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Nitrogen Metabolism and Antioxidant Capacity of Selected Vegetables from Organic and Conventional Crops

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Abstract: The study aimed to determine the level of selected indicators of nitrogen metabolism in vegetables from organic (organic food store) and conventional (supermarket and local market) crops. Nitrates, total chlorophyll content, and the activity of the nitrate biosynthesis pathway enzymes—nitrate reductase (NR) and glutamine synthetase (GS)—were determined in the leaves of selected species from different plant families. The research material consisted of dill, carrot, celery, beet, onion, Chinese and white cabbage, and rocket. The nitrate content was within the permissible limits, except for vegetables bought at a local market. In most cases, no significant differences in the level of nitrates between organic and conventional farming were observed. The analyses revealed the highest nitrate content in dill [2.16 mg $\times$ g^{-1}] and the lowest in onions [0.06 mg $\times$ g^{-1}] from conventional crops. The enzyme activities were related to the level of nitrates. The analysed species differed in phenolic compounds, ascorbate levels, and total antioxidant capacity (TCA). Positive correlations were found between TCA and antioxidants.

Keywords: nitrates; nitrate reductase; glutamine synthetase; total chlorophyll; phenolic compounds; ascorbate; total antioxidant capacity; vegetables

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1. Introduction

In recent years, organic farming methods have become an increasing competitor to conventional agriculture. Organic farming is as attractive as traditional farming in many European regions and countries [1,2]. Organic agriculture uses organic fertilisers, and diseases and pests are combated with biological preparations, positively affecting the environment, human health, and biodiversity protection [3–5]. In the case of conventional methods, it is the pesticides and other chemicals that maximise yields and accelerate plant growth. According to the available literature, organic food is characterised by a higher nutritional value and lower levels of nitrates, nitrites, pesticide residues, growth regulators, antibiotics, or synthetic food additives [6–12].

Excessive accumulation of nitrates in vegetables has been the subject of intensive research for many years. The amount of nitrogen in plants is mainly influenced by the cultivation method and environmental factors. Moreover, the content of nitrates is genetically controlled and may constitute a characteristic feature of a species or even a variety [13]. Therefore, the nitrate level in nine plant species from different cultivation systems was determined. Additionally, an attempt was made to understand and compare nitrogen metabolism based on the measurement of chlorophyll content and the activity of nitrate reductase (NR) and glutamine synthetase (GS).

Readily available nitrogen from mineral fertilisers can boost plant growth and the synthesis of nitrogen-containing compounds, but it may also limit the synthesis of carbon-based compounds, such as secondary metabolites [14]. Conversely, the absence of synthetic pesticides in organic farming may increase plant sensitivity to pests and pathogen attacks, thereby reinforcing phenolic compound (Phe) syntheses [15,16]. Their increased content

is essential for plant defence systems [17–20]. The concentration of phenolic compounds can be affected by environmental conditions such as light intensity, carbon dioxide levels, temperature, fertilisation, and biotic and abiotic factors [21].

Phenolic compounds remarkably affect human health [22–25]. They are of great interest because of their potential role in preventing cancer and heart disease. This role may be related to their antioxidant activity, which was shown to be higher than ascorbic acid (AsA) against reactive oxygen species [26]. Usually, plants with higher phenolic compound quantities prove their increased antioxidant capacity. According to the literature, the results regarding the antioxidant capacity of vegetables from conventional and organic farming vary according to plant type and the bioactive compound measured [27–31].

This study aimed to determine the level of selected indicators of nitrogen metabolism in vegetables from organic (organic food store) and conventional (supermarket and local market) crops and their antioxidant capacity. Research is being conducted worldwide to compare the quality of raw materials and products obtained from different cultivation systems. Constant food monitoring is crucial, regardless of the place of cultivation. Therefore, the issues discussed in this work seem to be fully justified.

2. Materials and Methods

2.1. The Origin of the Plant Material

The research material consisted of vegetable leaves belonging to the following:

- celery family *Apiaceae*:
  - dill [*Anethum graveolens* L.]—dill (marking on the charts),
  - common carrot [*Daucus carota* L.]—carrot,
  - celery [*Apium graveolens* L.]—celery,
  - parsley [*Petroselinum crispum* (Mill.)]—parsley,
- amaranth family *Amaranthaceae*:
  - beet [*Beta vulgaris* L.]—beet,
- amaryllis family *Amaryllidaceae:*
  - spring onions [*Allium cepa* L.]—onion,
- cabbage family *Brassicaceae:*
  - Chinese cabbage [*Brassica rapa* L. var. pekinensis]—ch. cabbage,
  - white head cabbage [*Brassica oleracea* var. capitata L.]—w. cabbage,
  - rocket [*Eruca vesicaria* subsp. Sativa]—rocket.

The vegetables that were available at retail were purchased in Poznań (one of the main cities in western Poland) in the organic food store (required certificates), in the supermarket, and at the local market (independent agricultural production) at the beginning of July. Each vegetable sample was randomly sampled from the market shelves, simulating consumer shopping behaviour. The vegetables were characterised by consumption maturity, i.e., the samples were fresh, not withered or spoiled. All analyses were performed on fresh material-leaf blades, ignoring the main vascular bundles in 3–5 independent repetitions. For dill, celery, parsley, beet, cabbage, and rocket, leaf samples were prepared by cutting discs (Ø 12 mm) using a cork borer. The number of tissue pieces taken for analysis depended on the thickness of the tissue leaves. Fennel, carrot, and onion leaves were cut with scissors into fragments of approx. 12 mm.

2.2. Nitrate Content

The content of nitrates was determined colourimetrically by adding a coloured substance to the product of the nitration reactions of salicylic acid [32]. The crushed plant material was placed in glass tubes, poured with 10 cm^3 of deionised water, and boiled in a water bath [Labnet] for 20 min in the device. The extracts were transferred to centrifuge tubes and centrifuged at 4.000 g for 15 min [Beckman J2-HC Centrifuge]. The supernatant (0.1 cm^3) was taken, and 0.4 cm^3 of 5% salicylic acid (dissolved in concentrated H_2SO_4) was

added. At the same time, a reference sample was prepared (distilled water instead of the extract). After a 20-min incubation in constant humidification, 9.5 cm^3 of 2 M NaOH was added to all tubes and cooled in an ice bath. The absorbance of the sample was measured at a wavelength of 410 nm relative to the reference sample. The content of nitrates was read from the standard curve prepared for KNO_3 and was expressed as micrograms of nitrites per gram of fresh weight [μg NO_3^- × g^{-1} FW].

