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Effect of Different Forms of Sulfur Fertilization on Bioactive Components and Antioxidant Activity of White Cabbage (*Brassica Oleracea* L.)

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Abstract: Cruciferous vegetables are very popular in latitudes corresponding to central and eastern Europe. They are rich in bioactive compounds such as chlorophylls, carotenoids, and polyphenols. The type and quality of fertilization has a significant impact on their chemical composition. The aim of this study was to determine the impact of specific forms of sulfur fertilization on the chemical properties of white cabbage, and to explore the effect of storage conditions on its bioactive components and antioxidant activity. The research material was the late cultivar of white cabbage 'Stonehead', fertilized with a dose of 30 kg S·ha^{-1}, administered in the form of elemental sulfur, ammonium sulfate, and potassium sulfate. Sulfur fertilization had a significant impact on the parameters selected for this study, and the form in which it was applied resulted in different effects on these individual parameters. For all parameters investigated, the effect of sulfur fertilization was detectable. Antioxidant properties, determined as both ABTS and DPPH radical-scavenging activities, were the highest in cabbage harvested from the plot treated with potassium sulfate

Keywords: cruciferous; white cabbage; sulfur fertilization; bioactive components; antioxidant activity

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1. Introduction

Sulfur, as an essential macronutrient, plays an important role in plant growth and development [1]. Photosynthetic organisms use sulfate (SO_4^{2-}) as a primary sulfur source to synthesize an array of S-containing metabolites, including the amino acids cysteine and methionine, the tripeptide glutathione, vitamins, and cofactors (such as thiamine, biotin, and coenzyme A), and chloroplastic sulfolipids [2,3]. Moreover, primary sulfur assimilation is a prerequisite for the synthesis of glucosinolates in Brassicales.

Sulfur deficiency in soil is an increasing problem in many parts of the world. Over the last three decades, continuously declining soil sulfur levels have been reported for many European countries. This is largely due to the reduction in sulfur dioxide emissions from industrial sources, the increasing use of low sulfur fertilizers, and the increasing size of crop yields due to technological advances [4]. All sulfur in soil—whether applied as elementary sulfur, manure, or sulfate—is taken up by plants in the form of sulfate. Sulfate from fertilizer is immediately available as a nutrient and is easily absorbed by plants. Sulfate is highly mobile in the soil and reaches plant roots quickly. Sulfur applied during early stages of development and periods of intense plant growth benefits from combination with other fertilizers, especially nitrogen. When applied as elementary sulfur, oxidization to sulfate by soil microbes is necessary, which takes time. Elementary sulfur also has a strong acidifying effect.

As an economically important member of the Brassicaceae family, white cabbage (*Brassica oleracea* var. capitata L.) is consumed worldwide and is considered a good source

of bioactive phytochemicals. The most striking feature of such Brassica crops is their high levels of glucosinolates, which exert chemoprotective and anticancer effects [1,2,5–7].

Due to its chemical composition, white-head cabbage has high nutritional value. It constitutes a valuable source of provitamin A, the B group vitamins, and vitamin C, as well as minerals—predominantly calcium, potassium, and iron. Beyond glucosinolates, it contains organic acids, carotenoids, proteins made of amino acids beneficial to the human body, and dietary fiber [8]. This vegetable is also rich in several bioactive compounds and is the simplest form of functional food [9,10]. Brassicas are rich in carotenoids; however, the coloration of these plants can be misleading, as carotenoid-rich vegetables/fruit are assumed to be yellow to red. In brassicas, the color of carotenoids is masked by the green of chlorophyll. The coloration of plants due to the presence of carotenoids is related to the structure of these compounds. They belong to the group of tetraterpenes, which have two cyclohexyl rings connected by a carbon chain including many conjugated double bonds. The color of plants is derived from the number of double bonds (e.g., yellow corresponds to a minimum of seven double bonds) [8].

