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INFLUENCE OF FERTILIZATION ON YIELD AND QUALITY OF SPINACH (*Spinacia oleracea* L.) DURING STORAGE UNDER CONTROLLED ATMOSPHERE

WPŁYW NAWOŻENIA AZOTEM NA PLON I JAKOŚĆ SZPINAKU (*Spinacia oleracea* L.) PODCZAS PRZECHOWYWANIA W KONTROLOWANEJ ATMOSFERZE

Abstract: The aim of the study was to examine the influence of nitrogen fertilization, mainly in the form of ammonium, containing the inhibitor of nitrification Dicyandiamide that delays the release of nitrogen into the soil, on optimal growth, yield production, and yield quality development of spinach as well as the suitability of spinach for storage compared with conventionally applied fertilizer. In addition to the influence of the applied kind of fertilizers, the dependency from the concentration of the fertilizer was studied. The experimental results revealed that the application of nitrogen in the form of Basammon-nitrogen resulted in a better color development of leaves, a higher content of chlorophyll and an in tendency higher crop yield compared with calcium-ammonium nitrate. Independent from the kind of fertilizer, the standard dose of 220 $kg \cdot ha^{-1}$ N proved to be optimal. Excessive fertilization with 330 $kg \cdot ha^{-1}$ N resulted in a decrease of sugar contents, enlarged concentrations of nitrate, and the decrease in taste values. At the same time it increased the costs for fertilization and the environmental risk due to rinsing of nitrates into the groundwater. A positive aspect was the more intensive color of leaves. The kind of fertilizer did not affect the suitability for storage of spinach. However excessive nitrogen caused greater losses of soluble carbohydrates during storage.

Keywords: *Spinacia oleracea*, calcium-ammonium nitrate, Basammon-nitrogen, quality, storage in controlled atmosphere

Spinach belongs to plants with large demands of nitrogen (220 $kg \cdot ha^{-1}$), which are necessary for the development of leaf mass as the consumed part of the plant and of the deep green color preferred by consumers. At the same time the weak assimilation capacity for nitrogen by spinach may cause leaching of nitric compounds. It may also create the risk of nitrate accumulation in leaves and a general decrease in spinach quality [1]. Because nitrogen plays a very important role for yield production and quality characteristics in spinach, it is frequently applied in excessive doses, which may lead to a reduced suitability for storage and impair quality parameters [2]. By applying

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the suitable cultivation treatment, risks of losses due to excessive accumulation of nitrates(V), disturbances in the development of plants and their metabolism, especially carbohydrate metabolism, modifications of tissues, flabbiness of plants, and enlarged susceptibility to diseases and insect attacks can be reduced [3]. One possibility represents the application of nitrification inhibitors, which limit the contamination of groundwater with nitrogen, diminish the degree of nitrate(V) accumulation in leaves, and also decrease the content of oxalates [4, 5]. Moreover, the application of nitrification inhibitors can positively affect (increase) the contents of carbohydrates and ascorbic acid [6] and result in a more attractive leaf color [4]. Sometimes, however, they can reduce yield.

In the experiments presented here, it was investigated, whether higher fertilizer concentrations applied as Basammon-nitrogen (BAS) in combination with Dicyandiamide, an inhibitor of nitrification processes, result in a better growth and improve quality parameters of spinach plants, when compared with the conventional fertilizer calcium-ammonium nitrate (KAS) under field conditions. In addition, the influence of different nitrogen concentrations per hectare for both kinds of fertilizers on the quality of spinach and its suitability for storage was investigated. This study contributed to the improvement of quality and storage suitability of spinach by resulting in recommendations for fertilization and storage systems.