2.3. Nitrate Reductase Activity

The in vivo determination of nitrate reductase (NR) activity was based on Jaworski [33]. Plant tissue fragments (250 mg) were incubated in 50 cm^3 glass flasks with a glass joint in 5 cm^3 of the incubation mixture into which the enzyme diffused. Enzyme activity was measured by the number of nitrites formed due to the reduction in nitrates in this mixture (0.1 M phosphate buffer, pH 7.5; 0.1 M KNO_3, 2% propanol). In parallel, flasks with an incubation mixture without nitrates were prepared. After one hour of incubation at 30 °C, one cm^3 of the solution was pipetted into glass tubes, then 1 cm^3 of SAA (1% sulfanilamide in 1 M HCl) and 1 cm^3 of NED (0.01% N-[1-naphthyl]-ethylenediamine dihydrochloride) were added. The tubes were left at room temperature for 15–30 min, after which the absorbance at 540 nm was measured (the reference sample was a control in which the tested solution was replaced with the incubation mixture). Based on the standard curve prepared for nitrites [NO_2^-], the number of nmoles of nitrites formed was calculated. The NR activity was given as the number of nmoles of the product [NO_2^-] formed in 1 h per gram of fresh weight [nmoles NO_2^- × h^{-1} × g^{-1} FW].

2.4. Glutamine Synthetase Activity

The glutamine synthetase (GS) activity was determined based on Hipkin and Syrret [34]. The measure of enzyme activity is the amount of γ-glutamyl hydroxamate formed, which gives a coloured reaction due to the reaction with the ferrous reagent. Plant material (1 g) was ground with the addition of 100 mg polyvinylpyrrolidone (PVP) and 3 cm^3 of cooled extraction buffer [0.05 M Tris HCl, pH 7.2, containing 10 mM $MnCl_2$, 1 mM dithiothreitol]. The obtained extracts were centrifuged at 15.000 g [Beckman J2-HC Centrifuge] for 15 min at 4 °C, and then enzyme activity was determined in the supernatants. The following were pipetted into centrifuge tubes: 0.2 cm^3 of incubation buffer (0.05 M Tris HCl, pH 7.2), 0.2 cm^3 of 7.5 mM adenosine diphosphate (ADP), 0.5 cm^3 of 140 mM glutamine, 0.1 cm^3 of 0.3 mM sodium edetate, and 1.0 cm^3 of extract. The control sample was prepared by replacing the glutamine solution with the incubation buffer. The samples were initially incubated for 10 min at 30 °C, then 0.2 cm^3 of hydroxylamine (10 mM, pH 7.2) was added to each and set again for 30 min at the same temperature. The reaction was stopped by adding 1 cm^3 of iron reagent ($FeCl_2$ in 4% trichloroacetic acid), and then the samples were cooled and centrifuged at 10.000 g for 10 min. Enzyme activity was determined in the supernatant at 500 nm using a reagent sample (2 cm^3 H_2O and 1 cm^3 iron reagent) as a reference. The difference between the absorbance of the test sample and the control sample corresponded to the amount of γ-glutamyl hydroxamate read from the standard curve. The activity of the GS enzyme was expressed in micromoles of γ-glutamyl hydroxamate formed within one hour, converted to g of fresh weight [μmoles γ-glutamyl hydroxamate × h^{-1} × g^{-1} FW].

2.5. Total Chlorophyll Content

Isolation and quantification of total chlorophyll (a + b) were based on Hiscox and Israelstram [35]. The crushed plant material (0.250 g) was placed in glass tubes and flooded with 10 cm^3 dimethylsulfoxide (DMSO). The samples were incubated for an hour in a water bath at 65 °C, then the samples were cooled, and the absorbance was measured at two wavelengths: 649 nm (chlorophyll a) and 665 nm (chlorophyll b). Total chlorophyll content was calculated according to Arnon's formula: 7.34 × A_{665} + 17.76 × A_{649} [36]. The concentration of dyes was expressed in μg per 1 cm^3 of the extract and then converted to a milligram of fresh weight [mg × g^{-1} FW].

2.6. Phenolic Compound Content

The phenolic content (Phe) was determined with the Folin–Ciocalteu reagent with some modifications [37]. Methanolic (80%) extracts of leaf samples (0.2 g) were centrifuged for 30 min at 10.000 g; after that, diluted extracts were mixed with diluted Folin–Ciocalteu phenol reagent (1:1 with water). The resulting solutions were left to rest for 5 min before adding 10% Na_2CO_3. After a 20-min reaction time at room temperature, the intensity of the blue colour was measured at 660 nm. The total phenolic amount was calculated from the standard curve (coumaric acid) and expressed in milligrams per gram of fresh weight [mg $\times$ g^{-1} FW].

2.7. Ascorbate Content

The ascorbic acid (AsA) content was determined by the Law et al. [38] and Costa et al. [39] methods with modifications. Leaves (0.2 g) were homogenised in 5% ice-cold trichloroacetic acid (TCA), then centrifuged at 17.000 g [Beckman J2-HC Centrifuge] at 4 °C for 20 min. The supernatants were used for the assay. Sodium hydroxide (10 µL, 5 M) was added to 400 µL of extract, mixed, and centrifuged at 3.500 g [Eppendorf Centrifuge 5415 R] for 2 min at 4 °C. A 100 µL sample of supernatant was added to 100 µL of 0.15 M NaH_2PO_4 buffer, pH 7.4, and 100 µL of water. Samples were vortex-mixed and incubated at room temperature for 30 s. To each sample, 200 µL of 5% TCA, 200 µL of 44% H_3PO_4, 200 µL of 4% bipyridyl in 70% ethanol, and 100 µL of 3% $FeCl_3$ were added. Samples were mixed for 30 s at room temperature and then incubated for one hour at 37 °C. This assay is based on the reduction in Fe^{3+} by AsA, followed by complex formation between Fe^{2+} and bipyridyl that absorbs at 525 nm. The ascorbic acid level was reported on a per-gram fresh weight basis. The results are given in nmol per gram of fresh weight [nmoles $\times$ g^{-1} FW].

