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Original Research

Effect of Cultivar and Harvest Date of Kale (*Brassica Oleracea* L. Var. *Acephala*) on Content of Nitrogen Compounds

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Abstract

The level of total nitrogen, protein nitrogen, nitrates (NO_3^-) and nitrites (NO_2^-) was measured in the leaves of kale of the cultivars Winterbor F_1, Redbor F_1 and Średnio Wysoki Zielony Kędzierzawy. The investigation was carried out in two successive years, the raw material being harvested three times each year, i.e. 10, 14 and 18 weeks after planting seedlings in the field. Depending on the year of the investigation, the cultivar and the date of harvest, the content found in 100 g fresh matter of kale was: 0.54-0.74 g total nitrogen and 0.46-0.50 g protein nitrogen; in 1000 g the content of nitrates (NO_3^-) was 248-2810 mg and of nitrites (NO_2^-) 0.14-0.95 mg. In both years the highest content of total nitrogen was found in leaves of Średnio Wysoki Zielony Kędzierzawy and of protein nitrogen in Winterbor F_1. The cultivar Redbor F_1 was characterized by the highest content of nitrates and nitrites. Comparing material from the three harvest dates, average values for the year and cultivar showed that the second harvest contained 9% more total nitrogen and 4% more protein nitrogen than the first, while the third harvest contained, respectively, 17% and 8% more than the first. Nitrate content fell by 67% and 83%, respectively, and nitrite increased by 5% (but later was reduced by 46%). Nitrate and nitrite are regulated in spinach and lettuce and for this reason understanding the accumulation of these compounds is critical if regulations are developed for kale.

Keywords: total nitrogen; protein nitrogen; nitrates; nitrites; kale; time of harvest

Introduction

Nitrogen (N) is one of the most important elements, particularly in agriculture, where it is decisive in determining the size and quality of yields [1, 2]. Increased additions of N usually result in increased yield of crop plants [3]. Nitrogen is found in the composition of numerous compounds such as amino acids, proteins, nucleic acids and nitrates and nitrites. Plants typically uptake nitrogen from the environment and assimilate it to produce amines and amides [4]. Protein is an indispensable constituent of every organism, and because of the numerous important functions of protein, deficiencies of this constituent can result in the deterioration of health [5, 6]. Protein is used to perform many functions in the body, including producing enzymes, hormone production, creating new cells, and carrying nutrients throughout the body [7].

From the standpoint of human health, nitrates and nitrites are significant nitrogen compounds and particular attention is paid to their occurrence in food products. According to a report by the European Comission [8], vegetables can supply 80% of nitrates consumed daily.

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Although nitrates are regarded as non-toxic constituents, their reduction to nitrites can present serious health risks [9-11]. The Food and Agriculture Organization World Health of the United Nations and World Health Organization Joint Expert Committee on Food Additives determined 3.7 and 0.07 mg/kg body weight, respectively, as the maximum acceptable daily intake of nitrates and nitrites (ADI) allowed in the human diet [12].

The level of nitrates in vegetables depends on genetic and environmental factors such as species, cultivar, fertilization, temperature, light intensity and geographical location [9]. Nitrates and ammonium are natural constituents taken up from the soil by vegetables. Plants reduce nitrate to nitrite in the cytosol via the light-dependent enzyme nitrate reductase [13]. The results of numerous experiments [14-18] show considerable differences in the ability of various vegetable species to accumulate nitrates. Leaf vegetables – including kale – accumulate fairly large quantities [11]. The data given in literature concerning nitrate content in kale range from 300 to 1283 mg/1kg fresh matter [11, 19].

The aim of the present investigation was to determine the level of total nitrogen, protein nitrogen, nitrates (NO_3^-) and nitrites (NO_2^-) according to the growing season, the cultivar and the time of harvest.

