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The influence of cultivation method on nitrate content in some lettuce samples

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ABSTRACT

The use of nitrogen fertilizers is one of the main effects for the accumulation of nitrates in plants. Conventional agriculture, in comparison to integrated and organic farming, causes greater environmental pollution and poorer quality of crops. Within the framework of the research, we studied the influence of the method of cultivation on the content of nitrates in the samples of lettuce (*Lactuca sativa* L.). The samples were received directly from growing areas from different parts of Slovenia and analysed in the laboratory for the nitrate content (NO_3^-) according to the accredited method. The samples from conventional cultivation showed the highest sample representation (51 %), with values in the highest concentration range (1000 - 2500 mg of NO_3^- kg^{-1}, one sample exceeded 2500 mg kg^{-1} fresh mass). Within the framework of integrated cultivation, there were less such samples (34 %), and among the samples from organic cultivation no sample exceeded 1000 mg of NO_3^- kg^{-1}. Of 88 analysed samples, one sample exceeded the statutory limit value applicable to lettuce of the type Iceberg ('Ljubljanska ledenka'). On average, the Iceberg samples contained more nitrates than other types of lettuce.

Key words: organic; conventional; integrated cultivation; nitrates; lettuce

IZVLEČEK

VPLIV NAČINA PRIDELAVE NA VSEBNOST NITRATOV V VZORCIH VRTNE SOLATE

Raba dušikovih gnojil je eden glavnih vplivov kopičenja nitratov v rastlinah. Konvencionalno kmetijstvo, v primerjavi z integriranim in ekološkim kmetijstvom povzroča večje onesnaženje okolja in slabšo kakovost pridelkov. V okviru raziskave smo ugotavljali vpliv načina pridelave na vsebnost nitratov v vzorcih vrtne solate (*Lactuca sativa* L.). Vzorce smo prejeli neposredno iz pridelovalnih površin iz različnih delov Slovenije. V laboratoriju smo vzorce analizirali na vsebnost nitrata (NO_3^-) po akreditirani metodi. Kot pričakovano, so vzorci iz konvencionalne pridelave pokazali največjo zastopanost vzorcev (51 %), z vrednostmi v najvišjem koncentracijskem območju (1000 – 2500 mg NO_3^- kg^{-1} sveže mase, en vzorec je presegel vrednost 2500 mg kg^{-1}). V okviru integrirane pridelave je bilo takšnih vzorcev manj (34 %), izmed vzorcev iz ekološke pridelave pa ni bilo vzorca, ki bi presegel vrednost 1000 mg NO_3^- kg^{-1}. Izmed 88 analiziranih vzorcev, je en vzorec presegel zakonsko predpisano mejno vrednost, ki velja za vrtno solato tipa ledenka ('Ljubljanska ledenka'). V povprečju so vzorci ledenk vsebovali več nitratov, kot druge vrste vrtne solate.

Ključne besede: ekološka; konvencionalna; integrirana pridelava; nitrat; vrtna solata

1 INTRODUCTION

Nitrogen is the most important element in plant nutrition. The plants need it as a nutrient for building amino acids, proteins, nucleic acids and other vital compounds. The plants receive the nitrogen in the form of nitrate (NO_3^-) and ammonium ion (NH_4^+). Due to the oxidation of the ammonium nitrogen form to nitrate, which takes place in the soil in the presence of bacteria, the nitrate is the predominant form received by the plant (Below, 1994).

The intensity of farming and the associated use of nitrogen fertilizers cause nitrates to accumulate in plants. Most of nitrate is accumulated in vegetables (especially leafy vegetables), less in field crops, and much less in fruits (Schuddeboom, 1993; Ysart et al., 1999; Zhong et al., 2002). Vegetables are the largest source of nitrate input in the human body; that is 70-80 % of the total NO_3^- input (Gangolli et al., 1994). Nitrate itselves is harmless to health, however, after ingestion, it is influenced by microbiological processes

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which convert it into nitrite that can cause "methemoglobinemia" (inability of oxygen transfer in organism). Nitrite is in the stomach precursor for the nitrosation of the compound (nitrosamines, nitrosamides), which are, according to the criteria of the International Agency for Research on Cancer, grouped into individual groups of carcinogenicity (Boink and Speijers, 1999, Santamaria, 2006).