2.8. Total Antioxidant Capacity

Total antioxidant capacity (TAC) was measured using the ability of antioxidants contained in the extract to reduce the cation ABTS+ [2,2′-azinobis-[3-ethyl-benzothiazoline-6-sulphonic acid]] according to the method described by Re et al. [40] and modified by Bartosz [41]. The ABTS+ solution was prepared by dissolving 19.5 mg of ABTS in 7 mL of 0.1 M potassium phosphate buffer (pH 7.4) and 3.3 mg of potassium persulfate. After thoroughly mixing, the solution was left dark for 12 h. Immediately before the determination, the ABTS+ solution was diluted with 0.1 M potassium phosphate buffer, pH 7.4, so absorbance at wavelength 414 nm was 1.0. To determine TAC, 500 mg of plant material was homogenised in 5% trichloroacetic acid (TCA). Next, samples were centrifuged at 15.000 $\times$ g for 30 min at 4 °C. The cuvette contained 1.9 mL of diluted ABTS+, and absorbance (A0) was measured at a wavelength of 414 nm, and then 100 µL of the extract was added. Absorbance was measured again after 10 s (A1). TAC dependent on fast antioxidant (ascorbic acid or glutathione) As = A1 − A0 was calculated. The calibration curve was plotted by adding to the diluted ABTS+, followed by 5 µL portions of 1 mM Trolox, and measuring the gradual decrease in absorbance. The final result of TAC was expressed as µmol Trolox per gram of fresh weight [µmol Trolox $\times$ g^{-1} FW].

All determinations were made spectrophotometrically [Jasco V-530 UV—VIS] in the Jasco—Quantitative Analysis computer program.

2.9. Statistical Analyses

Results were subjected to statistical analysis of variance (ANOVA) and Tukey's HSD multiple range test using the STATISTICA 13.3 package [Stat-Soft Inc., Tulsa, OK, USA]. Moreover, linear regression was determined for the relationship between nitrate reductase (NR) and glutamine synthetase (GS) activity and nitrate accumulation. Pearson's correlation analysis was performed for all physiological parameters. Additionally, the p-value was determined to assess whether the correlation was statistically significant. The results of Pearson coefficients (r) and p values (p) were tabulated. Correlation coefficients are significant at $p < 0.05$.

3. Results and Discussion

3.1. Nitrate Content

The issue of excessive accumulation of nitrates in plants has been the subject of intensive research worldwide for several decades. Nitrates are essential for plants because they are a nitrogen source and a building block of proteins. Specific amounts of nitrates in plants are related to the natural nitrogen cycle in nature [42]. Unfortunately, the intensification of plant production causes an excessive transfer of these compounds from the soil to the plants. When plants receive too much nitrogen from artificial or organic fertilisers, they cannot use it and store it as nitrites [43].

Consumption of vegetables with excess nitrates does not pose a severe threat to humans because these compounds are absorbed relatively quickly from the digestive tract and excreted from the body unchanged [43]. The harmfulness of nitrates increases after a reduction in nitrites, which occurs during processes occurring after harvesting vegetables and in the digestive tract [44,45].

The results of nitrate determinations in various types of vegetables are widely presented in the literature, which indicates a considerable scale of exceedances of permissible standards [45–47]. Polish standards regarding nitrate content for the analysed vegetables are, respectively, [0.4 mg $\times$ g^{-1}] for carrots, parsley, and celery, [0.75 mg $\times$ g^{-1}] for cabbage, onion, and rocket, and [1.5 mg $\times$ g^{-1}] for dill and beet [46,48]. Considering the above criteria, it can be concluded that the content of nitrogen compounds was within the permissible limits, except for carrots, celery, parsley, and dill bought in a local market, representing the *Apiaceae* family [Figure 1A]. The analyses carried out revealed the highest nitrate content in dill [2.16 mg $\times$ g^{-1}] and the lowest in onions [0.06 mg $\times$ g^{-1}] from conventional crops (local market). In the case of cabbage family vegetables, the lowest nitrate content was recorded for plants from independent agriculture production (local market).

Of course, it should be remembered that the distribution of various compounds in plants changes during the growing season. Their concentration in different parts of plants also varies. Moreover, the taller the plants, the fewer nitrates are typically found [49]. For example, the results obtained for beet do not coincide with the data of other authors, who classified beet as a vegetable with an exceptionally high nitrate content [42,46,47,50]. The nitrate levels in all these studies are much higher than the results obtained in this study because nitrate accumulation was examined only in the root. According to Krężel [51] and in subsequent studies by Krężel and Kołota [52], several times higher amounts of these compounds are collected than in the leaves. It is generally known that vegetables with edible roots, stems, and leaves have more nitrates than vegetables with edible fruit [53]. It has been shown that nitrate concentrations differ between edible plant parts [54]. The nitrate content decreases in the following order: leaf > stem > root > inflorescence > tuber > fruit > seeds [50]. The results of other authors quoted and discussed in the work usually concern the root part, which was not examined in this study.

It should be noted that the exact determination of the nitrate content in vegetables is complicated because it is determined by various factors acting simultaneously [55–57]. It turns out that the same vegetable, grown in a different place, may differ significantly in terms of nitrate concentration. The factors influencing their level include region and growing conditions, such as season, growth temperature, humidity, pH, and soil composition, including the content of microelements [58]. The plant's age and thermal treatment method have a significant impact, as mentioned in [45,59] and the works cited therein. The study examined young vegetables purchased in the spring and summer. Young plants are characterised by many of these compounds (in young leaves, their level may exceed even 1% of dry weight). Higher nitrate accumulation is typical for early varieties and plant species with a shorter vegetation period [44,50]. Additionally, younger leaves are characterised by a lower nitrate content than older leaves because nitrate reductase activity decreases with the age of the plant [60].