Material and Methods

Material

The investigated materials were fresh leaves of kale obtained in three harvests:

- first (I) - 10 weeks after planting seedlings in the field,
- second (II) - 14 weeks after planting seedlings in the field,
- third (III) - 18 weeks after planting seedlings in the field.

Two hybrid cultivars produced by Dutch Bejo Zaden b.d. breeders: Winterbor F_1 and Redbor F_1 and the cultivar Średnio Wysoki Zielony Kędzierzawy produced by Polish breeders were used in the experiment. Kale was grown in 2006 and 2007 in an experimental field lying in the western outskirts of Kraków (50º04’ N, 19º51’ W) on soil of good horticultural quality characterized by the following traits during the first and second years of the investigation: pH in H_2O 7.08 and 7.09; humus 1.96 and 1.55%; nitrogen 25 and 21 mg dm^{-3}; phosphorus 81 and 46 mg dm^{-3}; potassium 200 and 90 mg dm^{-3}; and calcium 1200 and 960 mg dm^{-3}. The kale was grown in the third year after manure fertilization, with leguminous vegetables preceding kale and a mixture of green manure plants used as a forecrop in the year of the experiment. Mineral fertilizers were applied, and the fertility of the soil and the nutritional requirements of the crop being taken into account, the following doses of fertilizer were used in the years of the investigation:

- Nitrogen before planting seedlings 40 kg N ha^{-1}, and 50 kg N ha^{-1} as a side dressing in the form of 34% ammonium nitrate;
- Phosphorus before planting seedlings 80 and 100 kg P_2O_5 in the form of 46% triple superphosphate (50% of the dose during ploughing and 50% during harrowing);
- Potassium before planting seedlings 150 and 200 kg K_2O in the form of 50% potassium sulfate (50% of the dose during ploughing and 50% during harrowing).

The crops were established by transplanting. Kale seeds were sown in boxes 1.0 cm deep on June 3. Seeds were germinated and grown in a greenhouse (23ºC day/15ºC night) under natural lighting conditions. After about 10 days the seedlings were moved to plastic pots 8 cm in diameter and height. The seedlings were planted in a field at the stage of 3-4 leaves, i.e. on June 25 in 2006 and on June 28 in 2007 at a spacing of 50 cm x 50 cm. The cultivation measures during plant growth included mechanical weed control, side dressing with nitrogen and protection against diseases and pests as necessary. Sprinkling irrigation was applied in the case of strongly reduced soil humidity.

The pattern of weather conditions in the period of kale growth is given in Table 1. In the two years of the investigation, the meteorological conditions were distinctly different. In 2006 maximum air temperatures were lower and minimum temperatures were higher, while atmospheric precipitation was usually higher in 2007, with the exception of much more abundant rainfall in July 2006.

The first harvest was carried out when the number of leaves of commercial value per plant was 25-30; this was observed during the first five days of September in both years of the experiment. The second harvest was carried out after four weeks and the third after a further four weeks. During harvest whole plants were cut. Yellowing leaves (usually growing at the base of the plant) and leaves less than 10 cm in length from the apical bud of the plant were discarded.

Methods

From marketable leaves, i.e. leaves of good colour, undamaged by diseases or pests, the main rib was removed and the material for analyses of chemical composition was sampled in four replications of 1000 g each.

The analysis covered total nitrogen, protein nitrogen; nitrates (NO_3^-) and nitrites (NO_2^-). The content of total nitrogen was determined using the Kjeldahl method [20], the Büchi unit for nitrogen measurement being applied.

The content of protein nitrogen was measured using the trichloroacetic acid (TCA) method [21]. The protein deposit was precipitated with 20% trichloroacetic acid. Non-protein nitrogen was removed by washing the protein deposit with 2% trichloroacetic acid. The content of protein nitrogen in the deposit was measured using the Kjeldahl method [20].

The content of nitrates (NO_3^-) and nitrites (NO_2^-) was determined using the colorimetric method [22]. Nitrates were reduced to nitrites with cadmium powder and measured by the colorimetric method after colour reaction with Griess reagent using a Shimadzu UV-160A spectrophotometer at 538 wavelengths.