Organic acids are widespread in fruit and vegetables. Their quantity and composition depend on biotic factors (i.e., species, variety, ontogeny) and abiotic factors (climate-related and soil conditions, etc.), while their content tends to decrease with plant maturation [11,12]. Vegetables generally contain low concentrations of organic acids but are of interest due to their important role as natural antibiotics, flavor enhancers, digestive gland stimulants, and intestinal peristalsis enhancers [13]. Of all vegetables, brussels sprouts (*Brassica oleracea* L. var. gemmifera (DC.)), parsnips (*Pastinaca sativa* L.), tomatoes (*Solanum lycopersicum* L.), and aubergines (*Solanum melongena* L.) have the highest amount of total organic acids (0.4–0.6 g·100 g^{-1} FM) [8].

The aim of this study was to determine the effect of the form of sulfur used for fertilization on the bioactive component content and antioxidant activity of white-head cabbage, and the effect of storage conditions on changes to its chemical composition.

2. Materials and Methods

2.1. Materials

The research material was the late cultivar of white cabbage 'Stonehead', grown at the Vegetables and Ornamental Plants Research and Training Station (51°11′5″ N, 17°1′53″ E) of the Department of Horticulture, Wrocław University of Environmental and Life Sciences. Chemical analyses were performed at the laboratory of the Department of Fruit, Vegetable and Plant Nutraceutical Technology, Wrocław University of Environmental and Life Sciences.

Stonehead is an old cultivar with good storage ability; it is resistant to many common cabbage diseases as well as cracking and splitting as it matures. It is also very popular in organic and traditional farms for use in the production of sauerkraut.

Plots were fertilized with a dose of 30 kg S·ha^{-1}, administered in the form of elemental sulfur (2), ammonium sulfate (3), and potassium sulfate (4), while trial (1) was a control subject with no fertilization. The selected forms of sulfur fertilizers were those most often used to treat vegetable crops. Cabbage heads were harvested 65 DAT (days after transplanting), when heads were compact and hard. After harvesting, cabbages selected for analyses were cleaned by removing the top leaves and those that were dirty or wilted. Samples were taken from five randomly selected heads per repetition. Vitamin C content was determined from the fresh material, while the remaining values were determined from samples crushed in a Thermomix machine to a uniform pulp, which was maintained frozen until required.

Five randomly selected heads from each treatment group were kept in cold storage conditions at a temperature of 0–4 °C and relative air humidity 90–96% for a period of 5 months.

2.2. Methods

The determination methods were as follows: vitamin C as L-ascorbic acid content was determined according to standard methods [14]; total polyphenol content, calculated as gallic acid, was determined according to the Folin–Ciocalteu method [15] (determination of total polyphenol content was performed in methanol extracts (80% *v/v* ratio of material to extraction reagent, 1:5)); and determination of chlorophyll and carotenoid content was performed according to the method described by Nawirska-Olszańska et al. [16], in which the chlorophyll and carotenoid content is calorimetrically determined by measuring absorbance at the absorbance maximum for these pigments using a Shimadzu UV-160A spectrophotometer.

The ABTS antioxidant assay was performed according to the method determined by Re et al. [17], using a Shimadzu UV-2401 PC spectrophotometer (Kyoto, Japan). All determinations were performed in triplicate.

The DPPH radical-scavenging activity of white cabbage was determined according to the method of Yen and Chen [18]. To each 0.5 mL supernatant, 0.5 mL of DPPH and 1.5 mL of ethanol was added. The mixture was shaken and left at room temperature for 10 min. The antioxidant capacity of each sample was measured separately by recording the absorbance at 517 nm in a spectrophotometer (Shimadzu UV-2401 PC). Ethanol was used as a blank. All determinations were performed in triplicate.