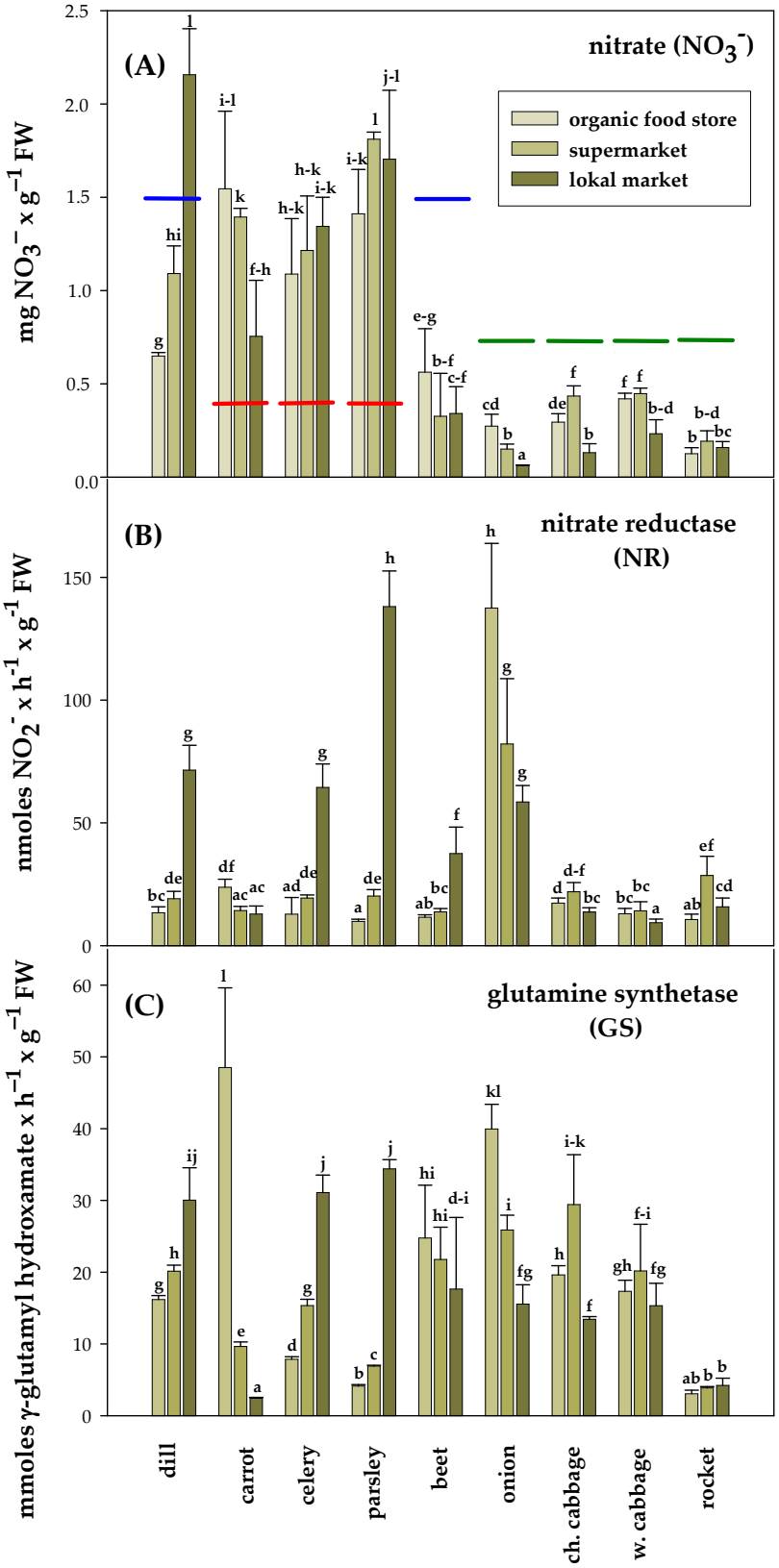

Figure 1. Nitrate content (**A**), nitrate reductase (**B**), and glutamine synthetase (**C**) activities in the leaves of selected plant species from organic and conventional crops. The permissible standards for nitrate content in Poland are marked with horizontal lines: red [0.4 mg × g^{-1}], green [0.75 mg × g^{-1}], blue [1.5 mg × g^{-1}]. Means with the same letter are not significantly different ($p < 0.05$, ANOVA followed by Tukey HSD test).

Differences in nitrate accumulation may also result from harvest time, growing season, and storage time [59,61,62]. It is believed that excessive accumulation of nitrates is characteristic of plant species with a shorter vegetation period or which are early varieties [63]. During storage, a gradual decrease in nitrates is observed, and delaying harvesting favours a reduction in their content [64].

It has been shown that the ability to accumulate nitrogen compounds may be genetically determined and is a characteristic feature of a given species or variety of vegetables [65]. The cultivation system, the nature of the farm, and the doses and types of fertilisers used are also important. When fertilising plants, the total nitrogen doses recommended in integrated production should be observed, and nitrogen fertilisation should not be applied too late in the growing season [66–69].

In the case of dill, parsley, and celery, the nitrate content in conventionally grown vegetables was higher than in organic vegetables. Still, these changes were not significant for parsley and celery. The exception is carrots, where a more substantial accumulation of nitrates was recorded in the leaves of plants from organic farming. Similar relationships were observed in beet and onion leaves. In general, the level of nitrates in plants of the *Apiaceae* family was 3–4 times higher than other families, regardless of the crop kind. For example, for dill from the *Apiaceae* family, the highest nitrate values were recorded for the local market [2.16 mg $\times$ g^{-1}], while the lowest was for plants from organic farming [0.65 mg $\times$ g^{-1}]. However, lower values were recorded for Chinese cabbage, a representative of the *Brassicaceae* family: local market [0.13 mg $\times$ g^{-1}] and organic food store [0.29 mg $\times$ g^{-1}]. The content of nitrates depended on species but did not depend on the cultivation system [Table 1]. The nitrate content of the analysed plant species was found to have no significant effect on the remaining analysed parameters, as evidenced by p values [Table 2].