Table 1. Mean daily air temperatures and total atmospheric precipitation during the growth of kale*.

Year of investigation	Month											
	July			August			September			October		
	temp. max.ºC	temp. min.ºC	rainfall mm	temp. max.ºC	temp. min.ºC	rainfall mm	temp. max.ºC	temp. min.ºC	rainfall mm	temp. max.ºC	temp. min.ºC	rainfall mm
2006	25.0	14.8	126.1	26.6	13.9	23.6	20.9	8.6	30.4	9.7	2.7	45.1
2007	30.8	8.0	97.4	32.5	8.6	77.2	26.6	4.1	36.1	24.8	-1.4	41.7

*data from Kraków-Balice weather station (50º05' N, 19º47'W).

Table 2. Content of total nitrogen* of three cultivars of kale, in fresh matter.

Year of investigation	Cultivar	Harvest date**			Mean
		I	II	III	
		g 100 g^{-1}	g 100 g^{-1}	g 100 g^{-1}	
2006	Winterbor F_1	0.63±0.01	0.62±0.03	0.67±0.02	0.64
	Średnio Wysoki Zielony Kędzierzawy	0.55±0.02	0.65±0.01	0.65±0.02	0.62
	Redbor F_1	0.54±0.02	0.58±0.03	0.67±0.01	0.60
	Mean	0.57	0.62	0.66	
	LSD P<0.01 factor a – 0.024 factor b – 0.024 interaction a x b – 0.041				
2007	Winterbor F_1	0.57±0.02	0.61±0.02	0.72±0.03	0.63
	Średnio Wysoki Zielony Kędzierzawy	0.65±0.02	0.71±0.03	0.74±0.03	0.70
	Redbor F_1	0.56±0.02	0.59±0.02	0.65±0.03	0.60
	Mean	0.59	0.64	0.70	
	LSD P<0.01 factor a – 0.027 factor b – 0.027 interaction a x b – n.s				

*Mean (n=4) ± standard deviation,
**Harvest date: I - after 10 weeks of vegetation, II - after 14 weeks of vegetation, III - after 18 weeks of vegetation, LSD: factor a – cultivar, factor b – harvest date, interaction a x b, n.s – not significant

Table 3. Content of protein nitrogen* of three cultivars of kale, in fresh matter.

Year of investigation	Cultivar	Harvest date **			Mean
		I	II	III	
		g 100 g^{-1}	g 100 g^{-1}	g 100 g^{-1}	
2006	Winterbor F_1	0.60±0.02	0.56±0.01	0.58±0.03	0.58
	Średnio Wysoki Zielony Kędzierzawy	0.47±0.01	0.59±0.02	0.51±0.02	0.52
	Redbor F_1	0.49±0.01	0.46±0.01	0.57±0.02	0.51
	Mean	0.52	0.54	0.55	
	LSD P<0.01 factor a – 0.019 factor b – 0.019 interaction a x b – 0.034				
2007	Winterbor F_1	0.53±0.01	0.51±0.01	0.60±0.02	0.55
	Średnio Wysoki Zielony Kędzierzawy	0.54±0.03	0.61±0.01	0.58±0.03	0.58
	Redbor F_1	0.50±0.02	0.48±0.01	0.51±0.06	0.50
	Mean	0.52	0.53	0.56	
	LSD P<0.01 factor a – 0.030 factor b – 0.030 interaction a x b – 0.052				

*Mean (n=4) ± standard deviation,
**Harvest date: I - after 10 weeks of vegetation, II - after 14 weeks of vegetation, III - after 18 weeks of vegetation, LSD: factor a – cultivar, factor b – harvest date, interaction a x b.

Table 4. Content of nitrate (NO_3^-)* of three cultivars of kale, in fresh matter.

