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Original scientific paper

NITRATE CONTENT AND NITRATE-REDUCTASE ACTIVITY IN VEGETABLES COLLECTED AT THE CITY MARKET IN BANJA LUKA AND THE RISK ASSESSMENT

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Summary

The high content of nitrates and nitrites in vegetables can have toxic and carcinogenic effects on the human body, and monitoring is necessary in order to apply appropriate protective measures. The aim of the research in this paper was to determine the nitrate content and monitor the activity of nitrate reductase in vegetables (chard, carrots, parsley, cabbage) taken from various stands of the city market in Banja Luka. The average concentration of nitrate in vegetable samples ranged from 1 to 650 $mgkg^{-1,}$ with the highest values obtained in chard and cabbage, and the lowest in parsley root. The nitrate reductase activity was increased in chard, while the lowest was found in parsley root. The nitrate content in the vegetable samples was within the limits prescribed by the World Health Organization so there is no potential danger to human health.

Key words: nitrates, nitrate reductase, vegetables, city market

INTRODUCTION

Recently, in many countries, especially industrialized ones, vegetables and drinking water contain higher concentrations of nitrates than in the past due to the increasing use of synthetic nitrogen and manure in intensive agriculture, which is becoming a global problem. These circumstances and the pursuit of increasing yields have led to the accumulation of nitrates in nature, which causes numerous environmental problems and directly endangers human and animal health (Santamaria, 2006; Chung *et al*., 2011; Afzali and Elahi, 2014). The estimated daily dose of nitrate that a person eats and ingests with food reaches 75–100 mg, 80–90% of which comes from vegetables and about 20% from drinking water (Razaei *et al*., 2014). According to the European Food Safety Authority - EFSA (2008), 11 to 41% of daily nitrate intake is ingested through vegetable and fruit. Nitrate is relatively non-toxic, but approximately 5% of all ingested nitrates are converted in the gastrointestinal tract to a toxic form (nitrite) that causes a disease known as methaemoglobinaemia in humans and animals (Santamaria, 2006; Feng *et al*., 2006; Shahlaei *et al*., 2007; Ayaz, 2007). Vegetables, such as lettuce, spinach, kale and celery, are considered true "nitrogen accumulators" (Gorenjak and Cenčić, 2013), especially leafy vegetables belonging to the families Brassicaceae (rocket,

radish, mustard), Chenopodiaceae (beets, beans, spinach), Amaranthaceae (pigweed), Asteraceae (lettuce) and Apiaceae (celery, parsley) (Santamaria, 2006; Gorenjak and Cenčić, 2013). In Europe, nitrate concentrations in vegetable are generally higher during winter due to lower light intensity and shorter days (FAO/WHO, 2003; EFSA 2008; Weightman *et al*., 2006). Fruit and vegetable from greenhouse cultivation have higher nitrate content due to lower light intensity and higher mineralization of the environment in which it grows. The difference in data between different countries is accounted for by different environmental conditions and increased use of fertilizers during the summer by growers (Chung *et al*., 2003).

As nitrates are a potential poison, both for the human and animal population, knowledge of the nitrate content in food, and especially in fresh vegetable and fruit, is very important. Since the content of nitrates in fresh vegetable matter should not exceed 400 $mgkg^{-1}$, and in food intended for children 250 $mgkg^{-1}$, it is very important to control these toxic forms of nitrogen in vegetable. On the other hand, the amounts of nitrite in fresh fruit and vegetable are much lower (1-20 $mgkg^{-1}$), so the main source of nitrite is considered to be endogenous conversion from nitrate (Gangolli *et al*., 1994; Kastori and Petrović, 2003; EFSA, 2008; Rezaei *et al*., 2014).

The aim of the study was to determine the nitrate content and monitor nitrate reductase activity in samples of seasonal vegetables (chard, carrots, parsley and cabbage), taken from various stands from the city market in Banja Luka. Based on the obtained results, an exposure assessment and risk characterization were conducted.

MATERIAL AND METHODS

Vegetables that are most often grown and consumed in the area of Banja Luka are chard, carrots, parsley and cabbage. We took samples of vegetables during the season (spring, summer and autumn, 2019) at the city market. Selected vegetables (about 1 kg) were collected once a month from three different stands, of which samples from stand I and II were resellers, while samples taken from stand III were produced in greenhouse (Figure 1). Vegetable samples were delivered to the laboratory where the following analyzes were performed: determination of nitrate reductase (NR) activity according to the standard spectrophotometric method (Maksimović and Pajević, 2002) and determination of nitrate content using the device SOEKS (NITRATE TESTER) NUK-019-1, whose method is based on electrical conductivity. The obtained values were compared with the standards prescribed by the World Health Organization (FAO/WHO, 2003) and the European Commission for Food Safety (EFSA, 2008).