The accumulation of nitrate in plants is influenced by various factors, such as the harvest time, the length of the growth age, soil fertility characteristics (pH), weather conditions (rainfall, light), cultivar type and to a great extent the use of nitrogen fertilizers (McCall and Willumsen, 1999; Dapoigny et al., 2000; Chen et al., 2004; Guadagnin et al., 2005; Weightman et al., 2006). The latter can be influenced by different methods of cultivation. Lately, great attention is being paid to the organic and integrated cultivation methods, which are, unlike conventional, more environmentally friendly.

Conventional cultivation allows the use of mineral fertilizers, plant protection products (FFS) and genetically modified organisms. Mineral fertilizers additionally improve the supply of the soil with nutrients and increase the fertility of the soil, but on the other hand pollute the environment (Kocjan Ačko, 2000).

Integrated cultivation represents a more friendly form of farming for the environment and for the consumer. This mode of cultivation is today widespread in most of Western European countries and ensures the consumers that vegetables do not contain harmful substances above the permitted limit (Osvald and Kogoj Osvald, 2003). Unlike conventional farming, integrated farming demands careful monitoring of the use of minerals and organic fertilizers, whereby the fertilization with organic fertilizers is preferred (livestock manure, animal or plant residues, compost). Soil analysis is needed before fertilization, from which we find out how much nutrient intake is needed. Plant protection products in integrated farming are used only when biological, mechanical and other measures do not work (Džuban, 2015).

Organic cultivation is the only sustainably oriented agricultural food production system. It is a way of farming, which also pays attention to the balance between living organisms. Also, among all the ways of farming, it is the least burdensome on the environment (Poštrak, 2010). Organic farming system prohibits the use of soluble mineral fertilizers, as well as the use of plant protection products and genetically modified organisms.

Crop plants absorb nitrogen mainly in the form of nitrate ions. Mineral nitrogen fertilizers used in conventional agriculture directly provide the nitrate, while many organic fertilizers gradually release their nitrogen content. The amount of nitrate absorbed by plants depends on the nitrate dissolved in the soil solution and, therefore, the type of fertilizer is not the only cause of nitrate accumulation in plant. Incorrect practices, such as overfertilization with nitrogen also favour this accumulation (Matallana Gonzàlez et al., 2010; Barker, 1975; Muramoto, 1999; Raigon et al., 2002).

Many studies have demonstrated that organically grown crops have lower nitrate content that integrated and conventionally grown crops (Muramoto, 1999; Pussemier et al., 2006, Merino et al., 2006), although this conclusion is not uniformly supported (De Martin and Restani, 2003; Guadagnin et al., 2005).

Woese et al. (1997) reviewed 41 comparative studies of nitrate content in conventional and organically grown vegetable and concluded that, in general, higher nitrate levels were found in leaf, root and tuber vegetables with mineral fertilization. Worthington (2001) summarized the results of 18 studies comparing nitrate levels of organic and conventional vegetable, and found that in 72 % of the cases nitrate levels were higher in the conventional products, while in 24 % of the cases nitrate levels were higher in the organic products. In Slovenia, a comparison between conventionally cultivated lettuce and organic lettuce was presented and confirmed 30 % higher values of nitrates using conventional method of cultivation (Hmeljak Gorenjak et al., 2012).

In recent years, organic farming is becoming an increasingly way of producing food. Consumers are becoming more aware of the importance of a healthy diet, and organic farming is thus becoming a fast growing industry in the world. The data show that the share of organic land in the EU is around 4 %. Some countries are more "ecologically aware", such as Liechtenstein (26 %), Austria (11 %), Switzerland (10 %), Italy (8 %), Sweden (6 %) (Poštrak, 2010; Repič, 2010). In 2016, 3518 Slovenian agricultural holdings, which represent 4.8 % of all Slovenian farms, were included in "Eco control – organic method of cultivation", however, the number of them is increasing every year (MKGP RS, 2016).

Within the framework of the research, we wanted to determine how the cultivation method (conventional, integrated or organic) affects the nitrate content in the cultivated vegetables of Slovenian producers. We focused on the samples of lettuce.

2 MATERIAL AND METHODS

2.1 Sampling

In the laboratory of the Agricultural Institute of Slovenia we received 88 samples of lettuce from different regions of Slovenia. The samples belongs to various lettuce cultivar groups, such as Iceberg lettuce ('Ljubljanska ledenka'), Crisphead lettuce ('Margord', 'Beldi' and 'Vegor'), Looseleaf lettuce ('Green Oakleaf'), Romaine lettuce ('Salakis'), Batavian lettuce ('Noisete' and 'Vanity'), Frilly open lettuce type with an iceberg bite ('Crystal') and Butterhead lettuce ('Zimska rjavka').

