared to its counterpart,

green cabbage (0.011 μM/g FW), in fertilizer-applied plants. Similarly, in controlled

conditions, the MDA content in red cabbage (0.146 μM/g FW) exceeded that of green

cabbage (0.008 μM/g FW). Consistently, red cabbage exhibited greater hydrogen peroxide

(H_2O_2) concentration compared to green cabbage under both fertilizer-treated (7.316 nM/g

FW vs. 3.143 nM/g FW) and controlled condition (4.83 nM/g FW vs. 2.104 nM/g FW)

conditions (Figure 2).

Figure 2. Reactive Oxygen Species (MDA and H_2O_2) in Red and Green Cabbage. Different

letters indicate statistical differences.

Red cabbage demonstrated significantly elevated POD activity at 0.089 µM/min/g FW in

contrast to green cabbage at 0.0198 µM/min/g FW when subjected to fertilizer doses. Even in

controlled conditions, red cabbage maintained higher POD levels at 0.018 µM/min/g FW

than green cabbage at 0.0019 µM/min/g FW. A parallel trend was observed in ascorbate

peroxidase and catalase activities. Red cabbage exhibited augmented APX activity at 1.069

µM/min/g FW and 0.2812 µM/min/g FW, respectively, surpassing green cabbage values of

0.0116 µM/min/g FW and 0.0049 µM/min/g FW, in both cultivated conditions. Likewise, red

cabbage displayed heightened CAT levels at 0.116 mM/min/g FW and 0.0514 mM/min/g FW

compared to green cabbage at 0.0366 mM/min/g FW and 0.00167 mM/min/g FW under both

experimental conditions.

Figure 3. Antioxidant Activity (POD, APX, and CAT) in Red and Green Cabbage. Different

letters indicate statistical differences.

Analysis of Variance

A two-way ANOVA was carried out to understand if there were any significant variations in

average performance among different parameters. For combined data, all the parameters were

found to express statistically significant variations in the case of genotype, treatment, and

genotype-treatment interaction at 0.1% and 1% level of significance except RWC% of leaf

which showed significant variation only at 1% level.

Table 1: Analysis of Variance (Mean Sum Square values with the level of significance)

Source of variations	RWC (%)		MDA	H_2O_2	POD	APX	CAT
	Leaf	Head	532 nm	390 nm	470 nm	290 nm	240 nm

Genotype	2.072**	13.3***	0.5129***	178.46***	0.027568***	6.673***	0.04937***
Treatment	11.397***	960.8***	0.0372***	46.61***	0.029221***	2.37***	0.02714***
Genotype × Treatment	1.048***	40.2***	0.0325***	7.84***	0.010399***	2.291***	0.00773***
Residuals	0.105	0.2	0.0001	0.07	0.000019	0.003	0.00007
***Significance at P<0.001, **Significance at P<0.01, *Significance at P<0.05							

Correlation Analysis

The correlation analysis for the RWC%, ROS, and antioxidant compounds was conducted separately for fertilizer treatments, and for their combined conditions (Figure 4).

In fertilizer-treated plants, the analysis revealed no strong positive or negative relationships (Figure 4A). However, in green cabbage, the RWC of leaf was in moderate positive correlation with POD while in negative (moderate) correlation with APX; MDA also exhibited a moderate positive relationship with CAT but a negative one with APX. Furthermore, H_2O_2 and POD are positively (moderate) correlated with each other. In the case of red cabbage, the RWC of leaf was in moderate positive correlation with APX while in negative (moderate) correlation with MDA whereas MDA exhibited a moderate positive relationship with CAT. H_2O_2 with POD and APX with CAT are positively (moderate) correlated with each other.