Table 1. Summary of ANOVA results for physiological parameters by species and cultivation system.

(A) Source of Variation	Nitrate			Nitrate Reductase		
	d.f.	F	*p*	d.f.	F	*p*
Species (S)	8	91.557	<0.0001	8	246.116	<0.0001
Cultivation (C)	2	1.451	NS	2	9.816	<0.0001
S × C	16	10.013	<0.0001	16	75.976	<0.0001
Error	54			111		
(B) Source of Variation	**Glutamine Synthetase**			**Total Chlorophyll**		
	d.f.	F	*p*	d.f.	F	*p*
Species (S)	8	38.898	<0.0001	8	70.755	<0.0001
Cultivation (C)	2	4.407	0.0153	2	73.39	<0.0001
S × C	16	53.052	<0.0001	16	25.106	<0.0001
Error	81			54		

NS—non-significant.

Table 2. Results of the Pearson coefficient (r) and p-value between physiological parameters. Correlation coefficients are significant at $p < 0.05$ (data marked in grey).

Variable	Nitrate	Nitrate Reductase	Glutamine Synthetase	Total Chlorophyll	Phenolic Compounds	Ascorbate	Total Antioxidant Capacity
N = 81 (exception correlation between enzyme activities N = 106)							
nitrate	---	r = −0.0182 p = 0.872	0.1669 0.137	0.1415 0.208	−0.1155 0.305	0.0045 0.968	0.036 0.749
nitrate reductase	---	---	r = 0.4669 p = 0.000	−0.0308 0.785	0.0251 0.824	−0.116 0.918	0.0342 0.762
glutamine synthetase	---	---	---	r = 0.0473 p = 0.675	0.0084 0.941	−0.1358 0.227	0.0173 0.878
total chlorophyll	---	---	---	---	r = −0.3617 p = 0.001	−0.3065 0.005	−0.3182 0.004
phenolic compounds	---	---	---	---	---	r = 0.8317 p = 0.00	0.8164 0.00
ascorbate	---	---	---	---	---	---	r = 0.8981 p = 0.00
t. antioxidant capacity	---	---	---	---	---	---	---

3.2. Nitrate Reductase Activity

The nitrate content in plants usually depends on the activity of two reducing enzymes: nitrate reductase (NR) and nitrite reductase (NiR). It was found that both enzymes are induced by the presence of nitrate ions in the environment [70–73], and light is necessary to obtain maximum activity [44,74]. Plants grown in low-light conditions tend to have low levels of nitrate reductase. Notably, leaf nitrate reductase activity in the daily cycle often decreases as the day progresses, which may reflect its degradation and/or synthesis blockade [75]. Thanks to NR, inorganic forms of nitrogen are effectively incorporated into organic compounds [76].

In the case of dill, celery, parsley, and beet, the enzyme activity in conventionally grown vegetables was significantly higher than in organic vegetables [Figure 1B], as shown: dill (local market—71.5 nmoles NO_2^- × h^{-1} × g^{-1} FW; organic food store—13.38 nmoles NO_2^- × h^{-1} × g^{-1} FW), celery (local market—64.47 nmoles NO_2^- × h^{-1} × g^{-1} FW; organic food store—12.83 nmoles NO_2^- × h^{-1} × g^{-1} FW), parsley (local market—138.15 nmoles NO_2^- × h^{-1} × g^{-1} FW; organic food store—9.88 nmoles NO_2^- × h^{-1} × g^{-1} FW), beet (local market—37.56 nmoles NO_2^- × h^{-1} × g^{-1} FW; organic food store—11.58 nmoles NO_2^- × h^{-1} × g^{-1} FW). This enzyme is considered an adaptive enzyme, so its lower activity in organic vegetables, where there should be fewer nitrates, is understandable. Only carrots and onions from an organic farm were characterised by higher enzyme activity than conventional farming vegetables. What is extremely surprising is the very high NR activity concerning the amount of determined nitrates in the case of onions. The literature data indicate that NR gene expression may also occur after depleting nitrogen sources [72]. The presence of nitrates is not an absolute condition for increased expression of the NR gene. Some available data indicate that tiny amounts of nitrate are sufficient to induce the enzyme [71,72]. Moreover, it is known that in the daily cycle, nitrate reductase activity in leaves often decreases as the day progresses, which may reflect its degradation and/or blocking of its synthesis [75].

The negative correlation between nitrate reductase activity and nitrate levels is presented in Figure 2. A linear correlation coefficient value close to 0 ($r = -0.0182$) does not always mean the absence of any correlation, only the lack of linear correlation [Table 2]. The most important thing is the significance of the correlation, which in this case is not significant ($p = 0.0892$).

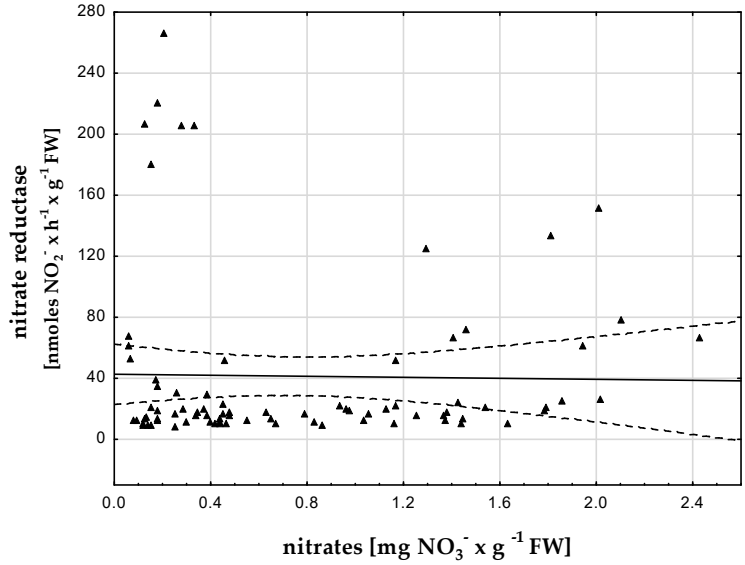

Figure 2. The relationship between nitrate accumulation and nitrate reductase activity.