Material and methods

Spinach plants (*Spinacia oleracea* L. cv. San Felix F1) were grown in experimental fields at the Marhof Research Station, Wesseling, of the University Bonn, Germany, under conditions in line with agricultural practice in a randomized block design with four replicates of each block (split-plot), where the area of single parcel was 11.25 m^2. The first factor was the applied form of fertilizer with 27 % nitrogen (calcium--ammonium nitrate (KAS) consisting of 13.5 % nitrate and 13.5 % ammonium and Basammon-nitrogen (BAS) consisting of 6.8 % nitrate(V), 18.2 % ammonium and 2 % nitrification blocker Dicyandiamide (DCD)). The second factor was the amount of applied nitrogen per hectare (standard supply of 220 kg and excessive supply of 330 kg). As control KAS with 220 kg $N \cdot ha^{-1}$ was used. Plants were cultivated on a brown soil (70–78 soil points) with a thickness of 0.75 to 1.20 m situated on top of sand and gravel, of middle water permeability and of middle to high water storage capacity with the groundwater level at 13 m depth. The soil (0–30 cm) was characterized by an pH_{KCl}-value of 6.6, P_2O_5 0.43 $g \cdot kg^{-1}$ soil, K_2O 0.24 $g \cdot kg^{-1}$ soil, and MgO 18 $g \cdot kg^{-1}$ soil. Contents of nitrate(V) and ammonia before fertilization were 39.36 ± 12.87 and 17.4 ± 9.0 kg $N \cdot ha^{-1}$, respectively. Spinach was sown in rows with a distance of 25 cm (500 seeds/m^2) at three dates: 29.04., 7.05., and 12.05. Two $kg \cdot ha^{-1}$ of Venzar 80 WP (lenacil 80 %), a pre-emergency herbicide, were used to control weeds. Other pesticides were not used during the cultivation. After 6 weeks of growing the mature plants were harvested as a rosette (so called root-spinach) and stored for 16 days at a temperature of 2–4 ^{o}C and a relative humidity between 95–100 % under a controlled atmosphere consisting of 3 % CO_2 and either 4 % or 18 % O_2, respectively (4 replicates with 20 plants each). Analyses were carried out at the beginning and at the end of the storage period to investigate quality changes. Investigated parameters were: the contents of dry matter, nitrates(V) [7], chlorophyll [8], soluble carbohydrates [9], and selected mineral substances [9]. Furthermore, changes of leaves color and chlorophyll fluorescence of PS II as indicators of freshness were measured [9]. The obtained data were analyzed with the SPSS 9.01 statistical program (SPSS Inc, 1989–1999). All data sets were tested for normal distribution and variance homogeneity ($p \leq 0.05$). Means were compared by Duncan test for homogenous variances ($p \leq 0.05$).

Results and discussion

At the end of the vegetative growth period, differences between the applied cultivation practices with respect to the development of fresh mass, dry mass and leaf area of spinach were not detected. The results did not confirm the observations of Ko et al [10] of a diminished reduction of fresh mass during storage under an atmosphere with a lowered oxygen content. During storage of spinach under controlled atmosphere slight losses of fresh mass were observed, which did not exceed the critical value of 3 % and were independent from the kind of fertilizer, the amount of nitrogen supplied and the concentration of oxygen in the storage atmosphere. Losses of dry mass could not be ascertained. The results indicated an even, well balanced supply of plants with mineral nutrients and did not confirm Lazo [11] or Kolbe and Zhang [12], who showed that fertilization with excessive nitrogen concentrations, particularly in the form of ammonium resulted in enlarged mass losses during storage. The reduction of storage temperature to 3–4 ^{o}C, the elevated relative humidity of up to 100 %, and the increased CO_2 concentration assured optimal conditions for the storage of spinach, resulting in decreased losses of fresh mass, which were smaller than 3 %.

High doses of nitrogen applied as KAS or BAS during the experiment indeed enlarged the concentration of nitrate(V) in leaves of spinach (Table 1). However nitrate content did not exceed the allowed limit of 2500 $mg \cdot kg^{-1}$ fresh mass [13]. The effect of excessive nitrogen was still visible during storage, which resulted in a further increase in leaf nitrate content as a result of transpiration processes. Neither cold storage nor the reduction of oxygen content in the storage atmosphere below 4 % did result in a reduction of nitrate content. The highest content of nitrates(V) was found in leaves of spinach fertilized with BAS (Table 1).

In plants fertilized with KAS, Ca contents were elevated before and after storage compared with plants fertilized with BAS (Table 1). This was the result of the additionally supplied calcium in form of calcium-ammonium nitrate fertilizer containing 7 % CaO. The excessive fertilization with nitrogen did not influence the accumulation of Ca ions in spinach leaves.

