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IMPACT OF NUTRITION ON THE QUALITY AND QUANTITY OF CAULIFLOWER FLORETS

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ABSTRACT

Cauliflower (*Brassica oleracea* var *botrytis*) as a member of the family Brassicaceae belongs to worldwide popular vegetable with using in all kinds of cuisine. The aim of the work was to find out the effects of nutrition and fertilization (in dependence on the amount of nitrogen) on the yield of florets as well as on selected qualitative characteristics - vitamin C, nitrate and sulforaphane content. Analyses were done by the help of liquid chromatograph (HPLC) with UV detector for separation. The trial was led in Nitra, Slovakia, in 2014 – 2015. Cauliflower variety CHARLOT F1 was selected for testing. Four variants have been examined in the trial: control (without application of fertilizers), $N_{150}S_{80}$ (application of nitrogen and sulphur at the supply level N: S = 150:80 kg.ha^{-1}), $N_{200}S_{80}$ and $N_{250}S_{80}$. Application of nitrogen led to significant increasing of the yields of primary cauliflower florets in case of the dose 200 and 250 kg N.ha^{-1} (increasing about 87% and 134% compared to control). Applied nitrogen nutrition caused significant nitrates increasing in monitored cauliflower florets in order of the variants: 1 (control) <2 (N = 150 kg.ha^{-1}) <3 (N = 200 kg.ha^{-1}) <4 (N = 250 kg.ha^{-1}), but the highest dosage of nitrates is still under the permissible standard according to Food codex of Slovak republic (700 mg.kg^{-1} of FM). The influence of differentiated nutrition on sulforaphane content was statistically confirmed in case of the 4th variant in comparison to control, where the decrease about 40% was noticed. Influence of nitrogen fertilizing according to used methodology on vitamin C (AA) content was not statistically confirmed. The nutrient concentration in the curds and stalks of cauliflower varied insignificantly with levels of nitrogen applied.

Keywords: cauliflowers; nitrogen fertilizers; vitamin C; nutrition

INTRODUCTION

Cauliflower (*Brassica oleracea* var *botrytis*) as a member of the family *Brassicaceae* belongs to worldwide popular vegetable with using in all kinds of cuisine. In the nutritional composition it has a high proportion of water, up to 91%, sugar in an amount of 4.5%, protein 2.5%, crude fibre 1.8%, and low fat, only 0.3%. From the minerals in the cauliflower are significantly represented potassium, phosphorus, calcium, sodium, magnesium, as well as iron and sulphur. Another part are the phytochemicals, vitamins (vitamin C, vitamin B 12, PP, etc.) **Uher et al. (2009)**. The vegetable when consumed confers health benefits to human as well as animals because of its richness in vitamins specially vitamin C, which is known to provide protection against certain types of cancer, help in lowering blood cholesterol, and serving as strong anti-oxidants **Batabyala et al. (2016)**. Sulforaphane (SFN) shows a range of biological activities and health benefits in humans, has been found to be a very promising chemopreventive agent against not only a variety of cancers such as breast, prostate, colon, skin, lung, stomach, and bladder but also against cardiovascular and neurodegenerative diseases and diabetes **Kim et al. (2016)**. Very important for human health as well as for optimal growth of the plant is nutrition which starts on the field. The scientific researches show that application of optimum dose of boron and nitrogen decrease head rot and hollow stem of broccoli and increase macronutrient and micronutrient concentrations such as nitrogen, phosphorus, potassium, iron and zinc in broccoli head those are useful and necessary for human's health **Yoldas et al. (2008)**.

Nitrogen and sulphur fertilization positively affected on crop yields and bioactive compounds (vitamin C, E1 and ß-carotene) content in cauliflower edible heads **(Uher et al., 2013)** as well as accumulation of sulforaphane in the cauliflower **(Čekey et al., 2011)**. The use of chemical fertilizers is essential in order to achieve the maximum potential of the genetic material and, as well as to succeed the highest possible yield and quality of the final product **(Theofanoudis et al., 2015)**. Nitrogen fertilizers are commonly used to ensure the yields in brassicas productions. Their insufficiency leads to quality and quantity reduction of the cauliflower florets which is not acceptable to growers. However, the application of N fertilizer will have a minimal effect on increasing yields if other factors are not properly managed (i.e., weather conditions and horticultural practice) **(Quiro´s et al., 2015)**. Cauliflower has high nitrogen (N) demand during growing, but due to its low N use efficiency and N-rich residues, it may cause N losses by leaching after harvest **(Xie and Kristensen, 2016a)**. Intake of ascorbic acid, pectin, fibre and certain minerals, which significantly suppress the conversion of nitrate to N-nitrous compounds, is also favourable **(Šrot, 2005)**.