Year of investigation	Cultivar	Harvest date **			Mean
		I	II	III	
		mg 1000 g⁻¹	mg 1000 g⁻¹	mg /1000 g⁻¹	
2006	Winterbor F_1	1326±102	462±24	305±11	498
	Średnio Wysoki Zielony Kędzierzawy	1054±38	436±30	272±22	587
	Redbor F_1	2810±147	704±37	313±37	1276
	Mean	1730	534	297	
	LSD P<0.01 factor a – 72.6 factor b – 72.6 interaction a x b – 125.7				
2007	Winterbor F_1	1523±61	523±17	248±11	765
	Średnio Wysoki Zielony Kędzierzawy	1544±75	641±29	263±10	816
	Redbor F_1	1862±111	591±16	361±12	938
	Mean	1643	585	291	
	LSD P<0.01 factor a – 57.7 factor b – 57.7 interaction a x b – 100.0				

*Mean (n=4) ± standard deviation,
**Harvest date: I - after 10 weeks of vegetation, II - after 14 weeks of vegetation, III - after 18 weeks of vegetation, LSD: factor a – cultivar, factor b – harvest date, interaction a x b.

Table 5. Content of nitrite (NO_2^-)* of three cultivars of kale, in fresh matter.

Year of investigation	Cultivar	Harvest date **			Mean
		I	II	III	
		mg 1000 g⁻¹	mg 1000 g⁻¹	mg 1000 g⁻¹	
2006	Winterbor F_1	0.17±0.01	0.32±0.02	0.15±0.01	0.21
	Średnio Wysoki Zielony Kędzierzawy	0.14±0.01	0.40±0.01	0.15±0.00	0.23
	Redbor F_1	0.80±0.06	0.67±0.03	0.41±0.02	0.63
	Mean	0.37	0.46	0.24	
	LSD P<0.01 factor a – 0.028 factor b – 0.028 interaction a x b – 0.048				
2007	Winterbor F_1	0.16±0.03	0.24±0.01	0.14±0.01	0.18
	Średnio Wysoki Zielony Kędzierzawy	0.25±0.03	0.32±0.03	0.15±0.01	0.24
	Redbor F_1	0.95±0.04	0.64±0.02	0.30±0.01	0.63
	Mean	0.45	0.40	0.20	
	LSD P<0.01 factor a – 0.027 factor b – 0.027 interaction a x b – 0.048				

*Mean (n=4) ± standard deviation,
**Harvest date: I - after 10 weeks of vegetation, II - after 14 weeks of vegetation, III - after 18 weeks of vegetation, LSD: factor a – cultivar, factor b – harvest date, interaction a x b.

Statistical Analysis

All analyses were conducted in four replications, each in two parallel samples. The results were statistically verified on the basis of analysis of variance, using the Snedecor F test and the Student T test, the least significant difference (LSD) being calculated for the probability level of $P < 0.01$. The Statistica 6.1 program was applied.

Results and Discussion

Total Nitrogen

Proteins are a basic nutritional constituent, being the main source of nitrogen compounds in food. Depending on the year of the investigation, the content of total nitrogen in kale varied from 0.54 to 0.74 g 100 g^{-1} fresh matter. In 2006 the highest mean content of total nitrogen was found in Winterbor F_1: 0.64 g 100 g^{-1}; a similar level in Średnio Wysoki Zielony Kędzierzawy – 0.62 g; while a significantly lower content of 0.60 g was found in Redbor F_1 (Table 2). In 2007 the level of total nitrogen was fairly similar, except for the cultivar Średnio Wysoki Zielony Kędzierzawy, which showed a significantly higher content of this constituent – 0.70 g 100 g^{-1} on average. Greater differences in the level of total nitrogen were noted between the harvest dates: the later the harvest, the higher the content of nitrogen. The average content of total nitrogen in the kale cultivars was, depending on the year of the investigation, 0.57-0.59 g in 100 g at the first harvest and at the two subsequent harvests the content was higher by 9-16% (2006) and 8-19% (2007), respectively. Lower levels of total nitrogen were recorded in such leafy vegetables as New Zealand spinach [23], lettuce and summer endive [24], and among brassicas, in broccoli [25], while in Brussels sprouts the content was higher [26]. On the basis of the results obtained in the present paper, kale is a good source of protein, as is confirmed by Almeida and Rosa [27]. The protein content in foodstuffs is estimated by multiplying the determined nitrogen content by a nitrogen-to-protein conversion factor (N x 6.25). This factor assumes the nitrogen content of proteins to be 16% [28]. Using the coefficient of nitrogen conversion into protein, the content of this constituent can be expressed as the amount of total protein, which in the kale varied in the range of 3.39 to 4.63 g 100 g^{-1} fresh matter, depending on the year of the investigation, the cultivar and the phase of growth.