3.3. Glutamine Synthetase Activity

Thanks to the activity of nitrate reductase and nitrite reductase, nitrates are reduced to ammonium ions, which are then incorporated into nitrogenous organic compounds. Ammonium ions can be built into amino acids with the participation of glutamate dehydrogenase or in a cycle catalysed by glutamine synthetase (GS) and glutamate synthase (GOGAT) [77–79]. As part of this study, the activity of glutamine synthetase (GS) was determined in all cases. The results indicate increased enzyme activity in celery vegetables (dill, celery, and parsley) from conventional cultivation, which is associated with a higher level of nitrates in these tissues [Figure 1C]. In contrast to nitrate reductase activity, the positive correlation between glutamine synthetase activity and nitrate levels is presented Figure 3. No linear relationship was observed (r = 0.1669). Moreover, the correlation's significance was insignificant [Table 2].

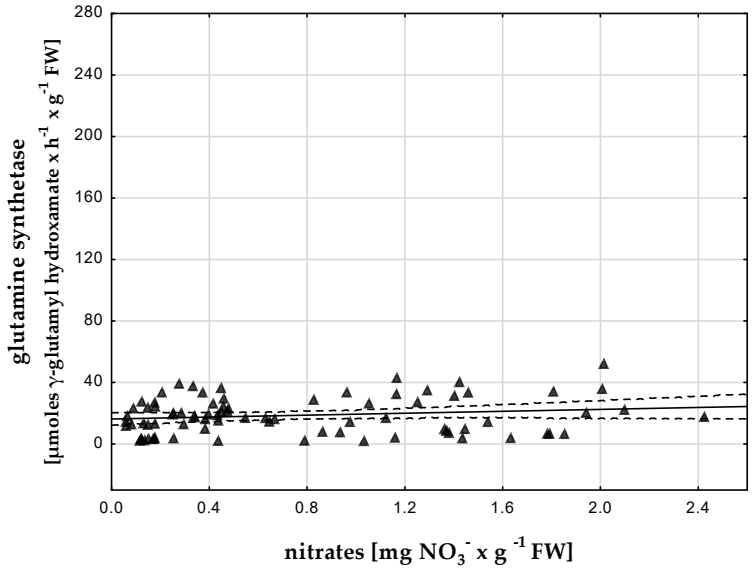

Figure 3. The relationship between nitrate accumulation and glutamine synthetase activity.

The opposite was true for carrots, beets, and onions, where higher activity was recorded for organic vegetables. The most significant differences in GS activity due to the place of purchase of vegetables were recorded for carrots. The enzyme activity in carrots from conventional cultivation was 20 times higher than the activity of vegetables bought at the market and 5 times higher than the enzyme activity in organically grown vegetables. The enzyme activity was as follows: in the organic food store [48.23 μmoles γ-glutamyl hydroxamate × h^{-1} × g^{-1} FW], in the supermarket [9.58 μmoles γ-glutamyl hydroxamate × h^{-1} × g^{-1} FW], and at the local market [2.47 μmoles γ-glutamyl hydroxamate × h^{-1} × g^{-1} FW]. In the case of beet and onion leaves, no significant differences in enzyme activity were observed due to the origin of the research plant material [Figure 1C]. The activity of both enzymes (NR) and (GS) depended on species and cultivation system [Table 1]. Positive correlations were found between nitrate synthase activity and glutamine synthetase activity [Table 2]. Moreover, the results were statistically significant ($p < 0.05$).

3.4. Total Chlorophyll Content

Chlorophyll is commonly found in vegetables, especially leafy ones. It also occurs in other green parts of plants exposed to light [80]. The dye obtained from plants synthesises haemoglobin and reduces cancer incidence with parallel exposure to carcinogenic compounds [81,82]. Its chemopreventive effect was also found with relatively low doses of carcinogens in the diet and high chlorophyll intake [83,84]. The level of chlorophyll in the leaves of the tested plants is shown in Figure 4. The lowest levels of chlorophyll were recorded for white and Chinese cabbage, which is related to the natural colour of these

vegetables (light green). Total chlorophyll content depended on species and cultivation system [Table 1]. A positive correlation was found between total chlorophyll content and total antioxidant capacity, phenolic compounds, and ascorbate content [Table 2]. Moreover, the results were statistically significant ($p < 0.05$).

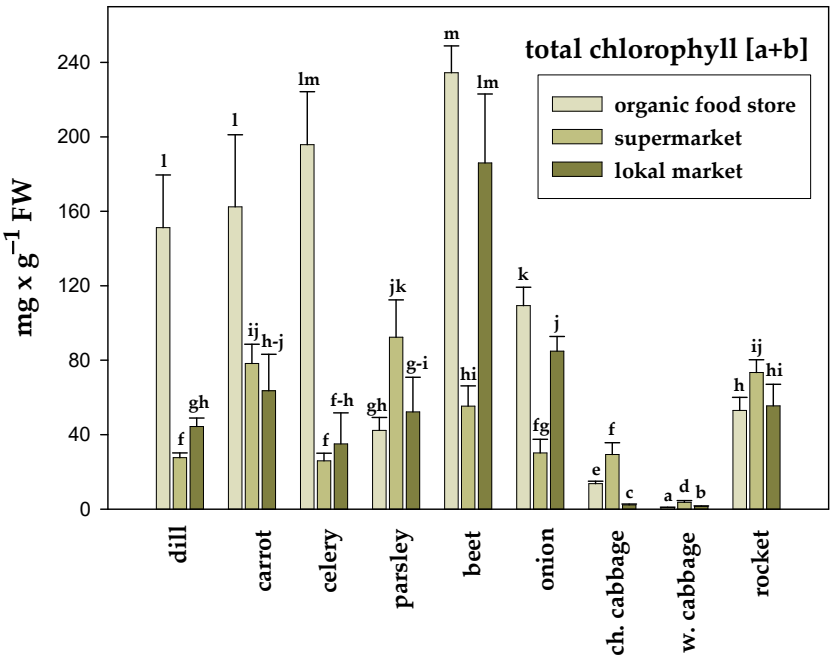

Figure 4. Total chlorophyll content in the leaves of selected plant species from organic and conventional crops. Means with the same letter are not significantly different ($p < 0.05$, ANOVA followed by Tukey HSD test).

