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THE EFFECT OF NITROGEN FERTILIZATION ON NUTRITIVE VALUE AND ANTIOXIDATIVE ACTIVITY OF RED CABBAGE

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Abstract. Phenolic compounds which occur in red cabbage, have strong antioxidant activity, and can be used as substances protecting human cells from oxidation. Field experiment conducted in the years 2007–2009 in Department of Horticulture at Wroclaw University of Environmental and Life Sciences involved research on the effect of different nitrogen doses on yield, chemical composition and antioxidant activity of red cabbage. Nitrogen, in the form of ammonium saltpeter, was introduced in the following doses: 50 kg N·ha^{-1}; 150 kg N·ha^{-1} and 250 (150+100) kg N·ha^{-1}. In the third decade of April red cabbage seeds of 'Langendijker' cv. were sown into multipots. The seedlings were planted on 5 m^2 – area plots at the end of May. Harvesting took place in the second week of September, when there was assessed marketable and total yield. In samples of red cabbage heads there were estimated the content of nitrates, vitamin C, total and reducing sugars, polyphenols, anthocyanins, as well as dry matter and soluble solids. In order to investigate antioxidant activity there were applied DPPH and ABTS tests. In experiment optimum nitrogen dose for red cabbage was 150 kg N·ha^{-1}. The highest amount of anthocyanins were determined in the leaves of red cabbage fertilized with moderate N dose up 150 kg N·ha^{-1}. The highest antioxidant activity proved to characterize edible parts of red cabbage fertilized with nitrogen in the dose of 50 kg N·ha^{-1}, while the level of total polyphenols featured the lowest values in the conditions of intensive nitrogen fertilization amounting 250 kg N·ha^{-1}.

Key words: poliphenols, doses of nitrogen fertilizer, ABTS, DPPH, vitaminum C, anthocyanins, nitrates

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INTRODUCTION

Nowadays, a considerable interest of scientists, has been focused on the role of antioxidants as the substances capable of improving human health and inhabiting a number of serious diseases [Basaga et al. 1997]. It is believed, that supplementing human diet with food products containing these compounds will provide for diminishing oxidative stress in an organism and will contribute to the improvement of people`s state of health. Red cabbage characterize high nutritive value and it belongs to a small group of vegetables featuring especially high content of anthocyanins classified as strong antioxidants. Bast and Haenen [2002] proved that chemical compounds contained in red cabbage prevent oxidative damage of DNA and also take part in the process of angiogenesis inhibition. Hagivara et al. [2002] stresses that these processes support neoplasm diseases control, while inhibition of angiogenesis alone brings about the reduction of tumors development. McDougall et al. [2007] points to the fact that substances contained in red cabbage have a beneficial influence on insulin excretion in pancreas cells and they feature anti-inflamatory properties as well. The latter ones are attributed to active antioxidants contained in red cabbage leaves. Proteggente et al. [2002] classify red cabbage to vegetables characterizing the highest antioxidant properties, even stronger than those featuring spinach, broccoli, onion or tomato.

The quantity of biologically active compounds in plants depends both on their genotype and cultivation conditions, as well as on environmental conditions. A significant role is also played by the stage of plant development and genetic factors [Pourmorad et al. 2006]. Yet it has not been much known about the effect of plant fertilization and cultivation method on the content of antioxidants in vegetables.

Research carried out in the years 2007–2009 aimed at determination of the effect of nitrogen fertilization dose on the content of antioxidant and nutritive compounds in the examined species.

MATERIALS AND METHODS

Field experiment was conducted in Experimental Station belonging to Department of Horticulture at Wroclaw University of Environmental and Life Sciences, on clay soil of pH = 7.6 containing 1.8% of organic matter, moreover 60 mg phosphorus, 180 mg potassium and 60 mg magnesium in 1 dm^3. In the experiment there was assessed the effect of diversified nitrogen doses on yield size and quality, as well as antioxidant activity of red cabbage of 'Langendijker' cultivar. Nitrogen in the form of ammonium saltpeter was applied in the total doses of 50 kg $N \cdot ha^{-1}$; 150 kg $N \cdot ha^{-1}$ and 250 (150 + 100) kg $N \cdot ha^{-1}$. Preplant fertilization was introduced one week before seedlings planting, and top dressing fertilization in the amount of 100 kg $N \cdot ha^{-1}$ was applied three weeks after planting date.