The analysis of organic acids was performed via high-performance liquid chromatography (HPLC), with 0.001 N sulfuric acid in isocratic mode, at 210 nm wavelength and with flow rate of 0.6 mL·min^{-1} [19]. The assay was performed using a Dionex liquid chromatograph (USA), adapted to an UltiMate 3000 detector with diode array. The detector was connected to an LPG-3400A pump, an EWPS-3000SI autosampler, a TCC-3000SD column thermostat, and the Chromeleon v.6.8 computer software. Separation was carried out on an Aminex HPX-87H column (300 × 7.8 mm) with a Bio-Red IG Cation H pre-column (30 × 4.6), at 65 °C. Determination of acids using HPLC required the following sample preparation: vegetable (10 g) was diluted to 50 mL with bidistilled water and clarified by centrifugation at 6000× *g* for 15 min. The extract was filtered through a 0.45 µm Millipore filter and a 20 µL sample was used for HPLC analysis of organic acid content.

Shredded samples were used for the determination of amino acid content. Analysis was carried out according to the protocols specified by the manufacturer of the amino acid analyzer. The amino acid composition was determined by ion-exchange chromatography after 24 h of hydrolysis with 6 N HCl at 110 °C. After cooling, filtering, and washing, the hydrolyte was evaporated in a vacuum evaporator at temperatures below 50 °C. The dry residue was dissolved in a buffer of pH 2.2. The prepared sample was analyzed via the ninhydrin method [20]. Buffers of pH 2.6, 3.0, 4.25, and 7.9 were applied. The ninhydrin solution was buffered at pH 5.5. The hydrolyzed amino acids were determined using an AAA-400 amino acid analyzer, supplied by INGOS, Czech Republic. A photometer, working with two wavelengths (440 and 570 nm), was used as the detector. The procedure also involved use of a 350 × 3.7 mm column packed with the ion exchanger Ostion LG ANB (INGOS), with column temperature maintained at 60–74 °C and detector temperature at 121 °C. Calculations were carried out according to the external standard. No analysis concerning tryptophan was conducted.

Statistical analysis was performed using one-way analysis of variance (ANOVA). Differences were evaluated using Duncan's test at significance level $p < 0.05$. All statistical calculations were performed using the computer software Statistica v.8.1. All analyses were performed in triplicate. Results are given per fresh weight of cabbage.

3. Results and Discussion

3.1. Antioxidant Activity and Vitamin C, Total Polyphenol, Chlorophyll, and Carotenoid Content

Table 1 presents the vitamin C, total polyphenol, chlorophyll a and b, and carotenoid content, alongside the antioxidant activity (determined as ABTS and DPPH) for fresh white cabbage and after five months of storage.

Table 1. Antioxidant activity and vitamin C, polyphenol, chlorophyll a and b, and carotenoid content for fresh and stored white cabbage.

	1	2	3	4	S1	S2	S3	S4
Vitamin C mg·100 g^{-1} FM	37.84 ± 1.09 ^{d}	39.02 ± 1.12 ^{c}	41.21 ± 0.99 ^{bc}	45.04 ± 1.15 ^{a}	34.16 ± 1.12 ^{e}	28.46 ± 1.05 ^{f}	37.44 ± 1.13 ^{d}	29.41 ± 1.09 ^{f}
Phenolics mg·100 g^{-1} FM	60.74 ± 094 ^{d}	65.94 ± 0.81 ^{bc}	58.89 ± 1.53 ^{d}	70.36 ± 2.63 ^{b}	69.53 ± 0.88 ^{b}	75.47 ± 0.74 ^{a}	69.80 ± 0.75 ^{b}	74.27 ± 2.04 ^{a}
Chlorophyll a + b mg·100 g^{-1} FM	116.9 ± 4.23 ^{e}	161.7 ± 4.57 ^{b}	128.6 ± 3.77 ^{d}	173.1 ± 6.29 ^{a}	106.1 ± 3.37 ^{f}	141.7 ± 2.36 ^{c}	115.5 ± 1.52 ^{e}	143.8 ± 6.29 ^{c}
Carotenoids mg·100 g^{-1} FM	4.94 ± 0.04 ^{f}	11.23 ± 1.03 ^{b}	13.87 ± 0.27 ^{a}	9.43 ±0.29 ^{c}	3.82 ± 0.09 ^{g}	10.92 ± 0.97 ^{b}	6.29 ± 0.42 ^{e}	8.54 ± 0.11 ^{d}
ABTSμMol·g^{-1} FM	1.26 ± 0.02 ^{e}	1.46 ± 0.02 ^{d}	1.70 ± 0.02 ^{c}	1.87 ± 0.04 ^{bc}	1.63 ± 0.01 ^{c}	2.06 ± 0.01 ^{b}	1.99 ± 0.01 ^{b}	2.63 ± 0.04 ^{a}
DPPHμMol·g^{-1} FM	6.33 ± 0.06 ^{e}	6.80 ± 0.02 ^{e}	12.57 ± 0.11 ^{b}	15.50 ± 0.04 ^{a}	7.99 ± 0.04 ^{d}	10.56 ± 0.11 ^{c}	13.54 ± 0.10 ^{b}	16.27 ± 0.08 ^{a}