Vegetable nitrate content is of interest to governments and regulators owing to the possible implications for health and to check that controls on the content are effective **(Santamaria, 2006)**. The key to reduce soil nitrate N without jeopardizing crop yields may be to identify suitable growing periods, sometimes combined with root pruning, for each LM (living mulches) species and system design **(Xie et al., 2016b)**. Nitrate per se is relatively non-toxic but approximately 5% of all ingested nitrate is converted in saliva and the gastrointestinal tract to the more toxic nitrite **(Santamaria, 2006)**. Nitrate metabolites may produce a number of health effects, some studies showed that nitrate exposure is correlated with gastric cancer risk due to the endogenous formation of N-nitroso compounds **(Nejatzadeh-Barandozi and Gholami-Borujeni, 2013)**. Approximately 80% of dietary nitrates are derived from vegetable consumption; sources of nitrites include vegetables, fruit, and processed meats **(Hord et al., 2009)**.

The aim of this work was to determine the effect of nutrition and fertilization (the amount of nitrogen) on the yield, the vitamin C (ascorbic acid) and sulforaphane content as well as on the nitrate content in the cauliflower florets.

MATERIAL AND METHODOLOGY

The trial realisation

The sowing of the cauliflower seeds was realised on May 15th 2014 and May 14th 2015 on free land (field conditions) at the Department of Vegetable FZKI in Nitra. Planting of the seedlings took place on June 16th 2014 and June 22nd 2015. The area of experimental plots of 1 repeat was 2.25 m²; in case of quadruplicate repetition the area of one experimental variant measured 9 m^2. In one repeat 9 seedlings of cauliflower were planted in spacing of 0.5 x 0.5 meters. The harvest of florets in physiological maturity was realised gradually starting from October 1st to October 28th in the frame of both observed years, whereby the 5 partial harvests were done. Analyses of qualitative parameters were made from the last harvest on case of both tested years. For sample preparation, cauliflower florets were harvested from different points of each treatment replication. The average sample from each treatment replication was prepared from 6 – 7 florets of cauliflower and it was taken from several points of floret and stalk. Samples prepared for sulforaphane determination were sequentially lyophilized at -58°C. The results were counted in fresh mass. The analyses of ascorbic acid and nitrate content were done at fresh samples of cauliflower.

In the experiment the cauliflower variety CHARLOT F1 was selected for testing. It is an excellent hybrid for early spring as well as for summer cultivation for direct consumption. It has solid white, good coverage rosette with very strong healthy foliage. It maintains its quality even in warm weather. This hybrid is resistant to yellowing of the florets. Vegetation period is 78 days.

Fertilisation

The variants with fertilization treatments were following:

0 – control (without application of fertilizers),

$N_{150}S_{80}$ – application of nitrogen and sulphur at the supply level N: S = 150:80 kg.ha^{-1},

$N_{200}S_{80}$ – application of nitrogen and sulphur at the supply level N: S = 200:80 kg.ha^{-1},

$N_{250}S_{80}$– application of nitrogen and sulphur at the supply level N: S = 250:80 kg.ha^{-1}

Figure 1 Cauliflower planting, Nitra 2015.

Table 1 Climate characteristics in experiment area, in 2014, 2015, Nitra.