Protein Nitrogen

Depending on the year of the investigation, the cultivar and the harvest date, the content of protein nitrogen in the evaluated material was 0.46-0.61 g 100 g^{-1}; and, if converted into protein, 2.88-3.81 g 100 g^{-1} (Table 3). The compared cultivars of kale showed some differences with respect to the level of protein nitrogen. In the first year of the study the cultivar Winterbor F_1 contained 0.58 g 100 g^{-1} on average, significantly exceeding, by 12% and 14% respectively, the levels found in Średnio Wysoki Zielony Kędzierzawy and Redbor F_1. In the following year the highest content of protein nitrogen was found in Średnio Wysoki Zielony Kędzierzawy: 0.58 g 100 g^{-1}, while in the remaining cultivars it was lower by 5% (Winterbor F_1) and 14% (Redbor F_1). At the first harvest date the mean level of protein nitrogen, as an average of all the cultivars over two years, was 0.52 g 100 g^{-1}. On the subsequent dates, i.e. after 14 and 18 weeks of growth, it increased by 4% and 8%, respectively. The highest proportion of protein nitrogen in total nitrogen, 91% (2006) and 87% (2007), was found in the cultivar Winterbor F_1; in the remaining cultivars it did not exceed 85%. No more comprehensive data were found in the literature concerning this constituent in kale. It can be quoted for comparison that Gębczyński [26] found mean protein nitrogen content of 0.39 g in 100 g fresh matter of Brussels sprouts; however, the 52% proportion of protein nitrogen in total nitrogen was much lower than that recorded in kale.

Nitrates

In the commercial production of vegetables one expects, on the one hand, high yields and sensory attractiveness, and on the other, high levels of nutritive constituents and low levels of undesirable compounds, such as nitrates and nitrites [18]. Vegetables, particularly leaf vegetables, are the main source of nitrates in a diet. Nitrates, however, do not present a direct risk for the health of consumers, the noxious constituents being nitrites generated after the partial reduction of nitrates [9].