There were also no strong positive or negative relationships were observed for both cabbage without fertilized conditions (Figure 4B). However, in green cabbage, the RWC of leaf was in moderate positive correlation with MDA, POD, and H_2O_2. Though the RWC of head was in moderately positive correlation with H_2O_2, it exhibited a negative one with CAT. Similarly, APX had in moderately positive correlation with H_2O_2, it exhibited a negative one with POD while MDA had in positive (moderate) correlation with POD. In red cabbage, the RWC of leaf was in moderate positive correlation with RWC of head, MDA, APX, and H_2O_2

whereas, H_2O_2 and CAT are positively (moderate) correlated with each other.

Figure 4: Correlation plot showing the relationship between RWC of leaf, RWC of head, MDA, H_2O_2, POD, APX, and CAT of green cabbage (lower matrix) and red cabbage (upper matrix) A) with fertilizer B) without fertilizer C) with and without fertilizer (combined) at $\alpha \leq$ 0.05.

In overall correlation, multiple sets of significant correlations were observed at $\alpha \leq 0.05$. Among them, a strong positive relationship between the RWC of the leaf and the RWC of the head whereas H_2O_2, POD, APX, and CAT have a strong positive correlation with MDA. Similarly, H_2O_2 possesses a strong positive correlation with POD, APX, and CAT. Moreover, a positive association was also observed in the case of POD with APX and CAT. Furthermore, APX displayed a highly positive correlation with CAT. No negative relationships were observed in this correlation study among these variables.

Discussions

This study provides a comprehensive comparison of the relative water content of the leaf and head and other antioxidant contents of red and green cabbage. These two types of cabbages were cultivated under both fertilizer and without fertilizer conditions. It was found that there were significant differences among the studied variables between these two varieties which is in line with several researchers (Yue et al. 2024; Zayed et al. 2023).

Green cabbage is the most common cabbage, but red cabbage has more nutritional value. Previous studies have also shown the health benefits of consuming chemoprotective substances found in red cabbage (Zayed et al. 2023; Mateljan 2001). In this study, it was also observed red cabbage possesses a higher amount of quality indices than green cabbage. Red cabbage held a higher amount of MDA, H_2O_2, POD, APX, and CAT content compared to green cabbage in both cultivated conditions. Red cabbage generally contains more anthocyanin pigments (Dyrby, Westergaard, and Stapelfeldt 2001), which are water-soluble and contribute to its vibrant color. These pigments are stored in the vacuoles of the cabbage cells, along with water due to this reason they contain more RWC than green. However, the relative water content of the leaf was found to be lower in red cabbage. The H_2O_2 content and MDA content can indicate oxidative stress levels in plant tissues, which can vary depending on factors such as environmental conditions, genotype, and developmental stage. Generally, higher levels of H_2O_2 and MDA suggest increased oxidative stress (Singh, Sharma, and Singh 2010). Studies have shown that red cabbage tends to have higher levels of anthocyanins compared to green cabbage. Anthocyanins are antioxidants that can help mitigate oxidative

stress. In terms of, research has indicated that red cabbage has higher levels of H_2O_2 content compared to green cabbage due to its richer antioxidant content depending on factors such as cultivar and growing conditions (Shiyab 2015), which is in line with our findings. The rich red color of red cabbage reflects its concentration of anthocyanin polyphenols, which contribute to red cabbage containing significantly more protective phytonutrients than green cabbage. A measure of the antioxidant capacity, of red cabbage is also six to eight times higher than that of green cabbage. Mainly due to the presence of a rich amount of catalase in red one, the antioxidant activities are higher. In our findings, the catalase activity is also significantly higher in red than the green cabbage. CAT is significant in guarding against harm caused by oxidative stress by accelerating the breakdown of hydrogen peroxide into water and oxygen. By effectively neutralizing free radicals, these enzymes contribute to upholding the cellular redox balance within plant cells (Rana and Saklani 2019). The prior findings also align with our current observation (Shiyab 2015; Singh, Sharma, and Singh 2010). They also found that the CAT and APX activity is higher in red cabbages compared to green. These compounds protect biological systems against oxidative stress in the development and progression of various human diseases. Findings of our results exhibited in the comparison between green and red cabbages in terms of RWC, ROS, and antioxidants red cabbage is ahead of green cabbage, which is in line with other researchers' findings (Zayed et al. 2023).