	2014				2015			
	Temperature (°C)		Rainfall (mm)		Temperature (°C)		Rainfall (mm)	
VI.	19.3	*H*	52.5	*N*	19.9	*H*	10.2	*ED*
VII.	21.8	*H*	64.1	*N*	23.6	*EH*	17.2	*VD*
VIII.	18.9	*N*	55.9	*N*	23.5	*EH*	57.7	*N*
IX.	16.8	*H*	122.0	*EW*	17.5	*H*	33.2	*N*
X.	12.1	*H*	34.6	*N*	10.5	*N*	54.8	*VW*
total	88.9		329.1		95.0		173.1	
mean	17.8		65.8		19.0		34.6	

Note: **Explanatory notes: evaluation of the months according to climatic normal 1961 – 1990, considering temperature: N – normal, H – hot, EH – extremely hot, considering rainfall: N – normal, EW- extremely wet, ED - extremely dry, VW – very wet, VD – very dry.*

Table 2 Agrochemical characteristics of the soil before the foundation of the experiment in 2014, 2015, Nitra.

	humus %	pH/KCl	Nutrients content in mg.kg^{-1} of the soil N	P	K	S	Ca	Mg
2014	3.46 Good	6.47 Sl. A	19.5 M	86.3 G	498 VH	26.25 M	610 H	816 VH
2015	3.25 Good	7.16 N	19.1 M	245 VH	149.5 M	7.5 VL	6340 H	643.5 VH

Note: *Explanatory notes: Nutrient content: VL – very low content, L – low content, M – medium content, G – good content, H – high content, VH- very high content, pH: pH - Sl. A - Slightly acidic, N - neutral.*

The doses of N and S were calculated on the basis of agrochemical soil analysis at the depth 0 – 0.30 m before experiment establishment (Table 2).

The fertilizer DASA 26/13 was applied three weeks before planting. The calculated dose of LAD 27 was applied in two terms - three (50%) and six weeks (50%) after planting. DASA 26/13 (26% of N and 13% of S; manufacturer: Duslo, a. s., Šaľa, Slovak republic) were used for N and S supply.

Quantity and quality parameters estimation

Yields

Quantitative parameters of the cauliflower were estimated in the frame of five harvests. Florets were harvested in consume maturity. Total reached yield was expressed in t. ha^{-1} after particular weighting in fresh mass.

Ascorbic acid (AA)

HPLC method of vitamin C content estimation **(Stan, et al., 2014)** was used by the help of liquid chromatograph with UV detector, for separation was used RP C18 column, mobile phase was methanol : water (5:95, v/v), UV detection was adjusted to 258 nm (HPLC fy. VARIAN).

Sulforaphane content

Sulforaphane content was measured by method of high pressure liquid chromatography (HPLC) at the certified laboratory. It was done according to the methods previously described by **(Sivakumar et al., 2007)**. The principle of the method is sulforaphane extraction by dichloromethane followed by HPLC analysis and subsequent detection with UV – VIS detector.

Nitrates content NO_3^-

Determination of nitrate was done according to methods described in **Hegedűs et al. (2010)**. It was performed with a Varian Analytical Instrument (Walnut Creek, CA, USA) equipped with a Solvent Delivery System (type 9012), used in isocratic elution mode for nitrate determination and a UV–VIS detector (Varian type 9050) at 215 nm, a Spark Holland Autosampler (Basic-Marathon type 816, Aj Emmen, The Netherlands) and a 20 µL sample loop. Varian Star Workstation software was used.

Statistical analyses

The obtained data were processed into tables in Microsoft Office Excel 2007. Then analysis of variance (ANOVA) were used by the help of the Tukey test (significance level α = 0.05) for statistical analyses in the program StatgraphicCenturion XVII (StatPointInc. USA).

RESULTS AND DISCUSSION

Yields

Table 3 Analysis of fertilising influence on yields (t.ha^{-1}) of cauliflower variety CHARLOT F1, Nitra, SR*.

Yields	2014^{A} (t.ha^{-1} of FM)	2015^{B} (t.ha^{-1} of FM)	2014 – 15 (t.ha^{-1} of FM)
Control	15.30 ±2.90	9.20 ±5.50	12.25 ±4.20^{a}
150 N	17.60 ±0.50	23.31 ±8.20	20.45 ±4.35^{ab}
200 N	17.40 ±0.10	28.47 ±6.80	22.94 ±3.45^{b}
250 N	18.00 ±0.90	39.32 ±7.36	28.66 ±4.13^{b}

Note: **Means ± standard deviation.*

Values with different italics letters are significantly different at P <0.05 by LSD in ANOVA.