In the third decade of April red cabbage seeds were sown in greenhouse to multipots of 76.5 cm^3 filled with peat substrate. The field experiment follow as a randomized block design in four replications. Seedlings were planted at the end of May, in spacing of 50 × 50 cm. Production was carried out after leek as the forecrop during experiment

duration. The harvest of cabbage for yield estimation was conducted from plot area of 5 m^2. Before the harvest done in the half of September there were collected plant samples for chemical analysis. In heads of red cabbage there were determined nitrates content using an ion-selective electrode, as well as vitamin C content – according to the Tillmans method (The Polish Standard PN-90/A-75101/11), total and reducing sugars – (Lane-Eynona method according to The Polish Standard PN-90/A-75101/07), polyphenols by Folin-Ciocalteau method [Slinghart and Singleton 1977] and anthocyanins – following the method by Fuleki and Francis [Fuleki and Francis 1968]. Dry matter was examined by drying to constant weight at 105^{o}C (PN-90/A-75101/03), while total soluble solids in degree Brix using refractometer (ATAGO-POCTEL). In order to determine antioxidative properties there were applied DPPH [Yen and Chen 1995] and ABTS [Re et al. 1999] tests.

The results were verified statistically. The significance of differences between mean values was estimated by Tukey's test at the significance level of $\alpha = 0.05$.

RESULTS AND DISCIUSSION

The weather conditions varied in the years of study (tab. 1). During the growth period of red cabbage the mean air temperatures were relatively higher in all years of experiment than long-term average. The mean precipitation in May and June of 2007 and 2009 were higher, than the long-term average but in 2008 there was noticed strong drought. The rainfall in July and August of the years of experiment were sufficient for cabbage growth but in September in all years of study there was observed low level of soil humidity.

Table 1. Mean temperatures and precipitation totals during the experimental period in Psary Experimental Station

Tabela 1. Średnie temperatury i sumy opadów w okresie badań w Stacji Doświadczalnej w Psarach

Month Miesiąc	Average monthly temperature Średnia temperatura miesiąca °C			Sum of monthly precipitation Miesięczna suma opadów mm			Mean value of monthly temperatures Średnia wieloletnia temperatura miesięczna	Mean value of monthly precipitation Średnia wieloletnia miesięczna suma opadów
	2007	2008	2009	2007	2008	2009	1981–2000	1981–2000
May Maj	15.9	16.0	15.8	53.4	1.4	54.2	14.2	57.1
June Czerwiec	19.7	20.7	17.0	101.1	23.5	79.4	16.9	78.7
July Lipiec	20.3	21.7	21.0	108.3	185.7	115.9	18.8	90.8
August Sierpień	19.7	20.3	21.3	60.6	96.6	94.0	18.0	64.0
September Wrzesień	13.8	14.6	16.9	19.8	27.8	7.0	13.6	50.6

The amount of nitrogen fertilization did significantly affect yield size of red cabbage (tab. 2). In 2007 the highest marketable yield was obtained due to nitrogen fertilization in the dose of 250 kg N·ha^{-1}, while in the remaining years optimum dose of this nutrient was 150 kg N·ha^{-1}. The highest total yield was gained in 2007 and 2009 with the use 250 kg N·ha^{-1} dose of nitrogen but in 2008 optimum dose of ammonium saltpeter for total yield was 150 kg N·ha^{-1}.

Table 2. Effect of nitrogen dose on total and marketable yield of red cabbage, t · ha^{-1}
Tabela 2. Wpływ dawki azotu na wielkość plonu całkowitego i handlowego kapusty czerwonej, t · ha^{-1}

Plon Yield	Dose of nitrogen Dawka azotu kg N·ha^{-1}	2007	2008	2009	Mean Średnia
Total Całkowity	50	53.10 a	26.86 a	25.36 a	36.10 a
	150	59.80 b	37.83 b	40.63 b	46.08 b
	250	67.60 c	36.15 b	45.25 c	49.66 c
Marketable Handlowy	50	41.70 a	15.34 a	19.87 a	25.63 a
	150	48.45 b	24.32 b	34.26 b	35.67 b
	250	57.53 c	21.86 b	32.57 b	37.32 b

