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Assessment of Nitrite Levels in Fruits, Vegetables and Herbs from Different Agricultural Sources

Cristina Stoian[1], Alexandra Virginia Bounegru[1], Veronica Filimon[2], Simona Butan[1]*

[1] *"Dunarea de Jos" University of Galati, Faculty of Sciences and Environment, 47 Domneasca Street, 800008, Galati, Romania, cristina.stoian@ugal.ro, ORCID: 0009-0009-7264-9259*
[1] *"Dunarea de Jos" University of Galati, Faculty of Sciences and Environment, 47 Domneasca Street, 800008, Galati, Romania, alexandra.meresescu@ugal.ro, ORCID: 0000-0002-6005-492X*
[1] *"Dunarea de Jos" University of Galati, Faculty of Sciences and Environment, 47 Domneasca Street, 800008, Galati, Romania, simona.patriche@ugal.ro, ORCID: 0009-0007-0554-2633*
[2] *"Dunarea de Jos" University of Galati, Cross-Border Faculty, 47 Domneasca Street, 800008, Galati, Romania, veronica.filimon@ugal.ro, ORCID: 0000-0001-8235-4918*

**Corresponding author: simona.patriche@ugal.ro*

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Abstract
This study aimed to evaluate nitrite concentrations in fruits, vegetables and leafy plants obtained from different agricultural sources, namely a local open market and a supermarket in Galați, Romania, in order to identify potential correlations between product origin, plant type and nitrite accumulation. Nitrite determination was performed using a spectrophotometric method based on the Griess reaction, which showed excellent linearity (R^2 = 0.9969) and high sensitivity (LOD = 0.0056 µg/mL; LOQ = 0.0188 µg/mL). The results revealed a wide variation in nitrite content, ranging from 5.9 to 80.5 ppm, with the highest concentrations detected in parsley, red beetroot and carrot. Statistical analysis indicated a non-uniform, right-skewed distribution of values, with predominance of low concentrations and isolated high levels. These findings highlight the influence of plant type, agricultural practices, and post-harvest conditions on nitrite accumulation and emphasize the importance of monitoring these compounds for food safety.
Keywords: nitrites, Griess reaction, plant products, food safety, spectrophotometry, agricultural practices, statistical analysis

1. Introduction

Nitrates and nitrites are inorganic compounds naturally found in the environment, assuring the food supply through multiple pathways, such as: soil fertilization with nitrogen-based fertilizers, natural nitrification processes within plants, meteorological conditions, and meat preservation methods [1, 2]. The highest concentrations of nitrates and nitrites can be accumulated in plant-based products compared to animal-based foods, especially when excessive amounts of nitrogen fertilizers are applied during cultivation [3-5]. Nitrate concentrations vary considerably depending on the plant organ examined, following a well-documented descending gradient: petiole> leaves> stem> root> inflorescence> tuber> bulb> fruit> seeds [6]. This distribution reflects the mechanisms of nitrogen transport within plants, from the root in direct contact with fertilized soil toward the other organs. An important food safety consideration is that, after harvest, nitrite concentrations can increase rapidly in vegetables through the microbial reduction of nitrates, particularly at room temperature, while nitrate content decreases accordingly. Furthermore, vegetables with a high nitrate content may suffer

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significant nitrite contamination during storage, due to the biological conversion of nitrates to nitrites. Therefore, it is recommended to store fresh vegetables with appropriate rapid cooling methods and in undamaged forms to prevent the risks of nitrite accumulation [7, 8].

Scientific interest in these compounds has grown substantially in recent years, driven by the risks they pose both directly and through the metabolites they generate in the body. Beyond these long-term effects, acute exposure to nitrates and nitrites can cause methemoglobinemia, a serious condition in which hemoglobin loses its ability to transport oxygen, posing a particular risk to infants - a phenomenon widely described in the literature as "blue baby syndrome" [9, 10]. Nitrites can react with secondary amines in the acidic gastric environment to produce nitrosamines - compounds with carcinogenic, mutagenic and teratogenic properties [11, 12].