European Commission Regulations [29] do not give admissible levels of nitrates in kale. The maximum level of these compounds was only quoted for fresh spinach and lettuce: 2500-3000 mg kg^{-1} and 2000-4500 mg kg^{-1}, respectively. Kale contained considerable amounts of nitrates. Depending on the year of growth, the cultivar and the harvest date, their level varied from 248 mg to 2810 mg kg^{-1} (Table 4). The compared cultivars differed significantly in the content of nitrates. Their highest mean level for any harvest dates was found in the cultivar Redbor F_1 – 1276 mg (2006) and 939 mg (2007) in 1000 g fresh matter – exceeding by 54% and 13%, respectively, the values recorded in Średnio Wysoki Zielony Kędzierzawy, and by 61% and 18% those in Winterbor F_1. According to Amr and Hadidi [30], the content of nitrates changes with the age of the plant. Young plants are characterized by a high content of these compounds, since the reduction process is still unable to keep up with their uptake [31]. In kale plants obtained in the first harvest, the content of nitrates was significantly higher compared with the remaining dates; it varied from 1643 to 1730 mg kg^{-1} on average, depending on the year of the investigation and the cultivar. Between the first and second harvest dates, the content of nitrates decreased by 69% (2006) and 64% (2007), and between first and third by 83% (2006) and 82% (2007). As Beis et al. [32] stress, the phase of growth also affected the content of nitrates in spinach plants. These authors showed the highest content of these compounds in the youngest leaves of this species. Gębczyński et al. [16], however, recorded an increasing level of nitrates in leaves of leaf red beet up to the third (August) harvest date, followed by their decrease. The content of nitrates found in kale must be regarded as high compared with that determined by Gębczyński [26] in Brussels sprouts: only 86-148 mg in 1000g fresh matter; or in broccoli: 127-232 mg 1000 g^{-1} [9]. The amounts of nitrates in lettuce found by Kim and Yoon [11] and in dill by Kmiecik et al. [18] were similar to those in kale, while Jaworska and Słupski [23] noted a much higher content of 3472 mg 1000 g^{-1} in New Zealand spinach.

Nitrites

Unlike nitrates, the content of nitrites in vegetables is distinctly low. According to Lisiewska and Kmiecik [33], the chief cause of nitrite accumulation is the inappropriate storage of vegetables, resulting in the microbiological reduction of nitrates. Prugar [34] cites the following as factors contributing to an increased risk of nitrates converting to nitrites in vegetables: high nitrate content in leaves, insufficient insulation or low temperatures during growth, and excessive use of nitrogen fertilizers. The raw material analyzed in the present work contained low amounts of nitrites, varying from 0.14 to 0.80 mg (2006) and 0.14-0.95 (2007) in 1000 g fresh matter (Table 5). The level of these compounds did not exceed 1 mg in 1000 g of a sample, this amount being regarded as normal for physiological transmutations of nitrogen compounds [35]. The highest content of nitrites was found in the cultivar Redbor F_1, whose mean content for the two years of the study and the investigated harvest dates was 0.63 mg nitrites in 1000 g, this being almost three times higher than the values found in Winterbor F_1 and Średnio Wysoki Zielony Kędzierzawy. The differences in nitrite content between the harvest dates were significant in both years of the investigation. The lowest level of nitrites found during the last harvest was 0.24 mg (2006) and 0.20 mg (2007) in 1000 g fresh matter, being 35% and 56% lower than the first harvest. A comparison of the level of nitrites in kale with that in other vegetable species shows that in cauliflower the content was distinctly lower [36], but higher in parsley leaves [37], spinach [17] and New Zealand spinach [23].

Conclusions

The compared cultivars of kale differed in the level of the nitrogen compounds analyzed. Throughout the whole period of the investigation, the mean content of total nitrogen in the cultivar Średnio Wysoki Zielony Kędzierzawy was 3% and 10% higher, respectively, than in the cultivars Winterbor F_1 and Redbor F_1. The content of protein nitrogen in Winterbor F_1 was 4% higher than in Średnio Wysoki Zielony Kędzierzawy and 12% higher than in Redbor F_1. The cultivar Redbor F_1 contained the highest levels of nitrates and nitrites. The content of total nitrogen and protein nitrogen increased with the growth of plants; that of nitrates decreased, while the level of nitrites varied. The average values for the two years and the three cultivars show that, compared with the first harvest kale obtained at the second harvest date contained 9% more total nitrogen and 4% more protein nitrogen; 67% fewer nitrates but 5% more nitrites. The material harvested on the third harvest date compared to the first harvest contained 17% more total nitrogen and 8% more protein nitrogen, while the content of nitrates and nitrites was 83% and 46% lower, respectively.