Values denoted with the same letter in columns do not differ significantly at α = 0.05
Wielkości oznaczone tą samą literą w kolumnach nie różnią się istotnie przy α = 0,05

The results obtained in the experiment prove that dry matter content and soluble solids in red cabbage leaves decreased under the influence of intensive nitrogen fertilization (tab. 3). Vitamin C content increased according to the increase in nitrogen dose. The highest quantity of vitamin C was recorded in 2009 in the treatment fertilized with nitrogen in the dose of 250 kg N·ha^{-1}, while in 2008 and 2007 – it was obtained when 150 kg N·ha^{-1}nitrogen dose was used. Many researchers reported inversely proportional relation in white head cabbage and kale cultivation [Nurzyński 1973, Sorensen 1984, Freyman et al. 1991]. Venter [1983] stated that high doses of mineral fertilization, especially nitrogen, result in considerable decrease in vitamin C level in vegetables. However, investigation by other authors regarding cultivation of garden lettuce, potato and leaf beet [Muller and Hippe 1987, Dzida 2004] proved that vitamin C content was dependent, to a high degree, on increased dose of nitrogen fertilization.

Lee and Kader [2000] explain that controversy by differentiation of the remaining environmental factors affecting plants development. In optimum conditions for plant growth sustainable NPK fertilization can provide for L – ascorbic acid accumulation in vegetables. According to Golcz and Kozik [2004], vitamin C content at increasing N level depended on the kind of medium.

The content of reducing and total sugars did decrease as a result of intensive nitrogen fertilization applied and it ranged, average, in the years of the experiment from 3.16% and 3.96% respectively in nitrogen fertilized treatment in the amount of 50 kg N·ha^{-1} to 2.74% and 3.29% at the dose of 250 kg N·ha^{-1}. These data remain in agreement with the results obtained by other authors [Takebe et al. 1995].

Increased dose of nitrogen did significantly affect nitrates content in red cabbage leaves, resulting in higher amounts of this component. On average at the dose of

50–150 kg N· ha^{-1} introduced before plant growing period nitrates level ranged from 324.64 to 389.48 mg·kg^{-1} weight of fresh matter, while split applied N dose of 250 kg N·ha^{-1} used as both preplant fertilization and top dressing, provided for nitrates level increased to 509.54 mg·kg^{-1}. At the highest nitrogen dose of 250 kg N·ha^{-1} the amount of nitrates in edible parts of red cabbage was lower than permissible values for cabbage equals 750 mg NO_3-N·kg f. m. (except in 2009).

Table 3. Effect of nitrogen dose on the content of reducing sugars, total sugars, vitamin C, dry matter, extracts and nitrates in red cabbage

Tabela 3. Wpływ dawki azotu na zawartość cukrów ogółem i redukujących, witaminy C, suchej masy, ekstraktu i azotanów w kapuście czerwonej

Content Zawartość	Dose of nitrogen Dawka azotu kg N·ha^{-1}	2007	2008	2009	Mean Średnia
Reducing sugars mg · 100 g^{-1} f.m. Cukry redukujące mg · 100 g^{-1} ś.m.	50	3.36b	3.11c	3.02b	3.16
	150	3.47b	2.76b	2.51a	2.91
	250	3.24a	2.54a	2.44a	2.74
Total sugars mg · 100 g^{-1} f.m. Cukry ogółem mg · 100 g^{-1} ś.m.	50	4.05b	3.96c	3.88c	3.96
	150	4.04b	3.56b	3.31b	3.64
	250	3.71a	3.11a	3.05a	3.29
Vitamin C mg · 100 g^{-1} f.m. Witamina C mg · 100 g^{-1} ś.m.	50	37.74a	59.34a	54.91a	50.66
	150	40.71b	70.68c	55.56a	55.65
	250	40.09b	65.38b	64.60b	56.69
Soluble solids Ekstrakt °Brix	50	5.90a	7.10b	7.20c	6.73
	150	5.70a	7.05b	7.10b	6.62
	250	6.10a	6.80a	6.90a	6.60
Dry matter Sucha masa %	50	9.81c	9.89c	9.55b	9.75
	150	8.91b	9.61b	9.84b	9.45
	250	8.68a	9.40a	8.71a	8.93
Nitrates mg · kg^{-1} f.m. Azotany mg · kg^{-1} ś.m.	50	98.10a	474.72a	401.10a	324.64
	150	98.01a	499.72b	570.72b	389.48
	250	112.84b	658.00c	757.77c	509.54