Table 4 Analysis of fertilising influence on ascorbic acid (AA) content of cauliflower variety CHARLOT F1, Nitra, SR*.

AA	2014^{A} (mg.kg^{-1} of FM)	2015^{B} (mg.kg^{-1} of FM)	2014 – 15 (mg.kg^{-1} of FM)
Control	477.33 ±17.62	135.50 ±30.50	306.42 ±24.06^{a}
150 N	330.33 ±75.69	218.33 ±80.50	274.33 ±78.10^{a}
200 N	380.33 ±147.55	211.00 ±65.37	295.67 ±106.46^{a}
250 N	380.67 ±23.29	251.00 ±90.57	315.83 ±56.93^{a}

Note: **Means ± standard deviation.*

Values with different italics letters are significantly different at p <0.05 by LSD in ANOVA.

The yields of primary cauliflower florets were increased by the influence of applied nutrition in all evaluated variants compared to a control variant without fertilization (Table 3) during the monitored period under the terms of methodology. The lowest yield reached the value of 12.25 t.ha^{-1} (control variant); the highest yield 28.66 t.h^{-1} was reached by the highest dose of fertiliser (250 N). Application of nitrogen led to significant yields increasing confirmed by statistical analysis in case of the dose 200 and 250 kg N.ha^{-1} (increasing about 87% and 134% compared to control). Increasing of the yields after nitrogen fertilizing corresponds with the results of **Nazrul and Shaheb (2016)**, when their results revealed that yields of cabbage and cauliflower responded significantly to fertilizer doses, levels of lime, and their combinations in all the locations.

According to **Bashyal (2011)** application of nitrogen along with the biofertilizer significantly increased yield as compared to application of nitrogen without biofertilizer. Cauliflower curd yield obtained at 120 kg nitrogen ha^{-1} did not significantly differ with the curd yield recorded at 60 kg nitrogen and 2 kg biofertilizer ha^{-1}. The effect of different levels of organic manures and conventional practices on growth, yield and quality of cauliflower was studied by **Prabhakar et al. (2015)**. The trial included five levels of organic manure nutrient and two inorganic nutrient supplies. The treatment which received recommended dose of farm yard manure along with recommended NPK produced the highest mean curd yield (21.23 t.ha^{-1}) followed by the treatments, which received 100 and 75 percent recommended dosage of nitrogen (RDN) through organics (19.36 and 18.42 t.ha^{-1}).

The influence of climate characteristics was very significant, according to used statistical analyses (Table 3). In 2015 the yields were higher in case of all three fertilised variants. Climate conditions in 2015 as an important external factor influenced the yields because there was higher total sum of temperature during the growing season and according to evaluation of the months according to climatic normal 1961-1990, the July and August was extremely hot (Table 2). The rainfall hasn't that influence, because the trial was irrigated as needed.

Ascorbic acid (AA)

Average value of AA (vitamin C) ranged in interval from 274.42 mg.kg^{-1} (in case of the dosage of 150 kg N.ha^{-1}) to 315.83 mg.kg^{-1} (the highest dosage of 250 kg N.ha^{-1}), while the value of control variant 306.42 mg.kg^{-1} moved between the limits (Table 4). Influence of nitrogen fertilizing according to used methodology on vitamin C (AA) content was not statistically confirmed. The nutrient concentration in the curds and stalks of cauliflower varied insignificantly with levels of nitrogen applied.

Growing cauliflower without additional sources of nutrients (control) caused significant decrease (9%) in total organic C (TOC) than that in the initial soil according to **Batabyal et al., (2016)** as they evaluated 15 nutrient management (NM) technologies for production of cauliflower taking its yield, quality, profitability, energy balance and environmental sustainability in terms of soil quality as the goal variables. **Bayshal, (2011)** noticed that after application of nitrogen along with the biofertilizer the vitamin C content was significantly increased and the highest vitamin C content of curds and the most attractive curd color were recorded at 60 kg nitrogen and 2 kg biofertilizer.ha^{-1}. Variable results in case of fertilizing effect on vitamin C were found by the **Hrabovskaet al. (2012)** in case of potato. The content of C vitamin was increased with the graduated nitrogen doses to the soil only till variant 4 and after that the content of vitamin C was decreased. The average content of C vitamin ranged in interval 3.786 – 6.225 mg.kg^{-1} of fresh matter. Also in case of potato in research of **Hamouz et al. (2009)** there was noticed, that after soil application of 180 kg N.ha^{-1} the level of vitamin C decreased about 12.4%, compared with soil application of 100 kg of N.ha^{-1}. When tested members of the family *brassicaceae* by **Lisiewska and Kmiecik 1996,** directly after harvesting, broccoli contained 116.3 – 116.4 mg of vitamin C in 100 g of fresh matter, and cauliflower contained 60.5 – 64.7 mg. Increasing the