The present study aims to evaluate the nitrite content in fruits, vegetables and leafy plants originating from different agricultural sources, namely an open local market and a supermarket in Galați, Romania, to identify potential correlations between product origin and the residual levels of nitrite ions. Additionally, the study sought to highlight trends in nitrite accumulation depending on plant type and the analyzed plant organ (roots, tubers, leaves, and fruits). The importance of this approach lies in its implications for food safety, as well as in emphasizing the role of agricultural practices, particularly organic farming, in limiting the accumulation of nitrogenous compounds and in obtaining safer plant-based products for consumption.

2. Materials and method

2.1. Reagents and Solutions

All determinations were performed using analytical-grade reagents supplied by Sigma-Aldrich (Merck, Darmstadt, Germany). The chemicals used included glacial acetic acid, sodium nitrite, sulfanilic acid, α-naphthylamine and metallic zinc powder. All solutions were prepared using bidistilled water in order to minimize potential interferences arising from impurities.

For nitrite quantification, a calibration curve was established by preparing a concentrated stock solution of sodium nitrite, obtained by dissolving a known mass of the substance in a defined volume of distilled water. From this stock solution, a series of dilutions were subsequently prepared and used for spectrophotometric determination.

The Peter–Griess reagent, essential for the colorimetric reaction, was freshly prepared prior to each set of determinations due to its limited stability over time. It was obtained by mixing equal volumes of two separate solutions: a sulfanilic acid solution in acetic acid medium and an α-naphthylamine solution, initially prepared in hot distilled water and subsequently acidified. To eliminate possible chromatic interferences, the final solution was treated with zinc powder followed by filtration, resulting in a clear and stable reagent throughout the analysis.

2.2. Equipment and Instrumentation

The analyses were carried out using standard laboratory glassware, including graduated pipettes, volumetric flasks, Erlenmeyer and Berzelius beakers, graduated cylinders, funnels, and glass stirring rods. Weighing operations were performed using an analytical laboratory balance (Sartorius LE 623 P), while heating and dissolution steps were conducted using a water bath and conventional heating sources.

Spectrophotometric measurements were performed using a double-beam UV–Vis spectrophotometer (Rayleigh UV201), which enabled the determination of solution absorbance at the characteristic wavelength of the colorimetric reaction. Reagent handling and experimental procedures were conducted under controlled conditions, using a fume hood to ensure both operator safety and experimental reproducibility.

2.3. Principle of the Analytical Method

Nitrite determination was performed using a spectrophotometric method based on the Griess reaction, widely employed in analytical chemistry for the identification and quantification of these anions. In acidic medium, nitrites are converted into nitrous acid, which reacts with sulfanilic acid to form a diazonium salt. This intermediate subsequently couples with α-naphthylamine, leading to the formation of an intensely pink-red azo compound.

The intensity of the developed color is directly proportional to the nitrite concentration in the sample, and quantitative determination is achieved by measuring the absorbance at a wavelength of 540 nm. The obtained values are then correlated with a calibration curve constructed under the same experimental conditions.

2.4. Sample Preparation

The study was conducted on fresh plant-derived samples characteristic of the spring season, purchased from two distinct commercial sources in Galați, Romania, namely a local open market and a supermarket, on 17 May 2025. Sample selection was carried out considering the degree of morphological development, with preference given to larger specimens, under the assumption that these may reflect a higher use of nitrogen-based fertilizers during cultivation.

A total of 20 plant samples representing 19 botanical varieties were analyzed, spanning five categories: fruits (four lots of strawberries and two of cherries from four geographical origins), root vegetables and tubers (carrot, red beetroot, new potatoes, celery), leafy vegetables (lettuce, spinach), commonly consumed salad vegetables (tomato, bell pepper, cucumber) and fresh herbs (parsley, dill, lovage, red orach, sorrel). In order to reduce variability associated with cultivation conditions, herb samples were obtained from the same producer, thus assuming a similar fertilization regime and comparable soil characteristics.