1. Control without sulfur fertilization; 2. 30 kg S·ha^{-1} elementary sulfur; 3. 30 kg S·ha^{-1} ammonium sulfate; 4. 30 kg S·ha^{-1} potassium sulfate; S1. control without sulfur fertilization; S2. 30 kg S·ha^{-1} elementary sulfur; S3. 30 kg S·ha^{-1} ammonium sulfate; and S4. 30 kg S·ha^{-1} potassium sulfate. Mean values with different letters (a–g) within the same row were statistically different (p = 0.05). Values expressed as the mean ± standard deviation.

White-head cabbage has moderate vitamin C content but, due to its large share of total vegetable consumption, it constitutes an important source of this vitamin for the body. In this study, the highest vitamin C content was found in cabbage subjected to fertilization with sulfur in the form of potassium sulfate (45.04 mg·100 g^{-1} FM), while the lowest content was seen in the control sample (37.84 mg·100 g^{-1} FM). A similar vitamin C content was found in a study of different varieties of late white cabbage by Gajewski and Radzanowska [21]. The highest levels of vitamin C were found in the Galaxy variety (40.9 mg·100 g^{-1} FM), and the lowest in the Alfama variety (36.8 mg·100 g^{-1} FM). In her literature review, Podsędek [22] noted significant differences in the vitamin C levels reported in different studies, ranging from 18.8 to 47.0 mg·100 g^{-1} FM. After 5 months of storage, the vitamin C content decreased. The greatest loss (of 35%) was recorded in cabbage grown on the plot treated with potassium sulfate, while the smallest loss (of 8%) was seen in cabbage grown on the plot treated with ammonium sulfate. Research by Hounsome et al. [23] revealed a significant loss (of up to 20% of initial content) of vitamin C after 3 months of storage, followed by a further loss of up to 50% after 5 months of storage. In the study by Godlewska at al. [24], the measured vitamin C content was higher than that determined in the present study for fresh cabbage, a difference which may be attributed to different weather conditions in the respective research years. However, the chlorophyll a and b and carotenoid contents were similar.

As with the vitamin C content, the highest total polyphenol content of was determined in cabbage grown with potassium sulfate as fertilizer, while the lowest was seen in cabbage grown on the plot where ammonium sulfate was used. During storage, there was an increase in the total polyphenol content. The highest increase was found in samples from the plot where elemental sulfur was used, with a change from 65.94 to 75.47 mg·100 g^{-1} FM. Statistical calculations revealed that there was no significant difference in the polyphenol content of cabbage from the control plot and from the plot where ammonium sulfate was used. The data collected in a literature review by Šamec et al. [25] demonstrate that the total polyphenol content of white cabbage is highly variable, depending on the extracting agent, the variety under analysis, and the region of origin, i.e., the growing conditions.

Chlorophyll content was highest in cabbage grown with potassium sulfate as fertilizer, and carotenoid content was highest in cabbage from plots where ammonium sulfate was used. Hence, we conclude that sulfur fertilization had a positive impact on vitamin C, chlorophyll, and carotenoid contents. Zhou et al. [26] observed that sulfur fertilization induced significant increases in the individual glucosinolate, carotenoid, chlorophyll, and total phenolic contents. The phenolic content of radish sprouts cultivated using 20, 60,

or 100 mg/L of sulfate were 20.7%, 40.4%, and 40.8% higher, respectively, than those of 7-day-old control sprouts. However, the authors observed no detectable effects on the 4-methoxy-glucobrassicin or vitamin C contents. Antioxidant activity was also higher in cabbages subjected to fertilization than in the control.