Plants were harvested at the stage of market acceptance (5 May and 23 October). The samples were grown outdoors, with the exception of one ('Marcord') that was from a greenhouse. They were cultivated using organic, integrated or conventional method. Among them, there were 31 samples which were produced conventionally, 53 were cultivated using integrated method and only 4 samples were organically produced. A similar ratio represents the actual state of organic, integrated and conventional farming in Slovenia (MKGP RS, 2016). This might be a reason for a small number of samples in organic farming.

At organic cultivation method farmers used fertilizers of organic origin, such as compost manure, green manure and places emphasis on techniques such as crop rotation and companion planting.

In the frame of integrated production system, farmers mostly used fertilizers of organic origin and mineral nitrogen fertilizers. Synthetic pesticides were not included in their way of production, as they can be in conventional method of cultivation.

2.2 Chemical analysis

One lettuce sample consisted of several heads of lettuce. The scrap leaves of each head were removed and all inner and outer leaves were homogenized and prepared for the analysis. Water was added to homogenized sample and the nitrate ions were extracted into the solution by shaking with the shaker. The content of nitrate in the extraction solution was determined photometrically with a segment flow analyser (San, Skalar). The nitrate in the sample was reduced to nitrite with hydrazine sulphate. The nitrite forms, in reaction with sulphanilamide, a diazo compound, the latter turning red after the addition of NEDD (N-naphthylethylenediamine dihydrochloride). The colour intensity was measured photometrically at a wavelength (λ = 540 nm) (EN 12014-7, 1998). The method for the determination of nitrate in vegetables is accredited at the Agricultural Institute of Slovenia (Kmecl and Žnidarčič, 2015).

2.3 Limit values of nitrates in lettuce in accordance with the legislation

Table 1: The prescribed threshold values for nitrates in lettuce (Commission Regulation (EU) No. 1258/2011)

Foodstuffs	Harvesting time/ Place of production	Maximum levels (mg NO_3^- kg^{-1})
1.1. Lettuce (*Lactuca sativa* L.) (protected and open-growth lettuce) excluding lettuce listed in point 1.2.	Harvested 1 October to 31 March:	
	Lettuce grown under cover	5000
	Lettuce grown in the open air	4000
	Harvested 1 April to 30 September:	
	Lettuce grown under cover	4000
	Lettuce grown in the open air	3000
1.2. Iceberg type lettuce	Lettuce grown under cover	2500
	Lettuce grown in the open air	2000

3 RESULTS AND DISCUSSION

The Commission Regulation (EU) No. 1258/2011 defines the maximum permissible levels of nitrate for the lettuce cultivar groups (*Lactuca sativa* L.) and among this groups separately defines the permissible value for Iceberg type lettuce, which naturally accumulates less nitrates. In the light of the above classification, we present the values of nitrates in analyzed samples of lettuce (Table 2 and 3).

Table 2: Concentration of nitrates in the samples of lettuce

Nitrates (NO_3^-)					
Sample	No. of samples	Min. (mg kg^{-1})	Max. (mg kg^{-1})	Average (mg kg^{-1})	Median (mg kg^{-1})
Iceberg lettuce	20	47	3211	968	912
Other types of lettuce	68	11	2451	841	780

Table 3: Number and percentage of samples in a particular concentration range

Number and percentage of samples in the concentration range of nitrates (NO_3^-)					
Sample	<200 (mg kg^{-1})	200-500 (mg kg^{-1})	500-1000 (mg kg^{-1})	1000-2500 (mg kg^{-1})	>2500 (mg kg^{-1})
Iceberg lettuce	1 (5 %)	4 (20 %)	6 (30 %)	8 (40 %)	1 (5 %)
Other types of lettuce	10 (15 %)	9 (13 %)	19 (28 %)	30 (44 %)	0 (0 %)

The amount of nitrates in vegetables depends on the type of cultivar, the weather conditions (light, quantity of rainfall), method of cultivation and harvesting time. Vegetables that are grown in winter or early spring and those grown in greenhouses contain more nitrates. Lettuce grown in a greenhouse in winter in reduced light can contain up to 3500 mg of NO_3^- kg^{-1}. If it is grown outdoors, at a time when the illumination is greater, the content of nitrates can be ten times smaller (Brown et al., 1993; Amr and Hadidi, 2001). In general, the cultivars of lettuce with looseleaf heads accumulate more nitrates than lettuce with tightly formed heads, such as Iceberg type lettuce (Commission Regulation (EU) No 1258/2011).