Different anatomical parts of the plants, such as roots, tubers, leaves, and fruits, were analyzed in order to evaluate the differential distribution of nitrites depending on the plant organ. All samples were purchased on the same day and the analyses were performed within a maximum of 24 hours. Prior to analysis, the samples were stored at controlled temperature (2°C) to minimize microbial contamination and compositional changes.

The samples were purchased and analyzed on the same day, precisely to eliminate inter-daily variability, as nitrates are enzymatically transformed into nitrites under the action of microorganisms, a process favored by ambient temperatures.

2.5. Experimental Procedure

The quantitative determination of nitrites involved a sequence of interdependent steps, starting with the preparation of working solutions and followed by the extraction of the analyte from plant samples. The resulting aqueous extracts were subsequently subjected to a purification step by filtration, in order to remove solid particles and potential interfering substances.

In the final stage, the samples were treated with the Peter–Griess reagent, and the absorbance of the resulting solutions was measured spectrophotometrically at 540 nm. Nitrite concentrations were determined by interpolation on the calibration curve obtained from standard solutions prepared under identical experimental conditions. The applied methodology provides good sensitivity and selectivity, making it suitable for the determination of nitrites in complex plant matrices.

3. Results and Discussion

3.1. Construction of the Calibration Curve and Determination of the Limit of Detection

The quantitative determination of nitrites was carried out using a calibration curve constructed from sodium nitrite standard solutions in the concentration range of 0.04–0.40 μg/mL, prepared according to the composition presented in Table 1.

Table 1. Composition of standard solutions for nitrite determination

No.	Volume of diluted $NaNO_2$ stock solution (mL)	$NaNO_2$ concentration (μg/mL)	Volume of Griess reagent (mL)	Volume of distilled water (mL)
1	1	0.04	1	23
2	2	0.08	1	22
3	3	0.12	1	21
4	4	0.16	1	20
5	5	0.20	1	19
6	6	0.24	1	18
7	7	0.28	1	17
8	8	0.32	1	16
9	9	0.34	1	15
10	10	0.40	1	14
Blank	0	0	1	24

The macroscopic analysis of the standard series (Figure 1) reveals a progressive increase in the intensity of the pink coloration with increasing nitrite concentration, confirming the formation of the azo compound characteristic of the Griess reaction.

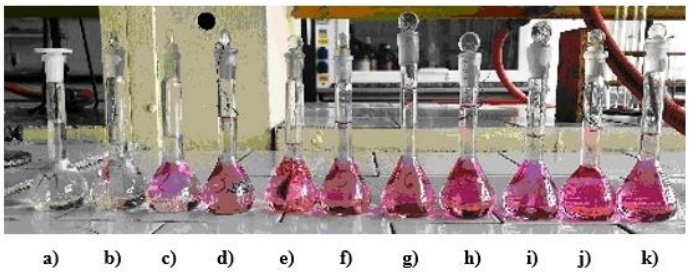


Figure 1. Series of standard solutions for nitrite determination: a) blank solution; b) standard solution 1; c) standard solution 2; d) standard solution 3; e) standard solution 4; f) standard solution 5; g) standard solution 6; h) standard solution 7; i) standard solution 8; j) standard solution 9; k) standard solution 10.

The absorption spectrum of the standard solutions showed a maximum absorbance at approximately 540 nm, in agreement with literature data [13]. Consequently, all spectrophotometric measurements were performed at this wavelength to ensure consistency between calibration and sample analysis.

The calibration curve exhibited a linear relationship between absorbance and nitrite concentration. The graphical representation of this relationship is presented in Figure 2. The high value of the regression coefficient (R^2 = 0.9969) indicates excellent linearity. Under these conditions, the quantification of nitrites in the samples was carried out by interpolation on this calibration line, without the need for additional dilutions.

