#### **DETERMINATION OF NITRATES IN FOOD PRODUCTS**

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#### Abstract

Nitrates are very toxic matters, which are stimulant methemoglobinemy and produce carcinogenic matters like nitrosoamines and nitrosoamides. The purpose of our research is to find out content of nitrates in different kinds of food products, which are produced in territory of the Republic of Macedonia and from import. Investigations were providing by an enzymatic method (reducing nitrates to nitrites) and after that, determining contents of nitrates spectrophotometricaly. All products are investigated by bioquant nitrate test. It was found that the medium values for nitrates in investigated products are less then maximum levels for nitrates in our country and less then regulation in WHO.

#### Key words: nitrates, determination, food, spectrophotometry

### 1. Introduction

Nitrates are very toxic matters, which reduce to nitrites at certain physiological conditions in the human body. They cause metchemoglobinemy and produce carcinogenic matters like nitrosoamines and different health disturbances as changes in vitamin levels, disturbance in thyroxin production; negative influence in reproduction; changes in the blood etc. Almost all food products contain nitrates, because nitrates are naturally present in the soil (e.g. humid matters), the water and the air (e.g. nitrous gases). Nitrates negative influence on human health starts with their conversion to nitrites, by an enzyme "nitrate reductase" [1]. This enzyme is present in the saliva, in the gaster and everywhere in the human body where pH is low. Nitrites react with hemoglobin and produce methemoglobin, which enables to transport of oxygen at the cellular level.

Newborn organisms are very sensitive to metchemoglobinemy because they have anmature "methemoglobin reductase system". If the metchemoglobin level in the blood is lower than 15 % than of the total chemoglobin, there is not any significant change in the human body. But, If the metchemoglobin level is 15 % or higher than the total chemoglobin levels then there is a condition like cyanosis and coma. If the level of metchemoglobin is 70 % or higher than it is lethal.

WHO recommendation for the daily intake of nitrates by food products is 300 mg kg<sup>-1</sup>, for baby's is 25 mg NO<sub>3</sub><sup>-</sup>-N<sub>2</sub> and for drinking water is 45 mg NO<sub>3</sub><sup>-</sup> [2]. The maximum permitted level of nitrates in our country is 10 mg l<sup>-1</sup> (NO<sub>3</sub><sup>-</sup>-N<sub>2</sub>) for water and 250 mg kg<sup>-1</sup> (NO<sub>3</sub><sup>-</sup>) for the industrial preparation of baby food from vegetables, meat and fruits. For other food products we have taken the nitrate maximum levels as proposed by the WHO [3].

The purpose of our research is to find out the content of nitrates in different kinds of food products (vegetables, milk products and dietetic products) by enzymatic method - bioquant nitrate test. When we know the quantity of nitrates, we can establish the health safety of food products.

#### 2. Experimental

We have been investigating many different food products such as vegetables (cabbage, carrots and beet root), several kinds of cheeses and dietetic products. In our investigation we used an enzymatic method by enzyme "nitrat reductase" - bioquant nitrate test (produced by Merck - Darmstad). The test kit contained: fad and buffer; NADH; lyophilized enzyme "nitrate reductase; potassium nitrate- standard solution and nitrate color reagent. Under the influence of nitrate reductase, nitrate is partially reduced to nitrite under defined conditions. Nitrite then reacts with sulphanilic acid and NED to form a red - violet Azo - compound.

Measurement takes place at 545,6 nm using Perkin-Elmer Lamda 12 spectrophotometer.

### Preparation of the samples

1. Homogenized vegetables a precisely weighed amount (10 g) were placed in a small quantity of redistilled water and heated under stirring for approximately 10 min at 80  $^{0}$ C, using a water bath. These were than all owed to cool filter and the level was make up to the correct level with redistilled water in a volumetric flask (100 ml).

2. Thoroughly homogenized cheese (5 g) sample was placed into a beaker then 50% trichloroacetic acid solution was added together with 30 ml of water and homogenize agent in a blender. The mixture was adjusted to pH 7.0 with sodium hydroxide solution (5 mol/l) and fat extraction was carried out by adding 5 ml petroleum benzin, shaking thoroughly and transferring to a 100 ml volumetric flask. Once the phases have separated the flask was filled up with water until the organic phase was above the mark. The solution was remixed and placed in a refrigerator for approximately 15 minutes. Carefully aspirate the upper petroleum benzin layer. Filter the lower aqueous layer through a fluited filter and use the filtrate for the test.

3.To analyze dietetic products from vegetables and fruits, add 30 ml redistilled water to 10 g. of the sample and heat under stirring for approximately 10 minutes to 80°C using a water bath. Allow to cool, filter and make up to the mark with redistilled water in a volumetric flask (100 ml).

#### 3. Results and Discussion

The results of our investigations are given in Tables 1 and 2 and on Figs. 1-3. The results show that the content of nitrates in all samples of vegetables were less than the maximum level for nitrates as recommended by the WHO and FAO - 2500 mg kg<sup>-1</sup>. This means that our food products are safe for consumption.

WHO and FAO recommend that the maximum level for nitrates in cheese is  $36 \text{ mg kg}^{-1}$ . The average values for nitrates in the samples of cheese that we our investigated was less than the recommended maximum level and we could say that they are safe for consumption.

The maximum recommended level for nitrates in these kind of dietetic products is  $250 \text{ mg kg}^{-1}$ . Investigated samples of dietetic products were less than the maximum level for nitrates, so, they are safe for consumption.

Table.1. Content of nitrates in vegetable								
Vegetable	$NO_3$ (mg kg <sup>-1</sup> )					Average		
	1	2	3	4	5	value		

Cabbage	120.5	133.9	146.9	141.8	164.5	141.5
Carrot	116.5	49.5	48.4	54.6	134.6	80.7
Beet root	1099.9	1542.5	1048.4	1530.6	616.7	1167.6



Fig. 1. Average values for nitrates in investigated vegetables

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Table.2. Content of nitrates in cheese							
Kind of		NO <sub>3</sub> -	$(mg kg^{-1})$			Average	
cheese	1	2	3	4	5	value	
Sheep-cheese (soft-white cheese)	8.5	11.8	11.4	10.7	10.9	10.66	
Cow-cheese (soft-white cheese)	13.2	20.9	21.6	21.3	12.9	17.98	



Fig. 2. Nitrate content in several kinds of cheeses



Fig. 3. Nitrate content in some kinds of dietetic products

# 4. Conclusions

1. The average values of nitrates in the products that were investigated were less than the maximum level for nitrates recommended by the World Health Organization (WHO) and FAO. This means that the types of food products that were investigated are safe for consumption

2. Milk products - cheeses are products with lowest content of nitrates.

3. From all investigated vegetables, beet root is the one with the highest content of nitrates.

## References

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