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Determination of Nitrates Levels in Spinach and Lettuce in Albanian Market

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Abstract

Vegetable consumption is constantly increasing since they provide substances such as minerals, vitamins, dietary fiber and bioactive substances useful for human health. At the same time their content of nitrates and nitrites has raise the consumer concern because when levels are in excess they can be a potential health risk. The purpose of this study was to analyze nitrate levels in spinach and lettuce produced in different regions of Albania and determines whether their estimated quantity exceeds allowed levels constituting a risk to human health and safety. Sampling of vegetables took place at random on retail market of Tirana and four other regions which are considered as the most important areas producing vegetables for consumption. The weight of the sample was at least 1 kg from different regions of the country. All the samples were put in paper bags and immediately submitted to the laboratory for analysis. In accordance with the procedure, the samples were homogenized within 24 hours of sampling. Most samples were prepared immediately after homogenization, while others were stored at low temperatures in the refrigerator. Highest nitrate levels were detected for both vegetables in Tirana and Berat markets compared to other regions. Taking into account the allowed levels of nitrate content in vegetables it was found that spinach and lettuce are within the permitted levels. Daily consumption of 100g of studied vegetables was found not to exceed acceptable daily intake (ADI) for nitrates. Our findings showed the need of further monitoring the content of nitrate and nitrite in vegetables in different regions, conditions and seasons.

Keywords: nitrate, spinach, lettuce.

1. Introduction

According to European Food Safety Authority (EFSA) the consumption of fruits and vegetables is 400 g/capita [3]. The consumption of vegetables is related to overall health and well-being, improving gastrointestinal functions and decreases the risks of heart diseases, brain, diabetes, anemia, arthritis, peptic ulcer and some forms of cancer [2, 3, 6, 11]. Nitrogen compounds are found on natural soil, but they can also grow through chemical and organic fertilization. Plants use the nitrates for their metabolism and produce protein compounds. Different plants have different capacities for the use and accumulation of nitrates in their organs. Nitrates can also flush down into groundwater especially by rainfall and so they can be found in drinking water. Nitrate levels in soil and drinking water can be considerable, depending on the type, land use extent, precipitation and excessive watering. The nitrate levels in plant products and in particular their metabolites, nitrites, are associated with methaemoglobinemia. Nitrites can also react with amines to form carcinogenic nitrosamines in the stomach [3].

High concentrations of nitrates in vegetables are a worldwide problem, especiallyin leafy vegetables where have been reported concentrations exceeding allowed rates. In vegetables that contain nitrates, microbiological or enzymatic activities can cause nitrate conversion to nitrite. This can happen due to inadequate storage, inappropriate conditions of transportation and lack of standard hygiene rules [9].

Nitrates in a typical diet come from drinking water (about 21%), vegetables and fruits (more than 70%) but can also be obtained from meat products (about

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6%) in which sodium nitrate is used as a preservative and color-enhancing agent [1].

The Joint Committee of Experts on Food and Agriculture (JECFA) and the Scientific Committee of the European Commission on Food (SCF) have set the values for taking nitrates and nitrites for the human Body [4, 12, 13]. The acceptable daily intake (ADI) for NO3- is 0-3.7mg/kg body weight per day and for NO_2-0-0.07mg/kg body weight per day. These values are equivalent to 222 mg of NO_3 or 4.2mg of NO_2 per day for an adult of 60kg.The ADI does not apply to infants below the age of 3 months [6, 7]. In Regulations (EC) 1881/2006 and (EU) no. 1258/2011 laying down maximum levels for certain contaminants in foods, maximum levels are set for nitrates in certain leafy vegetables [4, 5].

The purpose of this study was to analyze levels of nitrite and nitrite in spinach and lettuce and determine whether their estimated quantity exceeds allowed norms constituting a risk to human health and safety.