The latter statement could not be confirmed by our research. Iceberg type samples on average contained more nitrates (968 mg kg^{-1}), compared to other types of lettuce (841 mg kg^{-1}). The reason probably lies in the fact that a significant proportion of these samples were produced in a conventional way (35 %) and the rest of the samples used integrated cultivation method (65 %). No sample was produced organically. The highest concentration of nitrates was contained in a sample of Iceberg lettuce ('Ljubljanska ledenka'), 3211 mg kg^{-1} (grown outdoors in June, using conventional cultivation method). Among other varieties of lettuce we found the highest value in the sample of 'Marcord', which was cultivated in a greenhouse in spring (2451 mg NO_3^- kg^{-1}). The reason of so high value of nitrates is due to the conventional farming system and the way of cultivation in greenhouse. Individual samples contained very little nitrates (<50 mg NO_3^- kg^{-1}), which is likely the result of lower intensification of fertilization with nitrogen fertilizers or other aforementioned factors.

Most samples of all types of lettuce (43 %) contained nitrate in the concentration range from 1000 to 2500 mg kg^{-1} (38 samples). Of these, 4 samples exceeded 2000 mg NO_3 kg^{-1}. 28 % of the samples (25 samples) contained nitrate in the range of 500 to 1000 mg kg^{-1}, 15 % of the samples (13 samples) were in the range of 200 to 500 mg of NO_3^- kg^{-1}, and in 13 % of the samples (11 samples) we determined lower values (< 200 mg NO_3^- kg^{-1}). In one sample, the presence of nitrate was greater than 2500 mg of NO_3^- kg^{-1} (1 % of the samples).

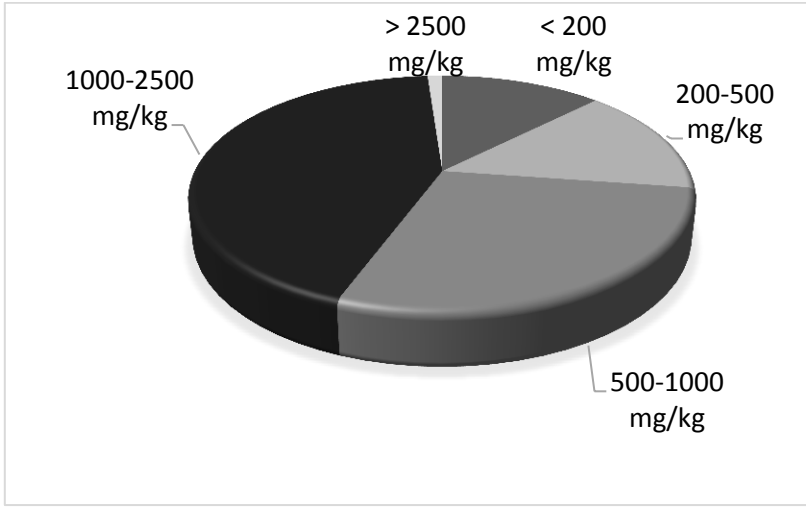

Figure 1: The proportion of samples in a particular concentration range of NO_3^-

Since 2011, the Slovenian legislation has prescribed nitrate limits for some leafy vegetables such as spinach, rocket and lettuce, and prepared baby food. For lettuce with looseleaf heads and tightly formed heads (Iceberg type), the limit values from 2000 to 5000 mg of NO_3^- kg^{-1} are prescribed. The values vary according to the harvest time (cultivation from October 1 to March 31, and from April 1 until September 30) and according to the method of cultivation (outdoor cultivation or in sheltered spaces). The standards are in accordance with the norms of the European Union (Commiss. Reg. (EU), 1258/2011).

All the samples that were analysed were growing the period from May to October, when the illumination of the plants is greater. During this time, less nitrogen accumulated in plants and NO_3^- values, which are prescribed by the legislation, were not exceeded. In winter, the light is less intense, which causes slower rates of photosynthesis, and at the same time greater accumulation of nitrate in certain parts of plants (Schuddeboom, 1993). Of the 88 analysed samples, 1 sample of Iceberg type with the value of 3211 mg of NO_3^- kg^{-1}, exceeded the legislative limit value (2000 mg NO_3^- kg^{-1}; for Iceberg type lettuce grown in an open air). Other samples did not exceed the limit values.

With this investigation we wanted to determine how the cultivation method (organic, integrated or conventional) affects the nitrate content in the crops. According to the producers' statements, most of the lettuce was grown using the integrated cultivation method (60 %), 35 % of the samples used the conventional method and only 5% of farmers cultivated lettuce organically.