Knowledge of the correlation between the variables is essential as this may help in constructing suitable selection criteria for further breeding purposes (Kibar, Karaağaç, and Kar 2014). To determine the relationships between the studied variables (RWC of leaf and head, MDA, H_2O_2, POD, APX, CAT) correlation coefficients were calculated, which indicates that multiple sets of associations exhibited significant positive correlations at $\alpha \leq 0.05$.

In our study, MDA exhibited a positive correlation with H_2O_2, POD, APX, and CAT. The correlation between MDA and H_2O_2 in cabbage is influenced by various factors. The S-methyl cysteine sulphoxide (SMCO) and thiocyanate ion (SCN−) contents in cabbage heads varied significantly, suggesting a potential impact on MDA and H_2O_2 levels (Bradshaw and Borzucki 2006). Higher MDA and H_2O_2 levels in the floral buds of a non-heading Chinese cabbage line were observed, indicating a positive correlation between these two compounds (Yuan et al. 2007). Prior studies found a positive correlation between POD and (MDA) accumulation in Brassica, with an increase in MDA under salinity stress (Hussain et al.

2024). This suggests that peroxidase activity may be linked to MDA production in response to stress. Catalase activity also showed a positive correlation with ascorbate peroxidase. Ascorbate peroxidase primarily scavenges hydrogen peroxide (H_2O_2) by using ascorbate as an electron donor, while catalase decomposes hydrogen peroxide into water and oxygen (Karyotou and Donaldson 2005). As the ROS levels are also found to be in a higher amount in our case the APX and CAT along with POD showed a positive association to cope with the upregulated ROS.

Conclusions and Recommendations

This study unveils the profound influence of cultivation conditions on the relative water content, ROS, and antioxidant profile of cabbage, particularly distinguishing between red and green varieties. Notably, due to fertilizer application, both cabbages displayed enhanced leaf and head relative water content, alongside elevated MDA, H_2O_2, POD, APX, and CAT levels, demonstrating their resilience and antioxidant potency. These findings underscore the potential of fertilizer application to amplify the antioxidant potential of red and green cabbage, positioning it as a promising strategy for enhancing antioxidant enrichment in agricultural produce. In comparison with red and green cabbages, this study presents red cabbage as a superior source of antioxidants compared to its green counterpart, outperforming it in multiple parameters, except for leaf relative water content. This suggests that red cabbage could be preferred for those seeking to maximize antioxidant intake from cabbage consumption. Future investigations should delve into a broader context considering its breeding program and practical implications at the farmer level.

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Novelty Statement

This study provides novel insights into the differential impact of fertilizer application on red and green cabbage varieties, highlighting that red cabbage exhibits superior antioxidant properties and ROS levels, whereas green cabbage demonstrates higher relative water content. Moreover, the research also identifies significant variations in the nutritional

properties of red and green cabbage, suggesting that red cabbage may be more beneficial for consumers seeking high antioxidant and ROS levels, thereby guiding future breeding programs to enhance these specific nutritional traits.

Authors' Contribution

Md. Rayhan Sojib: Experimental design, Field and Laboratory experiments, Data collection and analysis, Writing the manuscript (first draft)

Nusrat Jahan Methela: Experimental design, reviewing the manuscript, Supervising the whole experiment

Biswajit Das and Md Nure Adil Siddique: Writing the manuscript

Sadia Sultana, Ataul Karim, Rafiqul Islam: Data collection and Writing the manuscript (first draft)

Md Jahid Hasan Jone: Data analysis, visualization and editing of the manuscript

All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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A = With Fertilizer; **B** = Without Fertilizer; **C** = With & without Fertilizer (combined)