2. Material and Method

Sampling took place at random on retail chains and was based on local produce. The sample weight of two vegetables (spinach and lettuce), was at least 1 kg. All the samples were put in paper bags and, after sampling, immediately submitted to the laboratory for analysis. In accordance with the procedure described, the samples were homogenized within 24 hours of sampling. Most samples were prepared immediately after homogenization, while others were stored at low temperatures in the refrigerator. The sampling procedure, the sampling in the laboratory and the preparation of samples for analysis were carried out in accordance with the requirements of Directive 1882/2006 on sampling and analysis methods for the official control of nitrate levels in food [5]. Followed steps were:

- Prepare the sample for analysis

For the identification and determination of the amount of nitrate, high performance liquid chromatography (HPLC) techniques were used with UV [5, 8]. Before the analysis, the non-edible parts of each sample were removed. Each sample was then crushed and homogenized with the homogenizing apparatus, and immediately stored at -20^{o}C before analysis. 1g of the sample was placed in a volumetric container of 100 ml and added 50ml of deionized water. Then it was moved to a boiling water bath for 20 min. at 80^{o}C, with shaking and after this process was placed on the table until it cools down. After that it was diluted to a final volume of 100 ml with deionized water and filtered through a 0.45m syringe filter was followed. The first 3 ml filter was discarded and the following 1ml filters collected for determination of nitrate. All samples were analyzed immediately within 1 hour of their precipitation. Sample recovery rates were in an acceptable range of 90 to 120%.

- Prepare the mobile phase and standard solutions

The mobile phase with water/methanol concentrations (40%, v/v) and pH values (6.5-7.0) at a flow rate (0.5 ml/min) was used for the development of HPLC chromatograms. Subsequently a water methanol solution (20-40%, v/v) was prepared by adding a 0.01M octylamine-ortho-phosphate reagent solution followed by adjusting pH values (6.5~7.5) adding ortho-phosphoric acid and then filtering (Membrane Filter 0.45m) before injection.

To prepare the calibration curve, the peak area was determined for 5 different concentration levels (area method) with at least 3 repeats for each concentration level. The sample prepared for chromatographic analysis is placed on the HPLC auto sampler. Standard solutions were prepared with concentrations containing 10, 50, 100, 250 and 500 μg/ml of sodium nitrate prepared and stored at 4^{o}C for use. Solutions were prepared every 7 days.

- HPLC analysis

The mobile phase solution was allowed to pass through the HPLC column until a stable base signal was equilibrated. The flow rate was 0.5 ml/min and the length of the UV detecting wave was 213 nm. When standard solution injections yielded peak areas in the same time segment repeatedly, injection solutions were injected for analysis. Sample scores were identified by comparing them with the respective standards. Nitrate quantities in the test solution are calculated from peak areas using linear regression of nitrate returns. If the peak curve was greater than that of the maximum mass from the standard curve, the test solution was diluted in appropriate concentrations. The volume of the injection was 5 μl. At the end of the analysis, the HPLC column was refreshed by passing a water/methanol solution (1:1, v/v) for 4 hours at a flow rate of 0.5 ml/min.

Statistical analysis: The data were analyzed by analysis of variance and Tukey-Kramer test.

3. Result and discussion

Estimated standard curves and correlation coefficients are the indicator of the linearity within the tested concentration range. The method developed for the determination of nitrates and nitrites was well performed and this is clearly observed in the correlation with the coefficient R2 = 0.993 of the equation: y = 68659x + 87012.

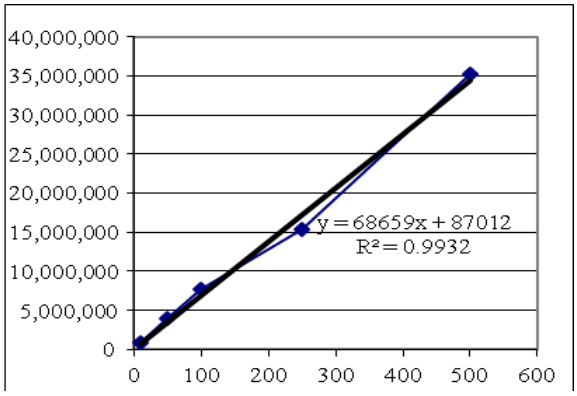

Figure 1. Correlation linearity of standard solutions and concentration of nitrate

To protect human health, most European countries have regulated the content of nitrates in food. The European Commission, 2011 amending Regulation EC No 1881/2006 by Regulation No 1258/2011 regulates the maximum levels for nitrates in vegetables, and these are also adopted by the Albanian Government [10].