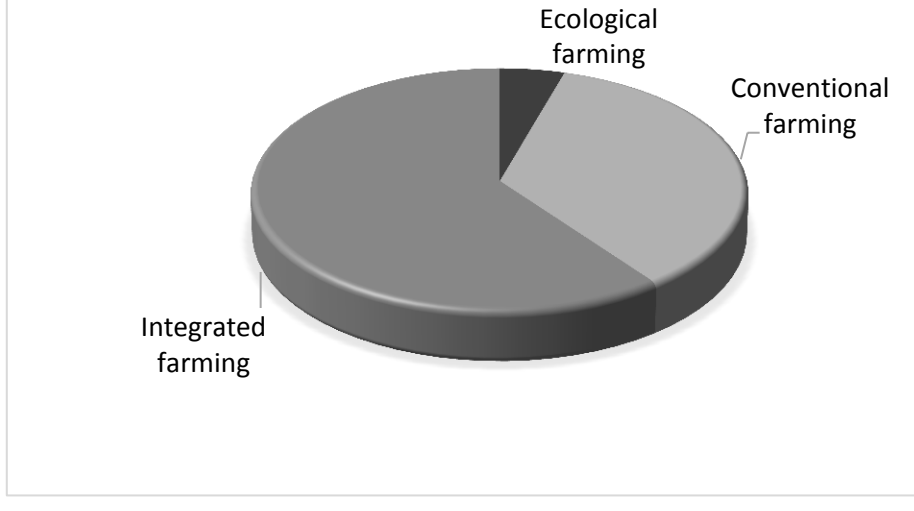

Figure 2: The proportion of organic, integrated or conventionally cultivated lettuce

A similar ratio represents the actual state of organic, integrated and conventional agriculture in Slovenia. Therefore, it is not surprising that we received only four samples of lettuce produced with organic method of cultivation (5 %), for which the producers used primarily organic fertilizers (compost, matured livestock manure, etc.). Most of the samples were received within the framework of the integrated cultivation method (53 samples), for which the use of mineral fertilizers (N, P_2O_5, K_2O fertilizers) is otherwise allowed, however, carefully monitored. A relatively large proportion of the samples (31) was received from the conventionally oriented farms, where the intensive farming method has been maintained, with the emphasis on the quantity of produce and less on the quality (greater soil stocking with mineral fertilizers).

3.1 Organic method of cultivation

Two of the 4 lettuce samples of organic production ('Green Oakleaf' and 'Zimska rjavka') contained below 500 mg of NO_3^- kg^{-1} (138 and 429 mg kg^{-1}). Two samples ranged from 500 to 1000 mg of NO_3^- kg^{-1} (783 and 969 mg kg^{-1}; 'Crystal' and 'Noisette'). Values above 1000 mg of NO_3^- kg^{-1} were not detected in any sample. It is interesting that organic cultivation did not result in very low levels of nitrates, as opposed to individual samples that came from integrated and also conventional cultivation.

3.2 Integrated method of cultivation

Within the framework of integrated production, 53 samples of lettuce were analysed ('Ljubljanska ledenka', 'Marcord', 'Beldi', 'Salakis', 'Noisette', 'Vegor', 'Vanity' and 'Green Oakleaf'). 34 % of samples contained nitrate in higher concentration range (from 1000 to 2500 mg of NO_3^- kg^{-1}). The highest proportion of samples (38 %) had values between 500 and 1000 mg kg^{-1}, while 28 % of the samples were in the <500 mg NO_3^- kg^{-1} range. The lowest measured value was 29 mg of NO_3^- kg^{-1} ('Marcord') and the highest 2200 mg of NO_3^- kg^{-1} ('Beldi').

3.3 Conventional method of cultivation

In the context of conventional production, 31 samples were analysed ('Ljubljanska ledenka', 'Noisette', 'Marcord' and 'Green Oakleaf'). The values of almost half of the samples (48 % or 15 samples) ranged from 1000 to 2500 mg of NO_3^- kg^{-1}. 23 % of the samples (7) ranged between 500 and 1000 mg kg^{-1}, 26 % of the samples (8) between 0 and 500 mg kg^{-1}. Two samples contained very little nitrate, which is unusual for conventionally grown lettuce (11 mg NO_3^- kg^{-1}, 'Noisette' and 47 mg NO_3^- kg^{-1}, 'Green Oakleaf'). One sample exceeded the value of 2500 mg NO_3^- kg^{-1} ('Ljubljanska ledenka').