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References

1. D'ANTUONO L.F., NERI R. Traditional crop revised: yield and quality of palm-tree kale, grown as a mechanised industrial crop, as a function of cutting height. Acta Hort. **598**, 123, **2003**.
2. PHUPAIBUL P., CHINOIM N., MATOH T. Nitrate concentration in Chinese kale sold at markets around Bangkok, Thailand. Thai J. Agric. Sci. **35**, 295, **2002**.
3. HOCHMUTH G.J., BRECHT J.K., BASSETT M.J. Nitrogen fertilization to maximize carrot yield and quality on a sandy soil. HortSci. **34**, 641, **1999**.
4. MOZOLEWSKI W., SMOCZYŃSKI S. Effect of Culinary Processes on the Content of Nitrates and Nitrites in Potatoe. Pakistan J. Nutr. **3**, 357, **2004**.
5. DELGHINGARO-AUGUSTO V., FERREIRA F., BORDIN S., COREZOLA do AMARAL M.E., TOYAMA M.H., BOSCHERO A.C., CARNEIRO E.M. A low protein diet alters gene expression in rat pancreatic islets. J. Nutr. **134**, 321, **2004**.
6. REEDS P.J., GARLIC P.J. Protein and amino acid requirements and the composition of complementary foods. J. Nutr. **133**, 2953S, **2003**.
7. ANDERSON G.H., MOORE S.E. Dietary proteins in the regulation of food intake and body weight in humans. J.Nutr. **134**, 974S, **2004**.
8. REPORTS OF THE SCIENTIFIC COMMITTEE FOR FOOD. Opinions of the scientific committee for food on: nitrates and nitrite, European Commission, thirty-eighth series, Luxembourg, **1997**.
9. GUADAGNIN S.G., RATH S., REYES F.G.R. Evaluation of the nitrate content in leaf vegetables produced through different agricultural systems. Food Addit. Contam. **22**, 1203, **2005**.
10. HUARTE-MENDICOA J.C., ASTIASARÁN I., BELLO J. Nitrate and nitrite levels in fresh and frozen broccoli. Effect of freezing and cooking. Food Chem. **58**, 39, **1997**.
11. Kim B.Y., Yoon S. Analysis of nitrate contents of Korean common foods. J.Korean Soc. Food Sci. Nutr. **32**, 779, **2003**.
12. WHO. Safety evaluation of certain food additives. Fifty-ninth Report of the Joint FAO/WHO Committee on Food Additives. Food Additives Series No. 50, Geneva: WHO, **2003**.
13. LEFSRUD M.G, KOPSELL D.A., KOPSELL D.E. Nitrogen levels influence biomass, element al accumulations, and pigment concentrations in spinach. J.Plant Nutr. **30**, 171, **2007**.
14. ABO-BAKR T.M., EL-IRAQUI S.M., HUISSEN, M.H. Nitrate and nitrite contents of some fresh and processed Egyptian vegetables. Food Chem. **19**, 265, **1986**.
15. FUJIHARA S., KASUGA A., AOYAGI Y. Nitrogen – to - protein conversion factors for common vegetables. J.Food Sci. **66**, 412, **2001**.
16. GĘBCZYŃSKI P., KMIECIK W., LISIEWSKA Z. Effect of harvest time, freezing, and storage of frozen chard on nitrate and nitrite content. Bromat. Chem. Toksykol. **32**, 81, **1999** [In Polish].
17. JAWORSKA G., KMIECIK W. Content of selected mineral compounds, nitrates III and V, and oxalates in spinach (*Spinacia oleracea* L.) and New Zealand spinach (*Tetragonia expansa* Murr.) from spring and autumn growing seasons. Elec. J. Pol. Agric.Univ. s. Food Sci. Technol.