Figure 1. Collecting vegetable samples from various stands at the city market in Banja Luka

RESULTS

The results presented in this paper display the nitrate content in vegetables sampled at the city market in Banja Luka during the spring, summer and autumn of 2019.

The average nitrate content in certain types of vegetables sampled in spring ranged from 10 $mgkg^{-1}$ (in parsley leaves and root) to 650 $mgkg^{-1}$ (in chard). A higher nitrate content was registered in vegetables sampled from stand III. According to EFSA (2008), the nitrate content in fresh vegetable should not exceed 400 $mgkg^{-1}$ for adults and 250 $mgkg^{-1}$ for children, for daily intake. Comparing the average values, the nitrate content determined in chard and cabbage according to EFSA (2008) was exceeded and those were also the highest recorded values of nitrate during the season.

Nitrate reductase activity ranged from 0.01 to 7.5 μM $NO_2^- g^{-1} h^{-1}$ with the highest activity recorded on average in chard and cabbage samples.

Table 1. Nitrate content ($mgkg^{-1}$) and nitrate reductase activity (μM $NO_2^- g^{-1} h^{-1}$) in vegetables sampled during spring from various stands of the city market in Banja Luka (SD-standard deviation)

	NITRATE					NITRATE REDUCTASE ACTIVITY				
Vegetables	I stand	II stand	III stand	Mean	SD	I stand	II stand	III stand	Mean	SD
Carrot	60	56	123	79.66	37.58	0.01	0.01	1.75	0.59	1.00
Chard	28	43	650	240.33	354.86	0.02	0.02	7.50	2.51	4.32
Cabbage	68	75	430	191.00	207.01	2.00	0.05	4.40	2.15	2.18
Parsley leaf	28	10	127	55.00	63.00	0.03	0.06	5.50	1.86	3.15
Parsley root	10	23	42	25.00	16.09	0.04	0.03	2.70	0.92	1.54

The average nitrate content during the summer (Table 2) in certain types of vegetables ranged from 1 $mgkg^{-1}$ (determined in the parsley and carrot root) to 68 $mgkg^{-1}$ (in cabbage). We recorded higher nitrate content in samples of vegetables from stand I.

Higher nitrate reductase activity was recorded in chard 0.62 μM $NO_2^- g^{-1} h^{-1}$ while lower was found in carrots (0.02 μM $NO_2^- g^{-1} h^{-1}$) and parsley leaves (0.01 μM $NO_2^- g^{-1} h^{-1}$).

Table 2. Nitrate content ($mgkg^{-1}$) and nitrate reductase activity (μM $NO_2^- g^{-1} h^{-1}$) in vegetables sampled during the summer from various stands of the city market in Banja Luka (SD-standard deviation)

	NITRATE					NITRATE REDUCTASE ACTIVITY				
Vegetables	I stand	II stand	III stand	Mean	SD	I stand	II stand	III stand	Mean	SD
Carrot	67	2	1	34.50	37.82	0.06	0.14	0.02	0.07	0.06
Chard	28	16	3	15.66	12.50	0.62	0.51	0.41	0.51	0.11
Cabbage	68	75	42	61.66	17.39	0.17	0.02	0.01	0.07	0.09
Parsley leaf	2	9	13	8.00	5.57	0.17	0.08	0.01	0.09	0.08
Parsley root	1	2	4	1.73	1.53	0.05	0.07	0.07	0.06	0.02

The nitrate content in fresh vegetable samples (Table 3) ranged from 1 $mgkg^{-1}$ in parsley root to 133 $mgkg^{-1}$ in cabbage.

Nitrate reductase activity in samples taken during the autumn ranged from 0.01 μM NO_2^- g^{-1} h^{-1} to 1.45 μM NO_2^- g^{-1} h^{-1}, with the highest activity found in chard and the smallest in carrots, in terms of mean values.