Chlorophyll and carotenoid content decreased after storage in all cases, but these losses were inconsistent. The highest chlorophyll loss (of 17%) was observed in cabbage grown using potassium sulfate, while the lowest was seen in cabbage grown using ammonium sulfate. A similar phenomenon was observed for vitamin C content, with the highest loss detected in stored cabbage grown using potassium sulfate, and the lowest in stored crops grown using ammonium sulfate. Exactly the opposite was found for the loss in carotenoids, with the highest decrease (of 61%) observed in crops from the plot treated with ammonium sulfate, and the lowest (of 16%) in crops whose fertilization method involved potassium sulfate.

Antioxidant properties, determined both as ABTS and DPPH radical-scavenging activities, were highest in cabbage harvested from the plot treated with potassium sulfate. Slightly lower activities were found in cabbage from the control plot, which also exhibited the lowest bioactive component content determined. Other studies have also reported that the antioxidant activity of Brassicaceae increased when sulfur was supplied to the plants [27]. Following storage, there was an increase in both ABTS and DPPH radical-scavenging activities. DPPH increased significantly in cabbages from plots where elemental sulfur was used as fertilizer. This was accompanied by a significant increase in the total polyphenol content.

Research conducted by Šamec et al. [28] suggests that the antioxidant activity of white cabbage decreases with maturity. According to research results published by Godlewska et al. [24], the measured ABTS radical-scavenging activity was the same as that determined in this study; however, the DPPH radical-scavenging activity was significantly less. This may be due to the existence of fewer DPPH-reactive components in cabbage sprayed with plant extracts [24]. A literature review by Podsędek [22] indicated that some procedures result in a decrease in antioxidant activity, while blanching results in its increase.

3.2. Organic Acids

The literature review available for this study included no results related to the organic acid content of white-head cabbage. In the 'Stonehead' cultivar studied here, we identified four acids, namely phytic acid, citric acid, malic acid, and fumaric acid (Table 2). The phytic acid content was found to be the highest, ranging from 87.7 to 36.8 g·kg^{-1} FM. The highest phytic acid content was found in crops from the plot where ammonium sulfate was used, while the lowest was detected in crops from the plot where elemental sulfur was used. The fumaric acid content was the lowest, and the citric acid content was only slightly higher. However, in cabbage from the plot enhanced with potassium sulfate, citric acid was not detected at all (Table 2).

Cabbage grown on the plot treated with ammonium sulfate proved to be the richest in organic acids, whereas cabbage grown using elemental sulfur yielded the lowest organic acid contents.

Table 2. Organic acid contents of fresh and stored white cabbage grown using different forms of sulfur fertilization (g·kg^{-1} FM).

	1	2	3	4	S1	S2	S3	S4
Phytic	36.840 ± 4.1 ^{g}	63.706 ± 1.12 ^{f}	87.711 ± 2.35 ^{c}	61.509 ± 1.21 ^{f}	69.906 ± 1.91 ^{e}	75.902 ± 2.13 ^{d}	107.000 ± 10.9 ^{b}	256.827 ± 10.5 ^{a}
Citric	0.084 ± 0.001 ^{b}	0.030 ± 0.001 ^{c}	0.120 ± 0.010 ^{a}	0 ± 0.00	0.022 ± 0.001 ^{d}	0.021 ± 0.000 ^{d}	0.018 ± 0.001 ^{d}	0.087 ± 0.001 ^{b}
Malic	0.110 ± 0.020 ^{b}	0.062 ± 0.001 ^{c}	0.139 ± 0.0 2 ^{a}	0.036 ± 0.001 ^{d}	0.095 ± 0.001 ^{b}	0.078 ± 0.001 ^{c}	0.104 ± 0.001 ^{b}	0.116 ± 0.005 ^{b}
Fumaric	0.006 ± 0.001 ^{b}	0.006 ± 0.001 ^{b}	0.015 ± 0.001 ^{a}	0.003 ± 0.000 ^{b}	0.007 ± 0.000 ^{b}	0.007 ± 0.000 ^{b}	0.007 ± 0.000 ^{b}	0.011 ± 0.002 ^{a}

Explanations see Table 1.