Table 5 Analysis of fertilising influence on sulforaphane content of cauliflower variety CHARLOT F1, Nitra, SR*.

Sulforaphane	2014^{A} (mg.kg^{-1} of FM)	2015^{B} (mg.kg^{-1} of FM)	2014 – 15 (mg.kg^{-1} of FM)
Control	3.80 ±0.30	9.02 ±0.40	6.41 ±0.35^{bc}
150 N	3.20 ±0.20	10.52 ±0.74	6.86 ±0.47^{c}
200 N	2.80 ±0.20	8.29 ±0.06	5.54 ±0.13^{b}
250 N	2.00 ±0.30	5.66 ±0.44	3.83 ±0.37^{a}

Note: **Means ± standard deviation.*
Values with different italics letters are significantly different at p < 0.05 by LSD in ANOVA.

Table 6 Analysis of fertilising influence on nitrates content of cauliflower variety CHARLOT F1, Nitra, SR*.

Nitrates	2014^{A} (mg.kg^{-1} of FM)	2015^{A} (mg.kg^{-1} of FM)	2014 – 15 (mg.kg^{-1} of FM)
Control	86.80 ±25.20	120.67 ±72.67	103.73 ±33.56^{a}
150 N	268.50 ±5.50	183.67 ±75.74	226.08 ±49.67^{b}
200 N	297.48 ±165.33	172.67 ±45.76	235.07 ±84.54^{bc}
250 N	391.00 ±59.01	279.67 ±96.70	335.33 ±26.66^{c}

Note: **Means ± standard deviation.*

amount of nitrogen fertilizer from 80 to 120 kg N ha^{-1} decreased the content of vitamin C only in cauliflower (by 7%). In our two years trial the influence of climate on vitamin C was confirmed (Table 4). **Açıkgöz (2012)** states that the vitamin C in vegetables w influenced by the seasons. He indicates in his work that the detected level of vitamin C in spring variant of mizuna (*Brassica rapa japonica*) was 702 mg.kg^{-1} and in autumn variant 576 mg.kg^{-1}.

Sulforaphane content

The lowest average value of sulforaphane content 3.83 mg.100 kg^{-1} was reached in the variant 4 (the variant with highest dosage N = 250 kg.ha^{-1}) <3 (N = 200 kg.ha^{-1}) <1 (control) <2 (N = 150 kg.ha^{-1}) with the highest average value 6.86 mg.100g^{-1} (Table 5). The influence of differentiated nutrition on sulforaphane content was statistically confirmed in case of the 4th variant in comparison to control, where the decrease about 40.24% was noticed.

The effect of four different variants of nitrogen on sulforaphane content in cauliflower florets was studied by **Čekey at al. (2011)** in the same conditions of fertilisation as in our trial. Their results varied in range from 2.50 mg.kg^{-1} to 2.96 mg.kg^{-1} of fresh mater and it increased in following variants order: 1 (control) <2 <3 <4. The highest sulforaphane content was ascertained at the variant 4 (2.96 mg.kg^{-1}). Similar trial was led by **Šlosár et al. (2013)** where they tested the nutrition influence on broccoli. The control variant (1) was free of any industrial fertilizers. In the nutrition variants 2, 3, 4, fertilizers LAD27 and DASA 26/13 were used to replenish supplies of N and S. The highest sulforaphane content was reached in variant with the highest dosage of both observed fertilisers: 48.43 – 50.93 mg.kg^{-1} of fresh mater; compared to the control variant, the increase of sulforaphane reached 5.2%. The N, S and Zn fertilization tended to the higher content of sulforaphane (SF) in broccoli florets according to **Šlosár et al. 2016**. The statistically significant increase of SF content was shown at all fertilized treatments in comparison with control treatment.