Independent of the kind and amount of fertilizer an increase of leaf Ca content in spinach leaves was observed during storage (Table 1), which was caused by the translocation of Ca from leaf petioles and apical shoots to leaves [14]. At the same time the concentration of K decreased, especially in plants fertilized with BAS. These changes resulted from the opposite translocation of Ca and K, the latter being transported to leaf petioles and meristematic cells of the growth cone [14]. In spinach plants fertilized with KAS the translocation of K to the growth cone and to leaf petioles was higher (data not showed). However, K contents of spinach leaves after harvest were not significantly affected by the fertilization.

Table 1

Content of nitrate(V), calcium and soluble carbohydrates in spinach leaves as affected by the kind of fertilizer and nitrogen supply as well as oxygen content in the atmosphere during storage, calculated in g · kg^{-1} d.m. (mean values of three experiments, n = 36)

Storage [days]	KAS 220 kg N · ha^{-1}	KAS 330 kg N · ha^{-1}	BAS 220 kg N · ha^{-1}	BAS 330 kg N · ha^{-1}
		Nitrate(V)		
0	0.43 ± 0.12 e	0.71 ± 0.29 abc	0.50 ± 0.16 de	0.73 ± 0.19 ab
16 (18 % O_2)	0.52 ± 0.23 cde	0.81 ± 0.21 a	0.53 ± 0.30 cde	0.80 ± 0.16 a
16 (4 % O_2)	0.60 ± 0.28 bcde	0.81 ± 0.17 a	0.66 ± 0.35 abcd	0.82 ± 0.11 a
		Ca		
0	14.5 ± 1.8 def	15.2 ± 2.2 ef	13.5 ± 1.0 ef	11.7 ± 1.0 f
16 (18 % O_2)	19.2 ± 2.4 bcd	19.7 ± 2.6 bc	13.7 ± 1.7 ef	16.1 ± 2.7 cde
16 (4 % O_2)	20.7 ± 2.6 ab	24.1 ± 2.5 a	16.5 ± 3.5 bcde	16.5 ± 3.3 bcde
		Total soluble carbohydrates		
0	97.4 ± 25.2 a	77.1 ± 20.8 b	105.1 ± 28.4 a	77.0 ± 18.5 b
16 (18 % O_2)	54.4 ± 16.8 cd	51.2 ± 17.5 cd	59.0 ± 14.1 c	56.0 ± 13.2 cd
16 (4 % O_2)	44.9 ± 21.6 de	33.6 ± 9.9 e	45.3 ± 12.5 cde	34.3 ± 9.7 e
		Sucrose		
0	57.2 ± 19.9 ab	46.5 ± 18.6 b	61.0 ± 24.5 a	55.0 ± 12.5 ab
16 (18 % O_2)	23.6 ± 19.3 c	23.1 ± 18.6 c	21.8 ± 17.4 c	21.8 ± 17.5 c
16 (4 % O_2)	16.5 ± 16.7 c	10.9 ± 3.5 c	15.9 ± 7.2 c	12.0 ± 2.5 c
		Glucose		
0	26.5 ± 7.2 ab	20.6 ± 8.2 bcd	29.1 ± 8.4 a	14.4 ± 10.8 def
16 (18 % O_2)	18.6 ± 4.4 cd	18.9 ± 5.7 cd	23.6 ± 7.0 abc	22.7 ± 3.7 bc
16 (4 % O_2)	12.6 ± 7.6 ef	8.7 ± 6.5 f	15.2 ± 7.7 de	11.5 ± 6.3 ef
		Fructose		
0	13.7 ± 6.2 abc	10.0 ± 7.0 bcd	15.0 ± 8.3 ab	7.6 ± 6.3 d
16 (18 % O_2)	12.4 ± 5.5 abcd	9.2 ± 5.8 cd	13.6 ± 7.5 abc	11.6 ± 6.4 abcd
16 (4 % O_2)	15.8 ± 5.1 a	14.0 ± 3.8 ab	14.2 ± 4.1 ab	10.9 ± 2.2 bc