The storage process resulted in a significant increase in phytic acid content, irrespective of the kind of fertilization used, as seen in Table 2. An increase in the content of all other acids was observed for the plot treated with potassium sulfate, while the other fertilization methods resulted in a decrease in the content of the remaining three acids. Additionally, cabbage from the plot where potassium sulfate was used as fertilizer was the most abundant in all acids after storage. In the other samples, a decrease in the acid contents after storage was typically seen, but without significant regularity.

Nawirska-Olszańska et al. [29] presented different findings based on their analysis of changes in the acid content of different varieties of pumpkin fruit after storage, noting a loss in organic acid content in all cases.

3.3. Amino Acids

The literature on the subject includes very few studies that focus on the specific amino acid content of cabbage [30,31], and, to date, no studies have focused on the form of sulfur used for fertilization and the impact of storage on parameters related to amino acids. According to [32], increasing the S content of soil reduced the cysteine and methionine content in kohlrabi by 16–28%, while the valine, tyrosine, aspartic acid, and serine values remained constant. With increasing soil S content, the levels of threonine, isoleucine, leucine, arginine, alanine, and the sum of essential amino acids decreased from 37% to 9%. The histidine concentration increased with increasing S fertilization.

Table 3 summarizes the results of content analysis for 16 of the amino acids commonly found in proteins. The protein content (i.e., the sum of individual amino acids) was 10.463–21.755 mg·g^{-1} FM.

Table 3. Amino acid content of fresh and stored white cabbage with different forms of sulfur used as fertilizer.