Nitrates content NO_3^-

Applied nitrogen nutrition caused significant nitrates increasing in monitored florets of the cauliflower according to ours expectations in order of the variants: 1 (control) <2 (N = 150 kg. ha^{-1}) <3 (N = 200 kg. ha^{-1}) <4 (N = 250 kg. ha^{-1}). According to statistical analyses there was found significant difference between the all variants in comparison to control (Table 6). Under the same conditions of fertilisation as in our trial the nutrition on cauliflower was applied in the trial of **Čekey et al. (2011)**. They observed also nitrates content and differentiated nutrition resulted in increased accumulation of nitrates in the cauliflower. The most increase of nitrate content, compared to the control variant, was also ascertained at the variant 4 (about 31.4%). **Lisiewska and Kmiecik (1996)** noticed in their trial with brassica vegetable and nutrition, that increasing the amount of nitrogen fertilizer from 80 to 120 kg N.ha^{-1} raised the level of nitrates by 44% in broccoli and by 33% in cauliflower. The effect of nitrogen (N), sulphur (S) and zinc (Zn) fertilization on the yield quantity and selected qualitative parameters of broccoli was studied in the experiment of **Šlosár et al. (2016).** The application of N fertilizers resulted in an increased accumulation of nitrates in broccoli florets. The nitrate content was ranged from 474.4 mg·kg^{-1} (control) to 632.8 mg·kg^{-1} of fresh weight ($N_{200}S_{80}$). At the treatment $N_{200}S_{80}$, the nitrate content was higher about 33.4% in comparison with control variant. The maximally highest acceptable amount of nitrates in Brassica vegetable species (700 mg·kg^{-1} of FW) as it is established by Food codex of Slovak republic **(SVFA SR, 2014)** was kept in case of all variants.

The climate characteristics influence wasn´t confirmed in case of nitrates, in time of florets creation there wasn´t found any extremes in temperature according to evaluation of the months according to climatic normal 1961 – 1990 (Table 2). The seasons as important nitrates influencing factor was confirmed by **Kudrnáčová and Kouřimská (2015)**. Overall, the nitrate content ranged from 221 to 334 mg.kg^{-1} in spring varieties and from 249 to 384 mg.kg^{-1} in autumn varieties of non-traditional vegetables from *brassicaceae*. In the favourable effect of light and heat on the reduction of nitrate content in plants was interested **Weightman et al. (2006)**. Their research resulted in some connection between observed characteristics: short-term shading (24 – 48 h) had no significant effects on mean tissue nitrate concentration (TNC), unlike the increase in TNC known to occur following dull periods 10 – 14 days before harvest. The effect on TNC of time of day harvested was significant, but there was no obvious pattern of diurnal variation.

CONCLUSION

The effect of fertilization (the amount of nitrogen) on quantity and quality of cauliflower florets was observed by the testing of four different variants of nutrition. From quantitative parameters the yields were evaluated as an important characteristic from economic point of view. There was found the significant, statistically confirmed increasing of the yields after the fertilisers using. The crop yields were depended on the increasing dosage. The highest yield was in variant with the highest dosage of applied nitrogen. Applied nitrogen nutrition caused significant nitrates increasing in monitored florets of the cauliflower, nitrates content increased from 103.73 mg.kg^{-1} of FM (control) to 335.33 mg.kg^{-1} of FM (4. variant). The dependence of increasing was statistically confirmed, but the highest dosage of nitrates is under the permissible standard according to Food codex of Slovak republic (700 mg·kg^{-1} of FM). All the used dosage can be recommended to farmers for using as a possibility of yields increasing. When evaluating quality, the highest dosage $N_{250}S_{80}$ (application of nitrogen and sulphur at the supply level N: S = 250:80 kg·ha^{-1}) caused decreasing of sulforaphane content according to used statistical analyses. The highest average value of sulforaphane content 6.86 mg. 100 kg^{-1} was reached in second variant ($N_{150}S_{80}$), but the influence of fertilizing wasn´t statistically confirmed on tested level. Dependence of vitamin C (AA) content increasing on nitrogen fertilizing according to used methodology was not statistically confirmed.

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