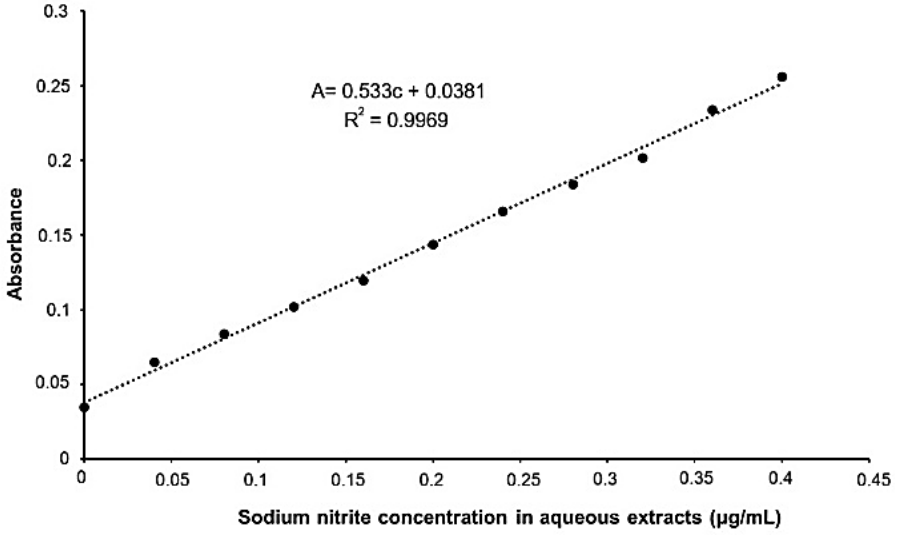


Figure 2. Calibration curve for sodium nitrite determination using the Griess method. The standard deviation (SD) of 3 replicate measurements ranged between 0.26 and 2.08 absorbance units.

The analytical performance of the method was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ), based on the standard deviation of the blank sample and the slope of the calibration curve.

The limit of detection was calculated using the following equations:

$$LOD = \frac{3 \cdot \sigma}{S} \quad (1)$$

and the limit of quantification was calculated according to the following relationship:

$$LOQ = \frac{10 \cdot \sigma}{S} \quad (2)$$

where σ represents the standard deviation of the blank sample, and S is the slope of the calibration curve.

Thus, the LOD corresponds to the lowest concentration that can be statistically distinguished from the blank signal, whereas the LOQ represents the lowest concentration that can be quantified with acceptable precision and accuracy.

The LOD and LOQ values were calculated based on Equations (1) and (2), and the obtained results are presented in Table 2.

Table 2. Analytical performance parameters of the spectrophotometric method for nitrite determination

Parameter	Value
Slope (S)	0.533
Standard deviation of blank (σ)	0.001
Limit of detection (LOD)	0.0056 µg/mL
Limit of quantification (LOQ)	0.0188 µg/mL

The obtained values indicate that the method is sufficiently sensitive for the detection and quantification of nitrites within the investigated concentration range.

3.2. Extraction and Spectrophotometric Determination of Nitrites in Samples

Approximately 5 g of homogenized plant sample were extracted with distilled water by heating in a boiling water bath, followed by cooling to room temperature (25°C). The extracts were filtered through quantitative filter paper, and the volume was

adjusted to 100 mL with distilled water. The obtained filtrate was used for spectrophotometric analysis.

For nitrite determination, 10 mL of the extract were transferred into a 25 mL volumetric flask, to which 0.5 mL of Griess reagent I and 0.5 mL of Griess reagent II were added. The solution was then brought to the mark, corresponding to a dilution factor of 2.5, and allowed to stand for 20 minutes to enable color development. Subsequently, the samples were filtered, and the absorbance was measured spectrophotometrically at the selected wavelength against a blank.

Nitrite concentrations were calculated using the calibration curve, taking into account the dilution factor and the initial sample mass. The aqueous extracts obtained after thermal treatment and filtration (Figure 3) exhibited color variations specific to the chemical composition of each plant product, due to the presence of natural pigments (carotenoids, phenolic compounds, betalains, etc.). These intrinsic colorations do not allow a direct assessment of nitrite content.