Figure 3 shows the distribution of nitrate content in lettuce according to the method of cultivation (in %). The highest values of nitrates (1000 to 2500 mg NO_3^- kg^{-1} and more) contain samples from the conventional production. In the case of the samples from integrated production, the proportion of such samples is lower, while the organically cultivated samples had values below 1000 mg of NO_3^- kg^{-1}.

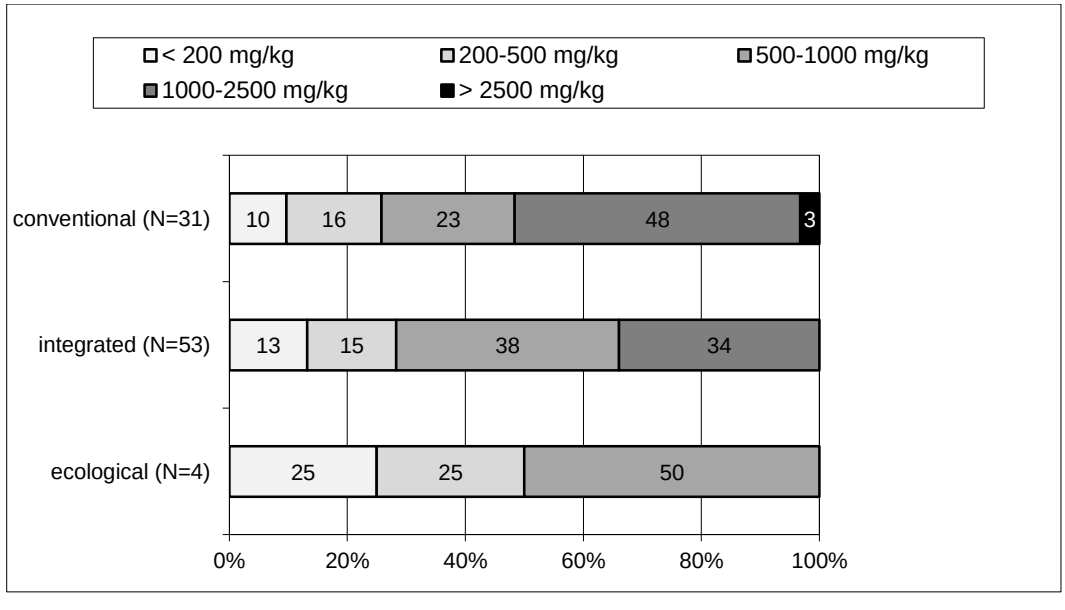

Figure 3: The distribution of nitrate content in lettuce according to the method of cultivation (in %)

4 CONCLUSION

Farmers advocated for a long time the conventional way of cultivation, where it was possible to produce more crops with less effort. Today, the opinion is different, especially with consumers who want healthy food and clean environment. In Slovenia, the share of producers who cultivate in the traditional way is still great. However, other methods of cultivation are becoming popular, such that use more environmentally friendly methods. The integrated cultivation of vegetables and fruits is becoming almost a demand on the Slovenian market, while more and more farmers are becoming interested in organic farming.

As part of our research we analysed the samples of lettuce of different types and cultivars. Of the 88 samples, there was 1 sample with a value of 3211 mg NO_3^- kg^{-1} which exceeded the statutory limit value of 2000 mg kg^{-1}, which applies to Iceberg type lettuce ('Ljubljanska ledenka'). The sample was cultivated outdoors and grown in the conventional way. Other samples did not exceed the statutory limit values, although for 43 % of the samples we determined relatively high values (from 1000 to 2500 mg of NO_3^- kg^{-1}). On average, Iceberg type samples contained more NO_3^- than other types of lettuce, which is probably because of different ways of cultivation and other factors that affect the accumulation of nitrates in plants (the use of nitrogen fertilizers, plant illumination, rainfall amount, soil fertility, length of growing, harvesting time and others).

Depending on the method of cultivation (conventional, integrated, organic), the laboratory received 35 % of the samples from conventional production, 60 % of samples from integrated and 4.5 % from organic production. Despite the fact that the number of analysed samples was relatively small, the results of the research confirm that the samples, cultivated in the conventional way, were the most contaminated with nitrates. On the other hand, it is surprising to know that the lowest values of nitrates (<50 NO_3^- mg kg^{-1}) were detected in individual samples from integrated and conventional cultivation and not in organic lettuce.

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