It is generally known that the chloroplast pigment content in plants increases with increasing nitrogen fertilisation levels [85]. Therefore, it depends on the nitrogen level. Excess nitrogen increases the production of chloroplasts in plant cells [86], while in conditions of its deficiency, the number of chloroplasts decreases [14]. In an ecological system, i.e., in an environment low in easily assimilable nitrogen, plants should intensively produce nitrogen-free pigments other than chlorophyll [87]. Unfortunately, our research is contrary. The organic celery family, beet, and onion recorded the highest total chlorophyll levels. The exception was parsley leaves, where vegetables from organic farming contained a smaller amount of this valuable ingredient. These results contrast the research of Wieczorek and Wieczorek [88] on eco-parsley from spring crops (May–June).

3.5. Phenolic Compound Content

Recent research studies have demonstrated that organic fruits and vegetables contain a wide variety of phenolic compounds and antioxidants, which could help enhance human immunity, eliminate free radicals, and have positive functions in anti-cancer immunomodulation [89–92]. Our results showed that the content of phenolic compounds remained at a higher level in the case of vegetables from the organic production system and local market compared to vegetables from the supermarket. This relationship was noted for dill, celery, Chinese and white cabbage, and rocket [Figure 5A]. It is known that the phenolic compound content is highly dependent on the cultivar and farming conditions [92]. In our research, phenolic compound content also depended on species and cultivation system [Table 3].

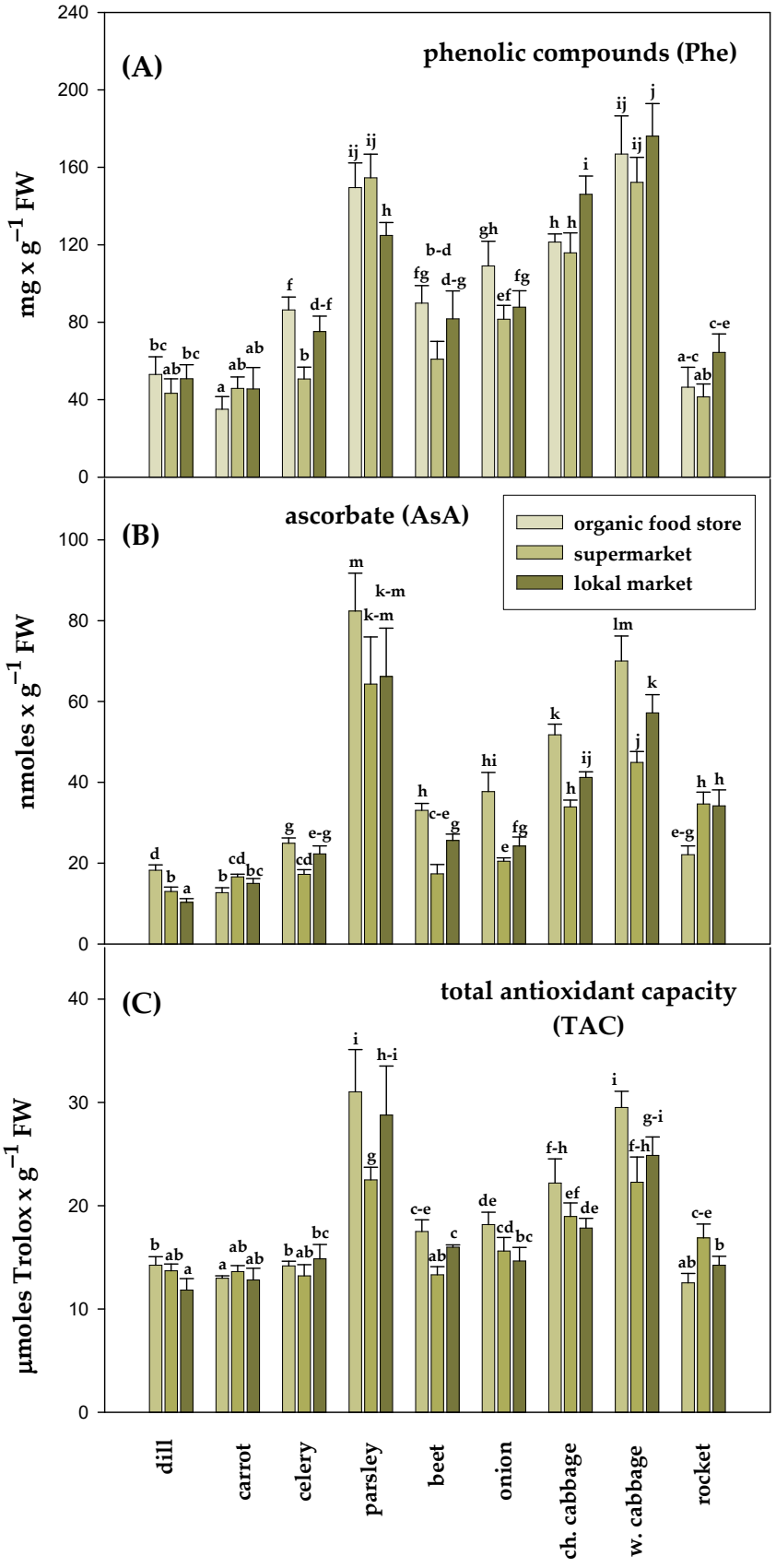

Figure 5. Phenolic compound content (**A**), ascorbate content (**B**), and total antioxidant capacity (**C**) in the leaves of selected plant species from organic and conventional crops. Means with the same letter are not significantly different ($p < 0.05$, ANOVA followed by Tukey HSD test).