Figure 3. Appearance of the samples after thermal treatment and removal of the plant matrix, coarsely dispersed prior to filtration—arranged from left to right according to the numbering on the Erlenmeyer flasks, corresponding to the following plant samples:1 – cherries; 2 – strawberries (Călărași); 3 – strawberries (Giurgiu); 4 – strawberries (Satu Mare), variety 1; 5 – strawberries (Satu Mare), variety 2; 6 – red beetroot; 7 – carrot; 8 – lettuce; 9 – spinach; 10 – lovage; 11 – celery; 12 – red orach; 13 – parsley; 14 – dill; 15 – sorrel; 16 – bell pepper; 17 – cucumber; 18 – new potatoes; 19 – tomato.

After treatment with the Peter–Griess reagent, all samples developed a characteristic pink-red coloration corresponding to the formation of the azo compound, the intensity of which is proportional to the nitrite concentration.

Macroscopic analysis revealed the presence of relatively high nitrite levels in all investigated fruit samples. For instance, elevated levels were also observed in red beetroot; the clear differences between the coloration of the extract after thermal treatment and that obtained following the reaction with the Griess–Peter reagent indicate that the analytical signal is specific to nitrites and is not significantly influenced by the natural pigments present in the matrix. Similarly, the parsley sample exhibited the most intense coloration characteristic of the reaction, suggesting the highest nitrite content among the analyzed samples. The absence of interfering coloration in the thermally treated extract confirms that subsequent determinations are not affected by naturally occurring chromophoric compounds.

The macroscopic observations are consistent with the spectrophotometric results, which show that the highest absorbance value was recorded for parsley, followed closely by red beetroot and carrot.

Based on the calibration curve equation, the mass of the analyzed samples, and the measured absorbance values, nitrite concentrations were calculated and expressed in ppm (Table 3).

Table 3. Nitrite concentrations in plant-derived samples (fruits, vegetables, and leafy plants) expressed as mean ± standard deviation (SD) and median values (ppm). Data represent the results obtained from triplicate determinations for each sample.

Plant product	**Concentration of nitrite (ppm)**	
	Mean ± SD	**Median**
Cherries (Greece)	51.00 ± 1.05	51.11
Cherries (Vrancea)	26.24 ± 1.11	26.3
Strawberries (Călărași)	70.05 ± 2.08	70.2
Strawberries (Giurgiu)	34.31 ± 1.43	34.5
Strawberries (Satu Mare), variety 1	5.95 ± 0.35	5.95
Strawberries (Satu Mare), variety 2	43.74 ± 0.82	43.8
Carrot	64.19 ± 1.04	64.3
Red beetroot	76.39 ± 0.26	76.5
Celery	6.55 ± 0.35	6.55
New potatoes	6.03 ± 0.33	6.05
Tomato	6.87 ± 0.37	6.87

Bell pepper	21.57 ± 0.95	21.6
Cucumber	5.90 ± 0.35	5.9
Lettuce	17.74 ± 0.84	17.75
Spinach	17.88 ± 0.87	17.9
Lovage	6.33 ± 0.33	6.33
Red orach	11.37 ± 0.56	11.4
Parsley	80.48 ± 1.47	80.5
Dill	6.76 ± 0.36	6.77
Sorrel	6.32 ± 0.32	6.32

The classification of samples according to nitrite concentration highlights four distinct ranges. Samples with low nitrite content (≈5.9–6.9 ppm) include cucumber, new potatoes, celery, dill, sorrel and several other products, suggesting either reduced fertilization or cultivation conditions closer to organic practices. Interestingly, some underground plant organs exhibited low values, contrary to theoretical expectations regarding nitrite accumulation.