Excessive fertilization with nitrogen resulted, independent of the kind of fertilizer, in a significant reduction of soluble carbohydrate contents in spinach leaves, particularly in reducing sugars such as glucose and fructose (Table 1). This effect persisted until the end of the shelf-life period. During this period a significant decrease of the sum of carbohydrates, caused by the decay of sucrose and the consumption of glucose in metabolic processes, was observed. Decreasing the concentration of oxygen to the 4 % level led to a quicker decrease of their concentrations (Table 1). In addition, plants fertilized with KAS were characterized by a slower reduction of the concentrations of soluble carbohydrates, except of fructose. According to Takebe et al [6] excessive fertilization with nitrogen negatively influenced carbohydrate metabolism, in particular it increased the degree of sugar degradation. These observations were confirmed by the present study. The excessive supply of nitrogen, irrespective of the applied fertilizer, resulted in a significant decrease of soluble carbohydrate contents.

The decrease of the oxygen concentration in the storage atmosphere resulted in the decrease of sugar content, particularly of sucrose and glucose. The concentration of oxygen in the atmosphere of about 4 % favored fermentative processes, probably as the result of a disadvantageous gas exchange among stored spinach plants and/or diffusion barriers within the tissue [10, 14]. In case of fermentation, carbohydrates were not completely oxidized to water and CO_2, but were degraded to ethanol. Hence, 19 times more sugar was needed for the synthesis of the same amount of ATP compared to the oxidative dissimilation. Oxygen deficiency in tissues of numerous vegetables and fruits has frequently been recorded at atmospheric oxygen concentration of ca 3 % and lower [15]. For spinach the so-called 'Extinction Point', at which fermentation processes occur, is known to correspond to 0.8 % O_2 at 0.5 % CO_2 [16]. The appearance of fermentative processes already at a concentration of 4 % O_2 was probably related to a rise in the CO_2 concentration in the storage atmosphere to the 3 % level.

At harvest, irrespective of the applied fertilizer treatment, optimum quantum yield (Fv/Fm) of photosystem II (PSII) recorded after dark adaptation reached levels of 0.82, which is typical of healthy, unimpaired plants (Table 2). Generally, storage, particularly under an atmosphere containing 4 % O_2, resulted in a significant decrease of optimum quantum yield and maximum fluorescence, while basal fluorescence rose simultaneously. The decrease of maximum fluorescence frequently indicates the occurrence of a reversible kind of stress, however an increase of basal fluorescence is interpreted as a damage of photosystem II (PSII) reaction centers at the acceptor side [17]. Changes in chlorophyll fluorescence generally reflect disturbances of chloroplast functions, a decrease of spinach quality and the loss of its freshness as a result of the 16-day storage period.

Content of total chlorophyll, Chl a and Chl b at time of crop harvest was related to nitrogen supply (Table 2). The excessive supply of nitrogen resulted in an increase of leaf chlorophyll content in spinach, independent of the kind of fertilizer. During storage of plants grown with BAS, total chlorophyll content and content of Chl *b* rose significantly (Table 2) independent of the amount of nitrogen fertilizer applied. In all cases the decrease of the oxygen concentration to the 4 % level improved the preservation of total chlorophyll content during storage (Table 2). These changes however, were not reflected in a better color appearance of leaves, expressed as a decreasing Chroma C* value (Table 2).

The present research demonstrated the essential influence of the applied fertilizer treatment on the intensity of leave color, expressed as the Chroma C* value (Table 2). The excessive fertilizations with nitrogen as BAS resulted in a more intensive color of spinach leaves. Storage under controlled atmosphere did not influence the intensity of leaf color. The experiments could not prove an impairment of leaf color during storage

(Table 2). This may be explained by adequate storage conditions, particularly the high relative humidity of up to 100 %, the low air temperature of about 2–4 ^{o}C, and the elevated CO_2 concentration in the storage atmosphere.