Values denoted with the same letter in columns do not differ significantly at $\alpha = 0.05$
Wielkości oznaczone tą samą literą w kolumnach nie różnią się istotnie przy $\alpha = 0,05$

The content of anthocyanins in red cabbage varied under the influence of cultivation conditions in particular years of the experiment (tab. 4). On average the highest amount of those pigments was determined in cabbage heads in 2008. There was not found explicit correlation between nitrogen dose and anthocyanins content in edible parts of red cabbage. In our own investigation conducted in 2007 and 2009 there was recorded diminished content of anthocyanins pigments as a result of intensive fertilization with nitrogen, while in 2008 moderate nitrogen fertilization in the amount of 150 kg N·ha^{-1} did not negatively affect the content of those pigments. Numerous research indicate that intensive nitrogen fertilization decrease anthocyanins level, among others, in grapes and

black chokeberry fruit [Jeppson 2000]. Nevertheless, Nguyen and Niemeyer [2008] reported that in red basil the content of those pigments was weakly correlated with nitrogen dose and strongly dependent on the course of cultivation conditions in particular season. Politycka and Golcz [2004] reported a tendency to increase in anthocyanins content in red basil of 'Dark Opal' cultivar under the influence of intensive nitrogen fertilization, especially at second term of harvesting.

Table 4. Effect of nitrogen dose on antioxidative activity, anthocyanins and polyphenols contents in red cabbage

Tabela 4. Wpływ dawki azotu na aktywność antyoksydacyjną, zawartość antocyjanów i polifenoli w kapuście czerwonej

Content Zawartość	Dawka azotu Dose of nitrogen kg N·ha^{-1}	2007	2008	2009	Mean Średnia
Anthocyanins mg · 100 g $^{-1}$ f.m. Antocyjany mg · 100 g $^{-1}$ ś.m.	50	111.16c	156.00a	178.55c	148.57
	150	99.09b	171.00b	141.55b	137.21
	250	83.81a	147.00a	84.25a	105.02
Polyphenols mg · 100 g $^{-1}$ f.m. Polifenole mg · 100 g $^{-1}$ ś.m.	50	319.18b	53.79b	259.59c	210.85
	150	309.22b	59.21c	208.24b	192.22
	250	276.61a	47.55a	184.11a	169.42
DPPH µM Trolox · g $^{-1}$ f.m. DPPH µM Trolox · g $^{-1}$ ś.m.	50	5.94c	4.65b	5.23b	5.27
	150	5.28b	4.85b	5.02a	5.05
	250	5.04a	3.85a	4.99a	4.63
ABTS µM Trolox · g $^{-1}$ f.m. ABTS µM Trolox · g $^{-1}$ ś.m.	50	21.37c	8.09b	5.37a	11.61
	150	19.83b	8.93b	7.02b	11.93
	250	17.06a	6.89a	8.39c	10.78

Values denoted with the same letter in columns do not differ significantly at α = 0.05
Wielkości oznaczone tą samą literą w kolumnach nie różnią się istotnie przy α = 0,05

The level of polyphenols in edible parts of red cabbage differed in particular years of the experiment. The highest amount of those compounds were determined in 2007 and 2009, considerably lower contents were assayed in 2008. In all the years of the experiment the increase in nitrogen dose contributed to the decrease in those group of compounds. A number of factors do affect polyphenols content in plants: genotype, cultivar, the stage of plants development, cultivation conditions including nitrogen form and dose [Parr and Bollwel 2000, Biesiada et al. 2006, 2008; Smoleń and Sady 2007].