	1	2	3	4	S1	S2	S3	S4
	mg·g^{-1} FM							
Asp	1.068 ± 0.114 ^{f}	1.065 ± 0.009 ^{f}	1.169 ± 0.019 ^{e}	1.148 ± 0.098 ^{e}	1.201 ± 0.017 ^{d}	1.359 ± 0.008 ^{c}	1.544 ± 0.011 ^{b}	2.526 ± 0.013 ^{a}
Thr	0.359 ± 0.001 ^{d}	0.348 ± 0.001 ^{e}	0.364 ± 0.001 ^{d}	0.342 ± 0.001 ^{e}	0.354 ± 0.001 ^{e}	0.426 ± 0.002 ^{b}	0.403 ± 0.001 ^{c}	0.696 ± 0.002 ^{a}
Ser	0.470 ± 0.002 ^{c}	0.479 ± 0.002 ^{c}	0.451 ± 0.002 ^{d}	0.454 ± 0.002 ^{d}	0.585 ± 0.003 ^{b}	0.469 ± 0.002 ^{c}	0.455 ± 0.002 ^{d}	0.986 ± 0.043 ^{a}
Glu	2.975 ± 0.112 ^{d}	3.388 ± 0.214 ^{c}	2.793 ± 0.124 ^{d}	3.052 ± 0.198 ^{d}	4.142 ± 0.254 ^{c}	3.873 ± 0.157 ^{c}	4.713 ± 0.321 ^{b}	7.490 ± 0.528 ^{a}
Pro	0.516 ± 0.004 ^{e}	0.609 ± 0.002 ^{b}	0.515 ± 0.013 ^{e}	0.561 ± 0.003 ^{d}	0.617 ± 0.003 ^{b}	0.606 ± 0.017 ^{b}	0.590 ± 0.002 ^{c}	0.848 ± 0.006 ^{a}
Gly	0.322 ± 0.002 ^{b}	0.290 ± 0.001 ^{c}	0.336 ± 0.011 ^{b}	0.314 ± 0.002 ^{b}	0.301 ± 0.001 ^{c}	0.320 ± 0.002 ^{b}	0.350 ± 0.003 ^{b}	0.611 ± 0.004 ^{a}
Ala	0.639 ± 0.005 ^{b}	0.497 ± 0.003 ^{d}	0.585 ± 0.014 ^{c}	0.605 ± 0.014 c	0.523 ± 0.002 ^{d}	0.608 ± 0.004 ^{c}	0.691 ± 0.005 ^{b}	0.801 ± 0.007 ^{a}
Cys	0	0	0	0	0	0	0	0
Val	0.395 ± 0.001 ^{e}	0.376 ± 0.001 ^{e}	0.452 ± 0.002 ^{c}	0.435 ± 0.001 ^{d}	0.475 ± 0.002 ^{b}	0.470 ± 0.002 ^{b}	0.478 ± 0.002 ^{b}	0.952 ± 0.005 ^{a}
Met	0.153 ± 0.001 ^{c}	0.134 ± 0.001 ^{d}	0.152 ± 0.001 ^{c}	0.153 ± 0.001 ^{c}	0.136 ± 0.001 ^{d}	0.160 ± 0.001 ^{b}	0.161 ± 0.001 ^{b}	0.313 ± 0.001 ^{a}
Ile	0.302 ± 0.002 ^{b}	0.262 ± 0.001 ^{c}	0.302 ± 0.001 ^{b}	0.296 ± 0.001 ^{b}	0.269 ± 0.001 ^{c}	0.304 ± 0.001 ^{b}	0.304 ± 0.001 ^{b}	0.464 ± 0.003 ^{a}
Leu	0.427 ± 0.003 ^{b}	0.370 ± 0.002 ^{d}	0.439 ± 0.003 ^{b}	0.371 ± 0.002 ^{d}	0.401 ± 0.002 ^{c}	0.400 ± 0.003 ^{c}	0.448 ± 0.002 ^{b}	0.637 ± 0.005 ^{a}
Tyr	0.886 ± 0.005 ^{c}	0.852 ± 0.005 ^{d}	0.974 ± 0.007 ^{b}	0.863 ± 0.007 ^{d}	0.918 ± 0.015 ^{c}	0.871 ± 0.011 ^{d}	0.998 ± 0.009 ^{b}	1.208 ± 0.014 ^{a}
Phe	0.263 ± 0.002 ^{d}	0.230 ± 0.002 ^{f}	0.217 ± 0.002 ^{g}	0.232 ± 0.002 ^{f}	0.249 ± 0.001 ^{e}	0.301 ± 0.002 ^{b}	0.283 ± 0.002 ^{c}	0.374 ± 0.002 ^{a}
His	0.250 ± 0.002 ^{d}	0.256 ± 0.002 ^{d}	0.263 ± 0.002 ^{c}	0.271 ± 0.002 ^{c}	0.270 ± 0.001 ^{c}	0.301 ± 0.002 ^{b}	0.294 ± 0.002 ^{b}	0.586 ± 0.004 ^{a}
Lys	0.464 ± 0.003 ^{d}	0.419 ± 0.003 ^{f}	0.509 ± 0.003 ^{c}	0.446 ± 0.003 ^{e}	0.435 ± 0.003 ^{e}	0.507 ± 0.004 ^{c}	0.566 ± 0.003 ^{b}	0.745 ± 0.005 ^{a}
Arg	0.974 ± 0.008 ^{e}	1.100 ± 0.010 ^{e}	1.089 ± 0.010 ^{e}	1.377 ± 0.012 ^{c}	1.461 ± 0.013 ^{c}	1.619 ± 0.015 ^{b}	1.228 ± 0.011 ^{d}	2.518 ± 0.019 ^{a}
Total	10.463 ^{d}	10.675 ^{d}	10.618 ^{d}	10.920 ^{d}	12.338 ^{c}	12.444 ^{c}	13.508 ^{b}	21.755 ^{a}

Abbreviations of amino acids: asp—asparagine; thr—threonine; ser—serine; glu—glutamine; pro—proline; gly—glycine; ala—alanine; cys—cysteine; val—valine; met—methionine; ile—isoleucine; leu—leucine; tyr—tyrosine; phe—phenylalanine; his—histidine; lys—lysine; and arg, arginine. Explanations see Table 1.