Table 3. Summary of ANOVA results for physiological parameters by species and cultivation system.

(C) Source of Variation	Phenolic Compounds			Ascorbate			Total Antioxidant Capacity		
	d.f.	F	*p*	d.f.	F	*p*	d.f.	F	*p*
Species (S)	8	170.24	<0.0001	8	176.275	< 0.0001	8	90.062	<0.0001
Cultivation (C)	2	12.499	<0.0001	2	35.823	< 0.0001	2	15.414	<0.0001
S × C	16	4.258	<0.0001	16	6.456	< 0.0001	16	4.993	<0.0001
Error	54			54			54		

NS—non-significant.

Compared with conventional foods, organic vegetables generally have a lower content of macronutrients (especially proteins) and a higher secondary metabolite concentration [27]. This may be the reason for the greater exposure of organic crops to nutritional stress as well as diseases and pests, which are a consequence of the ban on the use of pesticides and chemical fertilisers [93]. Studies on the effects of genotype and the kind of wheat cultivation system on nutritional quality have confirmed that these factors affect phenolic compound content [94]. Moreover, phenolic compounds' quantitative and qualitative composition significantly depended on the variability of environmental conditions, such as location or weather. Organic wheat yielded less than conventional wheat due to the nitrogen shortage.

3.6. Ascorbate Content

The ascorbate (AsA) level was also measured [Figure 5B]. The AsA content was predominantly higher and statistically significant in cruciferous and celery vegetables from the organic production system than in vegetables from conventional cultivation. The AsA content depends on plant variety, ripeness, growing conditions, and harvest time. These factors could lead to significant variations in results, both between studies and within studies [95]. In our research, ascorbate content also depended on species and cultivation systems [Table 3]. Ascorbic acid is one of the least durable vitamins and is very sensitive to storage and processing processes [96]. A relationship was found between the soil's nitrogen [N] content and the AsA level. The accumulation of ascorbic acid increases whenever the nitrogen available in the ground is low [97]. Zhang et al. [98] point to the critical role of N-level optimisation to maximise vitamin C content and provide a better understanding of the maturity stage-dependent N regulation on vitamin C anabolism. Generally, high nitrate provision also triggers the ascorbate–glutathione cycle gene expression [99]. The organic production system's research results regarding celery and parsley confirm the above relationship [Figures 1A and 5B]. Importantly, in most of the tested vegetable species (except carrot, celery, and rocket), the AsA level was significantly higher in vegetables from the organic production system. Additionally, the highest level of ascorbate [82.39 nmoles × g^{-1} FW] was recorded for organically grown parsley [Figure 5B].

3.7. Total Antioxidant Capacity

Typical antioxidants, such as ascorbate or glutathione, react very rapidly with ABTS+. Measuring the decrease in absorbance of the solution containing the extract after a very short time (10 s) is a measure of the antioxidant contents in a sample. Other substances (residues of tyrosine and tryptophan in proteins) react more slowly. We must stress the higher TAC, dependent on the pool of fast antioxidants in dill, parsley, beet, onion, ch. cabbage, w. cabbage, and rocket from an organic production system compared to vegetables from conventional cultivation [Figure 5C]. Similarly to the AsA level, the highest TAC value was obtained for organically grown parsley from the celery family [31.03 µmol Trolox × g^{-1} FW] and w. cabbage from the cabbage family [29.51 µmol Trolox × g^{-1} FW]. Importantly, in the case of AsA, Phe, and TAC levels, a similar relationship was noted in most of the tested vegetable species, i.e., higher levels of the tested indicators in organic farming than in vegetables from conventional farming [Figure 5A–C]. Studies comparing

products grown organically and conventionally appear to confirm the advantage of organic crops in terms of antioxidant properties [30]. Based on the results obtained, we can suggest that the cultivation system differently affects the level of phenolic compounds and antioxidant capacity depending on the examined vegetable species. In our research, total antioxidant capacity depended on species and cultivation system [Table 3]. Moreover, positive correlations were found between total antioxidant capacity and antioxidants, such as ascorbate and phenolic compounds [Table 2]. These results were statistically significant, as evidenced by $p < 0.05$.

There has been a growing trend to consume organic food instead of conventional food in recent years. The increased interest is associated with potential adverse health effects from consuming pesticides, fertilisers, hormones, and antibiotics commonly used in food production. However, environmental contamination is possible for both conventional and organic crops [29]. It turns out that the presence of anthropogenic pollution sources is a crucial issue affecting the occurrence of environmental pollutants in food, regardless of their origin. An extensive review of comparative studies of environmental contaminants, such as dioxins, mycotoxins, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, or trace elements in plants from both types of crops, evidences this. These results emphasise the need to constantly monitor the plant growth environment [100].

4. Conclusions

Nowadays, consumers are very concerned about food quality, nutritional composition, and the positive impact of their food on health. In this context, the preference for and consumption of organic products are increasing worldwide. Especially in developed countries, there is a much faster development of organic production compared to conventional production. The organic food market in Poland is also developing dynamically (10–20% per year). Our research confirmed that the tested vegetables' nitrogen metabolism and antioxidant capacity are influenced by more than just the cultivation method (ecological or conventional). The ambiguity of the obtained results may indicate that antioxidant properties and nitrogen metabolism depend largely on the cultivation system, the plant species, the examined organ, and environmental factors. It has been shown that changes in the level of the tested parameters are characteristic of a specific vegetable species. Most vegetable species were characterised by different antioxidant activities, which were influenced by various antioxidant components, such as vitamin C or phenolic compounds. When comparing vegetables from both types of agriculture, many statistical differences were observed in terms of the tested parameters. It is worth emphasising that in most of the tested vegetables in the celery and cabbage families, higher levels of AsA and TAC were recorded in organic vegetables compared to conventional ones. It can, therefore, be concluded that organic cultivation may be a suitable method of increasing the concentration of bioactive compounds with antioxidant properties in vegetables. Undoubtedly, extended research should be carried out to prove the superiority of the organic system over the conventional one because assessing the effects of organic farming is not easy and unambiguous.

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