Table 1. The maximum levels for nitrates content in vegetables (mg/kg FW)

Fresh spinach (Spinaciaoleracea)		3500
Preserved, deep-frozen or frozen spinach		2000
FreshLettuce (Lactuca sativa L.)	Harvested 1 October to 31 March	
	lettuce grown under cover	5 000
	lettuce grown in the open air	4 000
	Harvested 1 April to 30 September	
	lettuce grown under cover	4 000
	lettuce grown in the open air	3 000
"Iceberg" type lettuce	Lettuce grown under cover	2 500
	Lettuce grown in the open air	2 000

Vegetable consumption is constantly increasing last years. In our country there is no reliable data currently on the consumption of fresh vegetables per inhabitant, so data from the EFSA are used, according to which the consumption of fruits and vegetables is 400 g/capita.

Table 2. Nitrate level (mg/kg) in spinach on samples taken in 5 regions.

Locations	Samples					Total	Mean
	1	2	3	4	5		
Durres	97.6	81.28	46.56	30.96	75.92	332.32	66.464 B
Tirane	133.92	214.32	174.46	237.2	136.54	896.44	179.288 A
Fier	42.08	47.44	58.08	64.56	78.88	291.04	58.208 B
Vlore	84.96	82.24	75.52	98.62	88.76	430.1	86.02 B
Berat	268.86	192.64	165.64	224.34	212.46	1063.94	212.788 A
Mean	125.484	123.584	104.052	131.136	118.512		120.5536

* A = highest significance level for LSD = 2.96880 for p= 0.05 and C = lowest level for LSD = 2.96880 for p = 0.05 according to Tukey Kramer test.

Table 3. Statistical analysis for locations and repeats

Variation source	DF	Sum Squares	Mean Square	F Ratio F. fact	F stat 95%	99%
Locations	4	99810.71	24952.68	24.4073 **	3.0069	4.772
Repeats	4	2109.751	527.4379	0.51591 NS	3.0069	4.772
Error	16	16357.52	1022.345			
Total	24	118278				

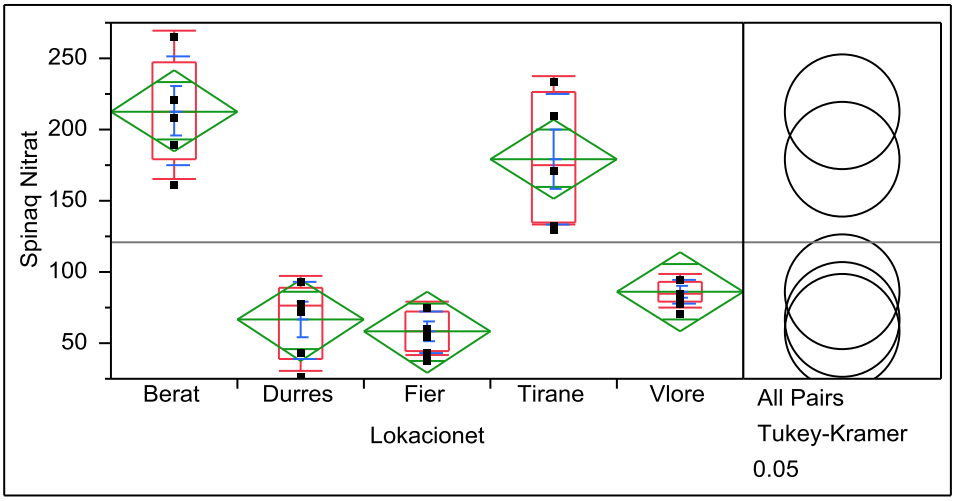

Figure 2. Nitrate content in five locations.

Table 5. Nitrate level (mg/kg) in lettuce on samples taken in 5 regions.

Locations	Samples					Total	Mean
	1	2	3	4	5		
Durres	109.52	61.44	109.36	75.76	75.92	432	86.4 B
Tirane	104.56	95.36	83.36	102.64	72.48	458.4	91.68 B
Fier	91.92	64.08	57.42	86.72	71.65	371.79	74.358 B
Vlore	74.22	84.92	62.24	93.36	88.44	403.18	80.636 B
Berat	168.88	162.64	165.68	196.72	184.48	878.4	175.68 A
Mean	109.82	93.688	95.612	111.04	98.594		101.7508

* A = highest significance level for LSD = 2.96880 for p= 0.05 and C = lowest level for LSD = 2.96880 for p = 0.05 ccording to Tukey Kramer test.