Glutamine was the major amino acid detected in the leaves of white-head cabbage Stonehead cv., followed by tyrosine, alanine, proline, serine, lysine, leucine, valine, and threonine. The results indicate that, with increasing soil S content, the sulfur amino acid methionine content decreased. These results correspond with the findings of Smatanová et al. [33] and Loshak et al. [32]. In our study, the second sulfur amino acid, cysteine, was not detected.

Regardless of treatment, fresh cabbage had similar amino acid content, ranging from 10.463 to 10.92 mg·g^{-1} FM, which suggests that fertilization had very little impact on amino acid levels in the research material. Following storage, the amino acid content

increased noticeably for each of the experimental scenarios, from 12.338 to as much as 21.755 mg·g^{-1} FM. In cabbage grown using potassium sulfate, this increase was twofold. The 'Stonehead' cultivar of white cabbage was characterized by the highest amino acid content being that of glutamine, and a very low methionine content. Such a relation was also noted by Park et al. [20], who determined the levels of 12 amino acids in green cabbage and 8 in red cabbage. The study by Wojciechowska et al. [31] demonstrated that sulfur fertilization resulted in an increase in free amino acid content following storage, which was confirmed by the results of this study.

Following an analysis of the correlation between antioxidant activity and the bioactive compound content of white cabbage, it was found that—in fresh cabbage—both activities demonstrated the highest correlation with vitamin C content, and the lowest with polyphenol content (Table 4). An equally significant correlation was found for amino acids, which was also more significant in relation to ABTS.

Table 4. Correlation of antioxidant activity and bioactive compound content in fresh and stored cabbage.

	Vitamins C	Phenolics	Chlorophyll a + b	Carotenoids	Amino Acid
ABTS	0.9590	0.4892	0.6158	0.5871	0.8649
DPPH	0.9626	0.3957	0.4527	0.4304	0.7787
After storage					
ABTS	−0.5392	0.6588	0.8281	0.6008	0.9075
DPPH	−0.1408	0.3385	0.5743	0.3798	0.8382

In stored cabbage, we found a negative correlation for vitamin C and concluded that, after storage, this parameter was most degraded. However, this correlation increased in relation to chlorophyll a and b and amino acids. We also noted the tendency by which the correlation of the determined bioactive compounds to ABTS was higher than that to DPPH (Table 4).

4. Conclusions

The form of sulfur used for fertilization had a significant impact on the parameters investigated in this study. For all tested parameters, the lowest results were recorded for the control sample without sulfur fertilization. We also noted that the form of sulfur applied affected individual parameters differently. Sulfur fertilization improved the nutritional value of cabbage. Higher levels of vitamin C, phenolics, and chlorophyll a and b were detected in cabbage treated with potassium sulfate, while a higher concentration of carotenoids was seen when elementary sulfur and ammonium sulfate were used. Elementary sulfur had a small effect on the antioxidant activity in comparison with potassium sulfate and ammonium sulfate. The highest phytic, citric, malic, and fumaric acid contents were found in crops treated with ammonium sulfate. Sulfur fertilization had very little impact on the total amino acid levels in the cabbage heads.

Irrespective of the method of sulfur fertilization, storage resulted in a decrease in vitamin C, chlorophyll a and b, and carotenoid contents, while polyphenol and amino acid contents increased, along with antioxidant activity.

After storage, the organic acid content in most cases decreased, and only phytic acid content increased for all plots in this study. By contrast, storage of cabbage grown using potassium sulfate as the fertilizer increased the content of all four organic acids.

Correlation analysis demonstrated that the correlation of bioactive compounds in white cabbage was higher in relation to the antioxidant activity in the ABTS assay than that in the DPPH assay.

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