Table 2

Optimum quantum yield, contents of total chlorophyll, Chl *a*, Chl *b*, the Chl *a/b* ratio and color values of spinach leaves as affected by the kind of fertilizer, amount of nitrogen supply and oxygen content in the atmosphere during storage (mean values of three experiments, n = 36)

Storage [days]	KAS 220 kg N · ha^{-1}	KAS 330 kg N · ha^{-1}	BAS 220 kg N · ha^{-1}	BAS 330 kg N · ha^{-1}
		Fv/Fm [rel. units]		
0	0.82 ± 0.08 a	0.83 ± 0.11 a	0.82 ± 0.09 a	0.82 ± 0.10 a
16 (18 % O_2)	0.58 ± 0.13 b	0.60 ± 0.11 b	0.64 ± 0.13 b	0.62 ± 0.12 b
16 (4 % O_2)	0.51 ± 0.09 c	0.50 ± 1.04 c	0.45 ± 0.14 c	0.49 ± 0.20 c
		Total chlorophyll [mg · kg^{-1} d.m.]		
0	9.61 ± 0.76 bc	9.77 ± 0.66 abc	9.27 ± 0.64 c	10.16 ± 0.63 abc
16 (18 % O_2)	9.24 ± 1.16 c	9.45 ± 0.82 c	9.90 ± 0.67 abc	9.75 ± 1.59 abc
16 (4 % O_2)	9.71 ± 0.55 abc	10.04 ± 1.04 abc	10.58 ± 1.02 ab	10.70 ± 0.99 a
		Chl *a* [mg · kg^{-1} d.m.]		
0	7.01 ± 0.48 ab	7.16 ± 0.45 ab	6.91 ± 0.49 ab	7.47 ± 0.49 a
16 (18 % O_2)	6.72 ± 0.88 b	6.84 ± 0.62 ab	7.18 ± 0.54 ab	7.04 ± 1.08 ab
16 (4 % O_2)	7.05 ± 0.42 ab	7.20 ± 0.75 ab	7.38 ± 0.68 ab	7.52 ± 0.71 a
		Chl *b* [mg · kg^{-1} d.m.]		
0	2.59 ± 0.29 bc	2.62 ± 0.29 bc	2.36 ± 0.22 c	2.70 ± 0.17bc
16 (18 % O_2)	2.52 ± 0.29 bc	2.61 ± 0.22 bc	2.73 ± 0.15 b	2.71 ± 0.53b
16 (4 % O_2)	2.66 ± 0.18 bc	2.85 ± 0.31 b	3.20 ± 0.51 a	3.18 ± 0.38a
		Chl *a/b* ratio [rel. units]		
0	2.71 ± 0.14 bc	2.76 ± 0.24 b	2.94 ± 0.23 a	2.77 ± 0.10 b
16 (18 % O_2)	2.66 ± 0.09 bc	2.62 ± 0.11 bc	2.63 ± 0.10 bc	2.61 ± 0.16 bc
16 (4 % O_2)	2.66 ± 0.15 bc	2.53 ± 0.13 cd	2.34 ± 0.29 e	2.38 ± 0.22 de
		Chroma C* [rel. units]		
0	20.06 ± 2.01 a	18.19 ± 0.94 ab	19.71 ± 1.33 a	15.69 ± 2.10 c
16 (18 % O_2)	19.92 ± 1.49 a	18.53 ± 1.71 a	19.59 ± 2.15 a	16.17 ± 3.17 bc
16 (4 % O_2)	20.14 ± 0.85 a	18.77 ± 2.13 a	19.94 ± 2.02 a	16.07 ± 2.96 bc

The choice of the suitable kind of fertilization in case of only small differences in growth and postharvest behavior should be based first of all on the profitability of the applied cultivation practice. Excessive fertilization, independent of the kind of applied fertilizer, resulted in additional costs and in the decrease of the yield per hectare (Table 3). In comparison with the standard supply, the excessive fertilization resulted in measurable losses and lowered profits in the case of KAS by about 6400 EUR and in the case of BAS by about 5540 EUR per hectare. In case of the standard nitrogen supply, fertilization in the form of BAS resulted in the greatest profit.