1999. (available at: http://www.ejpau.media.pl/series/volume2/issue2/ food/art- 03.html).
18. KMIECIK W., GĘBCZYŃSKI P. KORUS A. Effect of variety, type of the usable part and the growing period on the content of nitrates, nitrites and oxalates in dill. (Anethum graveolens). Bromat. Chem. Toksykol. **34**, 213, **2001** [In Polish].
19. CHWEYA J.A. Contents of nitrate-n and thiocyanate ions in kale (Brassica oleracea var. acephala. DC) leaves from kale-growing areas in Kenya. XII African Symposium on Horticultural Crops, ISHS Acta Hort. 218, **1988**.
20. AOAC. Official Methods of Analysis of Association of Official Analytical Chemists. 14th ed. Arlington, VA, USA, **1984**.
21. AWOLUMATE E.O. Accumulation and quality of storage protein in developing cowpea, mung bean and soya bean seeds. J. Sci. Food Agric. **34**, 1351, **1983**.
22. ISO/6635-2. Fruits, vegetables and derived products - Determination of nitrite and nitrate content. Molecular absorption spectrometric method. International Organization for Standardization, Case Postale 56, CH-1211, Geneve 20, Switzerland, **1984**.
23. JAWORSKA G., SŁUPSKI J. The value of New Zealand spinach for freezing. Żywność, **2**, 92, **2001** [In Polish].
24. WILLS R.B.H., LIM, J.S.K., GREENFIELD H. Composition of Australian foods 32. Leafy, stem and other vegetables. Food Technol. Austr. **38**, 416, **1986**.
25. WILLS R.B.H. Composition of Australian fresh fruit and vegetables. Food Technol. Austr. **39**, 523, **1987**.
26. GĘBCZYŃSKI P. Contents of selected nitrogenous substances in the fresh and frozen Brussels sprouts. Acta Sci. Pol. s. Technol. Alim., **1**, 27, **2002** [In Polish].
27. ALMEIDA D., ROSA E. Protein and mineral concentration of portuguese kale (*Brassica oleracea* var. *acephala*) related to soil composition. Acta Hort. **407**, 269, **1996**.
28. MARIOTTI F., TOME D., MIRAND P.P. Converting nitrogen into protein – beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. **48**, 177, **2008**.
29. European Commission Regulation 1822/2005/EC, of 8 November 2005, setting maximum levels for certain contaminants in foodstuffs. Off. J. Eur. Comm. **2005**. (available at: http://europa.edu.int).
30. AMR A., HADIDI N. Effect of cultivar and harvest date on nitrate (NO_3) and nitrite (NO_2) content of selected vegetables grown under open field and greenhouse conditions in Jordan. J. Food Comp. Anal. **14**, 59, **2001**.
31. ROŻEK S. Factors influence the nitrate levels in vegetable crops. Zesz. Nauk. AR Kraków **364**, 19, **2000** [In Polish].
32. BEIS G.H., SIMONS A.S., DOGRAS C.C. Spinach composition as affected by leaf age and plant part. Acta Hort. **579**, 653, **2002**.
33. LISIEWSKA Z. KMIECIK W. Nitrate and nitrite in vegetables. Part II. Changes of the levels of nitrates and nitrites in vegetables during short and long-lasting storage. Postępy Nauk Rol. **3**, 25, **1991b** [In Polish].
34. PRUGAR J. Zmeny obsahu dusicnanu a dusitanu pri skladovani rostlinnych produktu. Rostlinna Vyroba, **39**, 1155, **1993**.
35. LISIEWSKA Z., KMIECIK W. Nitrate and nitrite in vegetables. Part I. Effect of some factors on the levels of nitrates and nitrites in raw vegetables. Postępy Nauk Rol. **3**, 11, **1991a** [In Polish].
36. KOMOSA A., BREŚ W., GOLCZ A., KOZIK E., TYKSIŃSKI W. Nitrate and nitrite contents in vegetables grown in Poznań area. Prace Kom. Nauk Rol. i Kom.Nauk Leś. **75**, 91, **1993** [In Polish].
37. LISIEWSKA Z., KMIECIK W. Effect of freezing and storage on quality factors in Hamburg and leafy parsley. Food Chem. **60**, 633, **1997**.