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# Nitrite determination in spices

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**Abstract** The aim of this study is to determine the nitrite content in some spices: basil, pepper, dill, thyme, oregano, bay leaves, cinnamon, mint, mustard and cumin. The nitrite content was measured using UV-VIS spectrometric method with Griess reagent. The method was tested and partial validated. The nitrite content varied from 0.23 to 7.7 mg/Kg and these values are lower than those encountered in the literature.

*Keywords*: nitrite, spices, UV –VIS spectrometry, validation

## 1. Introduction

A spice is a dried seed, fruit, root, bark or vegetative substance used for several purposes since ancient times. Several uses of these plants are known for culinary purposes. In addition, they are also used in folk medicine as antiscorbutic, antispasmodic, tonic, carminative agents against bronchitis, ulcers and as diuretics, depuratives, vermifurges [1].

Vegetables are known as the major source of nitrate and nitrite intake in the human diet [2]. Several factors can influence the level of nitrite in various raw vegetables: type, amount, form of nitrogen fertilizer and light conditions [3]. Also the nitrite concentrations varied in the various edible plant parts. Nitrites are implicated in methaemoglobinaemia [4] and can cause gastric cancer [5]. On the other hand, $NO_2^-$ has been considered primarily as a stable precursor of NO in the body [6]. The bioefficacy of $NO_2^-$ has been demonstrated in many studies [7,8].

Limited studies were carried out on nitrite content of spices growing in Romania. The aim of this work was to establish the nitrite content of several spices: basil, pepper, dill, thyme, oregano, bay leaves, cinnamon, mint, mustard and cumin used in Romania. The nitrite content was measured using UV-VIS spectrometric method with Griess reagent. This method was tested and partial validated.

## 2. Experimental

### *2.1. Reagents and solutions*

All reagents were of analytical-reagent grade (Merck and Fluka) and all solutions were prepared using distilled-deionized water.

Griess reagent was prepared by mixing equal volumes from A solution (sulphanilic acid) and B solution (α-naphthylamine). A solution is obtained from 0.5 g sulphanilic acid in 150 mL acetic acid 12%. B solution is prepared by dissolving 0.2 α-naphthylamine in 20 mL of hot distilled water and then adding 150 mL acetic acid 12%.

### *2.2. Instrumentation*

The weightings were made with Metler Toledo analytical balance with ±0.0001g accuracy. A molecular absorbtion spectrometer DR 2800 LANGE UV-VIZ with single beam optical system was used.

### *2.3. Samples*

Spice samples used in research represent several representative Romanian spice sorts: basil, pepper, dill, thyme, oregano, bay leaves, cinnamon, mint,

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mustard and cumin. These samples were commercially available on the market.

*2.4. Nitrite determination*

Ten grams each of the homogenized samples by drying and grinding were blended with 250 mL hot distilled water. The extract was kept in a refrigerator for 12 h and then filtered through filter paper and the filtrate was used for nitrite determinations.

The Griess method (diazotisation method) was used in the determination of nitrite.

Nitrite is detected and analyzed by formation of a red pink colour upon treatment of a nitrite-containing sample with the Griess reagent. When sulphanilic acid is added, the nitrites form a diazonium salt. Then the azo dye agent (alpha-naphthylamine) is added and a pink colour is developed.

25 mL of each sample filtrate was pipetted into a conical flask. A 5 mL Griess reagent was added to each flask and left to stand for 15 min for the full development of the characteristic pinkish-red color. The absorbance was measured at a wavelength of 525 nm on spectrophotometer against distilled water blank. A standard curve was prepared using $7.14\times10^{-6}$M sodium nitrite and sample nitrite concentrations were computed directly from curve. The calibration curve was linear over the range 0.006-0.108 mg/L with a correlation coefficient of 0.9982.

## 3. Results and discussions

*3.1.Validation procedure*

The validation of an analytical procedure, according to international guidelines is necessary to demonstrate its suitability for the intended purpose.

In this work, linearity and precision (repeatability) were verified to evaluate the performance of the applied spectrophotometric method in the particular case of Romanian spices.

In order to verify the significant differences between the lowest and the highest concentration values of proposed concentration range (0.006-0.108 mg/L) was applied the homogeneity of variances test [9].

The results showed that no significant differences were found between the variances of the concentration range limits. Thus, it means that the working range was correctly choosen.

Precision was primarily expressed as a percentage relative standard deviation (RSD%). It can be discussed at three different levels: repeatability, intermediate precision and reproducibility, but in this study will address only the repeatability.

Repeatability was demonstrated by measuring a standard solution of nitrite concentration (0.025 mg/L) and each spice samples by six times.

The results obtained (RSD%) for the six measurements of standard solution and spice samples were between 3.02 and 24.82%. These values were lower than those required in Horwitz equation: $RSD< 2^{(1-0,5\lg c)}$, for studied working range.

*3.2. Nitrite contents of spice samples*

Nitrite amounts of foods are of great importance regarding the consumers' health. Nitrite content in Romanian spices (basil, pepper, dill, thyme, oregano, bay leaves, cinnamon, mint, mustard and cumin) are given in **Fig. 1.** Data were reported as mean±standard deviation. These values were established to vary widely depending on the different plant species.

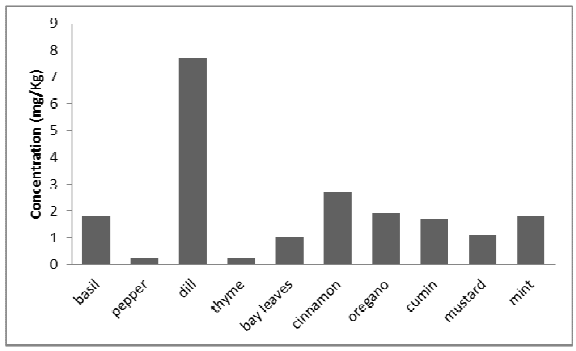


**Fig.1**. Nitrite content in spice samples

The nitrite content varied from 0.23±0.07 to 7.7±0.1 mg/Kg in spice samples and these values are lower than those encountered in the literature. According to Ozcan and Akbulut nitrite content of myrtle, basil, sumac, sater, bitter fennel and pickling

herb were from 22.06 mg/kg to 52.70 mg/kg [1]. Taras established nitrite contents in the range from 44 to166 mg/kg in vegetables [10].

Similar levels of nitrite (6.9 mg/Kg) were reported by Jaworska in New Zeeland spinach [11].

The level of nitrites increased by 8–78% after freezing and by 8–41% after sterilization, according to Jaworska [12].

Foods like vegetables contribute to dietary intake of nitrite. The European Commission has established a Temporary Acceptable Daily intake of 0 - 0.1 mg of nitrite per kg b.w. (expressed as sodium nitrate). This daily intake include human intake from all sources.

Our results were found to be lower compared with Committee values [13].

The nitrate and nitrite contents of agriculture products vary depending on plant species, fertilization form and amount and harvested time, use together with organic fertilizer of industry fertilizer [1].

As a result, nitrite amounts in spices and vegetables vary widely according to literature findings.

## 4. Conclusions

The nitrite content varied from 0.23 to 7.7 mg/Kg and these values are lower than those encountered in the literature.

The nitrite concentrations varied depending on the different plant species.

Although the levels of nitrite detected in this study were relatively low, it is necessary to investigate the spices to estimate the intake of nitrite.

The well-known Griess method was partially validated for determination of nitrite content in spices. Further research needs to be carried out in order to complete validation.

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