Table 6. Statistical analysis for locations and repeats.

Variation source	DF	Sum Squares	Mean quare	F Ratio F. fact	F. stat. 95%	99%
Locations	4	34993.97	8748.494	38.42216 **	3.0069	4.772
Repeats	4	1320.301	330.0753	1.449645 Ns	3.0069	4.772
Error	16	3643.103	227.6939			
Total	24	118278				

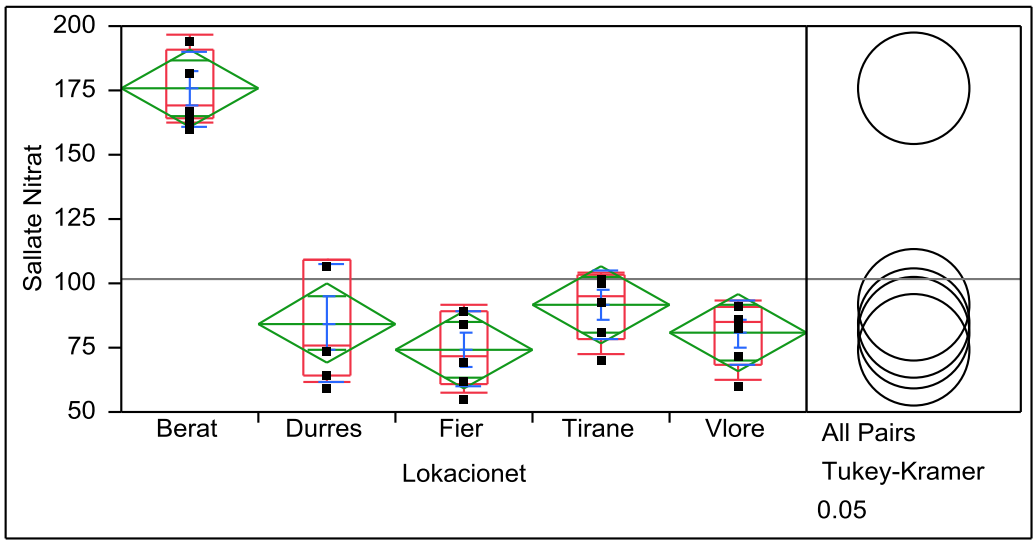

Figure 3. Nitrate content in five locations.

As noted the measurements of these samples resulted in low nitrate values. The study results show that the lowest levels of nitrate were detected in regions of Fier, Vlore and Durres for spinach and Fier, Tirane Vlore for lettuce, while the highest levels were found in Berat region for both vegetables. Vegetables were sampled in the autumn. Taking into account the permitted levels of nitrate content in vegetables from these measurements, resulted that spinach and lettuce levels are within permitted levels. The data obtained was compared with the levels allowed by EFSA and WHO and ADI in percentage was calculated. Regarding analyzes performed by us, the calculation of the percentage of ADI per 100g of consumable vegetables (spinach and lettuce) is as shown in the Table 7.

Table 7. Acceptable Daily Intake (ADI)

Regions	Spinach	ADI%	Lettuce	ADI %
Durres	66.464 B	2.99	86.4 B	3.89
Tirane	179.288 A	8.07	91.68 B	4.12
Fier	58.208 B	2.62	74.358 B	3.34
Vlore	86.02 B	3.87	80.636 B	3.63

The levels of nitrate found in the vegetables included in this study were such that they did not present an immediate health risk to the general population. However, further study related to factors which can increase nitrate content making them dangerous to consumers and especially for infants consuming unprocessed vegetables need to be undertaken. Current knowledge and evidence show that there are strong beneficial effects on the consumption of vegetables. To have these benefits from vegetable consumption, measures should be taken to reduce exposure to nitrates.

4. Conclusions

-Levels of nitrates from vegetables included in this study are lower than permitted limits by EU and considered safe for human consumption.

-Further study is needed related to the different factors such as characteristics of the region, conditions, cultivation techniques, consumer traditions as the customer can get higher levels of nitrates.

-Farmers are advised to apply good agricultural practices (GAP) that reduce the high concentrations of nitrates in vegetables.

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