Effects 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 (NO3−) and nitrites (NO2−) was measured in the leaves of kale of the cultivars Winterbor F1, Redbor F1 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 (NO3−) was 248-2810 mg and of nitrites (NO2−) 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 F1. The cultivar Redbor F1 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\textsubscript{3}\textsuperscript{−}) and nitrites (NO\textsubscript{2}\textsuperscript{−}) 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\textsubscript{1} and Redbor F\textsubscript{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 quality 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\textsubscript{3}\textsuperscript{−}) and nitrites (NO\textsubscript{2}\textsuperscript{−}) according to the growing season, the cultivar and the time of harvest.

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\textsubscript{3}\textsuperscript{−}) and nitrites (NO\textsubscript{2}\textsuperscript{−}). 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\textsubscript{3}\textsuperscript{−}) and nitrites (NO\textsubscript{2}\textsuperscript{−}) 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*.

<table>
<thead>
<tr>
<th>Year of investigation</th>
<th>Month</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>July</td>
<td>August</td>
<td>September</td>
<td>October</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>temp. max.ºC</td>
<td>temp. min.ºC</td>
<td>rainfall mm</td>
<td>temp. max.ºC</td>
<td>temp. min.ºC</td>
<td>rainfall mm</td>
<td>temp. max.ºC</td>
<td>temp. min.ºC</td>
</tr>
<tr>
<td>2006</td>
<td>25.0</td>
<td>14.8</td>
<td>126.1</td>
<td>26.6</td>
<td>13.9</td>
<td>23.6</td>
<td>20.9</td>
<td>8.6</td>
</tr>
<tr>
<td>2007</td>
<td>30.8</td>
<td>8.0</td>
<td>97.4</td>
<td>32.5</td>
<td>8.6</td>
<td>77.2</td>
<td>26.6</td>
<td>4.1</td>
</tr>
</tbody>
</table>

*data from Kraków-Balice weather station (50°05' N, 19°47'W).

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

<table>
<thead>
<tr>
<th>Year of investigation</th>
<th>Cultivar</th>
<th>Harvest date**</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g 100 g⁻¹</td>
<td>g 100 g⁻¹</td>
</tr>
<tr>
<td>2006</td>
<td>Winterbor F₁</td>
<td>0.63±0.01</td>
<td>0.62±0.03</td>
</tr>
<tr>
<td></td>
<td>Średnio Wysoki Zielony Kędzierzawy</td>
<td>0.55±0.02</td>
<td>0.65±0.01</td>
</tr>
<tr>
<td></td>
<td>Redbor F₁</td>
<td>0.54±0.02</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.57</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>LSD P&lt;0.01 factor a – 0.024 factor b – 0.024 interaction a x b – 0.041</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Year of investigation</th>
<th>Cultivar</th>
<th>Harvest date**</th>
<th>Mean</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g 100 g⁻¹</td>
<td>g 100 g⁻¹</td>
</tr>
<tr>
<td>2007</td>
<td>Winterbor F₁</td>
<td>0.57±0.02</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td></td>
<td>Średnio Wysoki Zielony Kędzierzawy</td>
<td>0.65±0.02</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td></td>
<td>Redbor F₁</td>
<td>0.56±0.02</td>
<td>0.59±0.02</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.59</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>LSD P&lt;0.01 factor a – 0.027 factor b – 0.027 interaction a x b – n.s</td>
<td></td>
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</tr>
</tbody>
</table>

*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.

<table>
<thead>
<tr>
<th>Year of investigation</th>
<th>Cultivar</th>
<th>Harvest date **</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g 100 g⁻¹</td>
<td>g 100 g⁻¹</td>
</tr>
<tr>
<td>2006</td>
<td>Winterbor F₁</td>
<td>0.60±0.02</td>
<td>0.56±0.01</td>
</tr>
<tr>
<td></td>
<td>Średnio Wysoki Zielony Kędzierzawy</td>
<td>0.47±0.01</td>
<td>0.59±0.02</td>
</tr>
<tr>
<td></td>
<td>Redbor F₁</td>
<td>0.49±0.01</td>
<td>0.46±0.01</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.52</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>LSD P&lt;0.01 factor a – 0.019 factor b – 0.019 interaction a x b – 0.034</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Year of investigation</th>
<th>Cultivar</th>
<th>Harvest date **</th>
<th>Mean</th>
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<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td></td>
<td>g 100 g⁻¹</td>
<td>g 100 g⁻¹</td>
</tr>
<tr>
<td>2007</td>
<td>Winterbor F₁</td>
<td>0.53±0.01</td>
<td>0.51±0.01</td>
</tr>
<tr>
<td></td>
<td>Średnio Wysoki Zielony Kędzierzawy</td>
<td>0.54±0.03</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td></td>
<td>Redbor F₁</td>
<td>0.50±0.02</td>
<td>0.48±0.01</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.52</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>LSD P&lt;0.01 factor a – 0.030 factor b – 0.030 interaction a x b – 0.052</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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.
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.

### Statistical Analysis

### 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 F₁: 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 F₁ (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 F₁ 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 F₁. 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 F₁) and 14% (Redbor F₁). 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 F₁; 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 date was found in the cultivar Redbor F₁ – 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 F₁. 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 Kniecik et al. [18] were similar to those in kale, while Jaworska and Slupski [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 Kniecik [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 nitrates, 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 nitrates was found in the cultivar Redbor F1, 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 F1 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 nitrates 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 nitrates 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 F1 and Redbor F1. The content of protein nitrogen in Winterbor F1 was 4% higher than in Średnio Wysoki Zielony Kędzierzawy and 12% higher than in Redbor F1. The cultivar Redbor F1 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 nitrites 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.

Acknowledgements

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