Our own investigation have proved that the increase in nitrogen dose in the course of red cabbage cultivation provides for generally not high, yet significant decrease in antioxidant activity measured with the use of DPPH and ABTS tests. In 2007, after application of nitrogen at rate of 50 kg N·ha^{-1}, the activity showed, respectively, the following levels: 5.94 for DPPH test and 21.37 for ABTS test, while in 2008 it amounted 4.65 and 8.09 µM Trolox · g^{-1} f.m. At the dose of 250 kg N·ha^{-1} the mentioned activity decreased to: 5.04 and 17.06 in 2007, then it ranged 3.85 and 6.89 in 2008 respectively for

DPPH and ABTS tests (tab. 4). Yet in 2009 antioxidative activity, measured by ABTS test, did increase according to increased nitrogen dose. Leja et al. [2007] also found that cultivation conditions affect antioxidant activity to a higher degree than experimental factors.

Jamroz et al. [2006], determining antioxidant properties of hop cultivars, proved strict positive correlation between polyphenols content and anitoxidative acttivity. Similarly, in our own investigation it was possible to confirm high antioxidant activity of red cabbage at considerably elevated level of phenolic compounds. These results were also in agreement with the data obtained by Katsube et al. [2003] in research involving berry fruits.

CONCLUSIONS

1. Optimum nitrogen dose for red cabbage of 'Langendijker' cultivar amounted 150 kg $N \cdot ha^{-1}$.

2. The increase in nitrogen dose did contribute to increased nitrates level and to the decrease in the contents of dry matter, extract, reducing and total sugars. The highest quantity of vitamin C accumulated red cabbage fertilized with nitrogen in the dose of 250 kg $N \cdot ha^{-1}$.

3. The highest content of anthocyanins were found in the leaves of red cabbage fertilized with moderate nitrogen dose, up to 150 kg $N \cdot ha^{-1}$, while total polyphenols level showed the lowest value in the conditions of intensive nitrogen fertilization 250 kg $N \cdot ha^{-1}$.

4. There was determined the highest antioxidant activity in edible parts of red cabbage fertilized with 50 kg $N \cdot ha^{-1}$ dose of nitrogen.

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WPŁYW NAWOŻENIA AZOTEM NA WARTOŚĆ ODŻYWCZĄ I AKTYWNOŚĆ ANTYOKSYDACYJNĄ KAPUSTY CZERWONEJ

Streszczenie. Związki polifenolowe zawarte w kapuście czerwonej charakteryzują się dużą aktywnością antyutleniającą, dzięki czemu chronią komórki organizmu ludzkiego przed zmianami oksydacyjnymi. W doświadczeniu polowym przeprowadzonym w latach 2007–2009 w Katedrze Ogrodnictwa Uniwersytetu Przyrodniczego we Wrocławiu badano wpływ zróżnicowanych dawek azotu na plon, skład chemiczny i aktywność antyoksydacyjną kapusty czerwonej. Azot w postaci saletry amonowej był stosowany w dawkach: 50 kg N·ha^{-1}; 150 kg N·ha^{-1} i 250 (150 + 100) kg N·ha^{-1}. W połowie maja nasiona kapusty czerwonej odmiany Langendijker zostały wysiane punktowo do wielodoniczek. Rozsadę sadzono pod koniec maja na poletka o powierzchni 5 m^2. We wrześniu przeprowadzono zbiór roślin, oceniając plon handlowy i plon ogólny. W próbach kapusty czerwonej oceniano zarówno zawartość azotanów, witaminy C, cukry ogółem i redukujące, polifenole, antocyjany, jak i suchą masę i ekstrakt. Do zbadania właściwości antyoksydacyjnych wykorzystano testy DPPH i ABTS. Stwierdzono, że optymalną dawką azotu dla kapusty głowiastej czerwonej odmiany Langendijker było 150 kg N·ha^{-1}. Najwięcej antocyjanów stwierdzono w liściach kapusty czerwonej nawożonej umiarkowaną dawką N do 150 kg N·ha^{-1}. Największą aktywność antyoksydacyjną stwierdzono w częściach jadalnych kapusty czerwonej nawożonej azotem w dawce 50 kg N·ha^{-1}, natomiast poziom zawartości polifenoli był najniższy przy nawożeniu azotem w dawce 250 kg N·ha^{-1}.

Słowa kluczowe: polifenole, dawki nawozu azotowego, ABTS, DPPH, witamina C, antocyjany, azotany

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