Table 3. Nitrate content (mgkg^{-1}) and nitrate reductase activity (μM NO_2^- g^{-1} h^{-1}) in vegetables sampled during autumn from different stands of the city market in Banja Luka (SD-standard deviation)

	NITRATE					NITRATE REDUCTASE ACTIVITY				
Vegetables	I stand	II stand	III stand	Mean	SD	I stand	II stand	III stand	Mean	SD
Carrot	4	48	58	36.60	28.73	0.04	0.02	0.03	0.03	0.01
Chard	22	19	53	31.33	18.82	1.00	1.45	0.20	0.15	0.63
Cabbage	133	74	42	83.00	46.16	0.02	0.01	0.28	0.10	0.15
Parsley leaf	44	46	17	35.60	16.20	0.18	0.13	0.10	0.14	0.04
Parsley root	10	9	1	6.60	4.93	0.13	0.04	0.06	0.08	0.05

DISCUSSION

This is the first study related to the determination of nitrate content in various samples of seasonal vegetables taken from the city market in Banja Luka. In the examined vegetable samples, we found higher concentrations of nitrate in chard and cabbage compared to carrots and parsley. Comparing the results obtained in this paper with the research conducted by the European Food Safety Commission EFSA (EFSA, 2008) and conducted in the Republic of Croatia (Miloš *et al*., 2016), it can be concluded that the nitrate content in vegetable samples taken from the city market in Banja Luci was at a much lower level. In this paper, the nitrate content in vegetables was compared with the activity of nitrate reductase. We noticed that the enzyme activity was positively correlated with the nitrate content, and it increased with the increase of nitrate content.

The difference in data on nitrate concentration in vegetables among different countries is primarily due to different environmental conditions and the increased use of fertilizers during the summer by growers (Chung *et al*., 2003), which may be related to the results recorded in this paper.

Our results suggest that the nitrate content was higher in spring compared to summer and autumn. A similar study in Iran indicated that nitrate concentrations in vegetables grown during the winter (cabbage, lettuce, radishes and carrots) were lower than those grown during the summer, while (Shahlaei *et al*., 2007) the nitrate content in seasonal vegetables was lower in spring than in winter. Greenhouse fruit and vegetable have higher nitrate content due to lower light intensity and higher mineralization of the environment in which they grow (Gangolli *et al*., 1994), which is probably one of the reasons for significantly higher nitrate content in vegetables in samples taken from the stand III.

The Federal Institute of Germany (BfR) also found that vegetables grown in season have a significantly lower nitrate concentration, requiring less fertilizer than those grown out of season in greenhouses (BfR, 2009) which can be related to the results obtained in this paper. Researchers Ayaz *et al*. (2007) in Ankara monitored the level of supply of nitrates and nitrites in vegetables taken from the market. The research was conducted on 258 samples on seven different types of vegetables (carrots, tomatoes, two types of lettuce, cabbage, spinach

and parsley), in the period from January 2001 to April 2002. Leafy vegetables, both parsley and spinach, contained most nitrate, while carrots and tomatoes contained the least amount of nitrate. In addition, they found that the differences in nitrate accumulation were the result of different harvest periods, the amount of nutrients in the soil, the amount and type of fertilizer used, and temperature, as well as other external factors. Iammarino *et al*. (2014) monitored nitrate and nitrite concentrations on 75 fresh and frozen spinach and lettuce samples, with fresh spinach samples having increased nitrate and nitrite content compared to frozen samples, while lettuce had a higher content compared to spinach. Razaei *et al.* (2014) conducted a similar study on 323 different samples at a city market in Iran, with nitrates and nitrites detected in all samples, and excessive concentrations found for celery. Studies in Hong Kong (Chung *et al*., 2011) on 73 different types of vegetables showed the presence of different nitrate concentrations, and increased ones were recorded in spinach (from 3-700-6300 $mgkg^{-1}$) and cabbage.

Nitrate reductase is considered a limiting factor in the assimilation of nitrates in higher plants, and it is induced by the presence of nitrates. However, the relationship between nitrate reductase and nitrate concentration is still uncertain (Chen *et al*., 2004). Some studies showed that nitrate reductase activity increased with decreasing nitrate (Chen *et al*., 2004) while most studies suggested that nitrate reductase was an inductive enzyme, i.e. its activity increased with nitrate content (Reddy and Menary, 1990; Ivashikina and Sokolov, 1997).

In this paper, the content of nitrate in vegetables was compared with the activity of nitrate reductase, and the results suggested that the activity of NR was positively correlated with the content of nitrate, i. e. it increased along with the increase of nitrate content.

CONCLUSION

In all tested samples of vegetables, the presence of nitrates was recorded, provided that the obtained values were lower than the standards set by the World Health Organization and that there was no danger to the health of the population. However, according to the European Food Safety Authority (EFSA), the nitrate content in fresh vegetable matter should not exceed 400 $mgkg^{-1}$ for adults and 250 $mgkg^{-1}$ for children, so that chard and cabbage samples collected in spring exceeded permissible nitrate concentrations. Due to insufficient data on nitrate levels in food, this study is the basis for future research that would include monitoring of a wider area with a larger number of samples in order to obtain reliable data on potential exposure and degree of risk.

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