Table 3

Summarized calculation of costs, losses and profit by the application of KAS and BAS in spinach cultivation based on spinach yield harvested from 1 ha and stored for 16-days under controlled atmosphere

Combination	Yield [Mg · ha^{-1}, EUR · ha^{-1}]	Storage losses [%, EUR · ha^{-1}]		Fertilizer [EUR · ha^{-1}]	Profit [EUR · ha^{-1}]	
		18 % O_2	4 % O_2		18 % O_2	4 % O_2
KAS 220 kg · N/ha	30.30 60600	1.97 1194	1.25 758	1062.5	58343.5	58779.5
KAS 330 kg · N/ha	27.42 54840	2.52 1382	1.87 1026	1527.7	51930.3	52286.3
BAS 220 kg · N/ha	30.84 61680	1.55 956	1.83 1129	366.6	60357.4	60184.4
BAS 330 kg · N/ha	28.14 56280	1.62 912	2.27 1278	550.1	54817.9	54451.9

Assumption: mean prize for (2008): a) 1 Mg of spinach: 2000 EUR, b) 1 Mg KAS: 1250 EUR, c) 1 Mg BAS: 450 EUR.

Conclusions

1. During storage under controlled atmosphere both of the tested fertilizers, KAS and BAS, showed similar influences on spinach quality, especially on losses of fresh mass, which did not reached the allowed limit of losses of 3 %.

2. The positive influence of fertilization with BAS resulted in a more intensive leaf color and higher contents of chlorophyll.

3. Fertilization in the form of KAS resulted in a greater leaf mass and a higher concentration of Ca in the plant.

4. Excessive nitrogen fertilization improved leaves color, lowered the content of sugars, and enhanced the accumulation of nitrates(V), especially during storage. Worthy of note, excessive nitrogen fertilization resulted in higher costs and caused leaching of nitrogen into the groundwater.

5. The reduction of oxygen content in the storage atmosphere from 18 % to 4 % at a CO_2 concentration of 3 % was unfavorable for spinach plants due to the enhanced consumption of sugars and production of ethanol, which resulted in a quicker loss of spinach quality.

6. Based on the results of the present experiment it can be concluded that spinach should be stored at a low temperature of 0–5 ^{o}C, high humidity of more than 98 % and in an atmosphere consisting of 3–5 % CO_2 with a partially reduced oxygen concentration not lower than 7–10 %.

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WPŁYW NAWOŻENIA AZOTEM NA PLON I JAKOŚĆ SZPINAKU (*Spinacia oleracea* L.) PODCZAS PRZECHOWYWANIA W KONTROLOWANEJ ATMOSFERZE

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Abstrakt: Celem pracy było przebadanie wpływu nawozów azotowych z przewagą związków amonowych zawierających inhibitor nitryfikacji (DCD) opóźniający uwalnianie azotu do gleby na rozwój, plonowanie i jakość plonu oraz zdolność przechowalniczą szpinaku w porównaniu z konwencjonalnym nawozem stosowanym w jego uprawie. Ponadto wpływ zastosowanych nawozów przebadano w zależności od ilości ich dawki. Badania wykazały, że zastosowanie azotu w postaci Basammonu spowodowało lepsze wybarwienie liści, większą zawartość chlorofilu i tendencyjnie wyższy plon w porównaniu do saletry wapniowo--amonowej. Niezależnie od rodzaju nawozu optymalną okazała się dawka standardowa w ilości N 220 $kg \cdot ha^{-1}$. Luksusowe nawożenie azotem w ilości N 330 $kg \cdot ha^{-1}$ spowodowało obniżenie zawartości cukrów, zwiększoną koncentrację azotanów i obniżenie walorów smakowych, przy jednoczesnym, niepożądanym wzroście kosztów zabiegów i zanieczyszczenia środowiska na skutek wypłukiwania azotanów do wód gruntowych. Pozytywnym aspektem okazało się zwiększenie intensywności wybarwienia liści. Rodzaj nawozu nie wpłynął na zdolność przechowalniczą szpinaku, natomiast nadmiar azotu spowodował większe straty węglowodanów rozpuszczalnych w czasie przechowywania.

Słowa kluczowe: *Spinacia oleracea*, saletra wapniowo-amonowa, Basammon, jakość, przechowywanie w kontrolowanej atmosferze