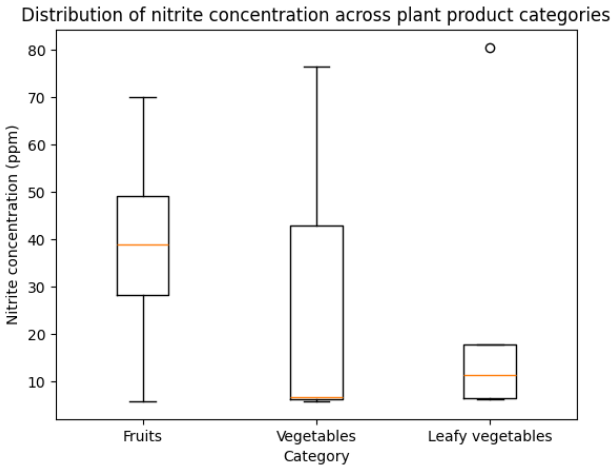
The medium content category (≈11–26 ppm) includes red orach, lettuce, spinach and bell pepper. Samples with high nitrite content (≈34–51 ppm), such as cherries from Greece and certain strawberry varieties, indicate significant differences between sources of origin, suggesting the influence of agricultural practices and fertilization regimes.

The highest values, corresponding to very high nitrite content (≈64–80 ppm), were determined for parsley, red beetroot, and carrot.

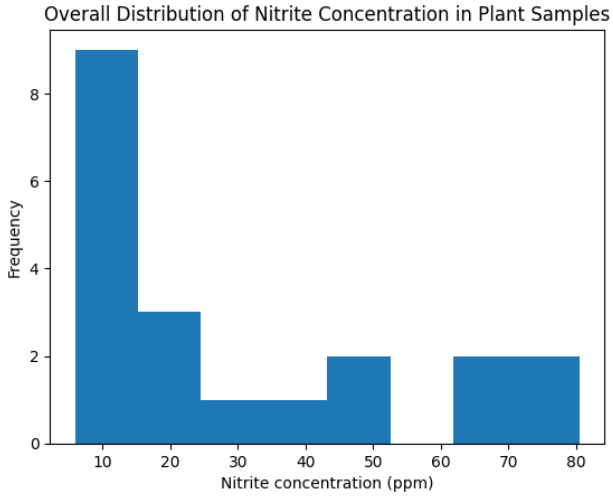
The distribution of nitrite concentrations in the analyzed samples highlights a pronounced variability among different plant products. The histogram shows a right-skewed distribution, characterized by the predominance of lower values (below 20 ppm) and the presence of a limited number of samples with high concentrations exceeding 50 ppm (Figure 4 a). This behavior indicates that nitrite accumulation is not uniform but occurs sporadically, being influenced by factors specific to each sample.

The box plot analysis confirms these observations, highlighting differences in value distribution among product categories. Fruits exhibit the highest median values along with considerable variability, while non-leafy vegetables show a strongly asymmetric distribution driven by high values in certain samples (e.g., red beetroot, carrot). In the case of leafy vegetables, median values are moderate; however, the presence of significant outliers is evident, particularly parsley, which recorded the highest nitrite concentration among all analyzed samples.

It can be observed that herbs (especially parsley) and certain root vegetables (red beetroot, carrot) exhibit the highest concentrations, which is consistent with literature data indicating an increased capacity of these plant organs to accumulate nitrogen compounds due to direct uptake from the soil and active nitrogen metabolism [3,15–16]. In contrast, fruits and vegetables with high water content (e.g., cucumber, tomato) show lower values, suggesting a dilution effect and a more limited accumulation.



(a)



(b)

Figure 4. (a) Distribution of nitrite concentration across plant product categories (fruits, vegetables, and leafy vegetables), presented as box plots indicating median values, interquartile ranges, and outliers; (b) overall distribution of nitrite concentration in plant samples, illustrated as a histogram showing the frequency of values across the analyzed concentration range.

The elevated nitrite concentrations observed in the analyzed samples may be explained by the prior accumulation of nitrates as a result of nitrogen fertilization, followed by their reduction to nitrites under the action of microorganisms and/or favorable storage and processing conditions—factors that have been shown in recent studies to significantly influence nitrite levels in plant matrices [2]. Additionally, excessive use of nitrogen-based fertilizers, as well as interspecies differences in nitrogen metabolism, may contribute to this variability, highlighting the need for careful monitoring of these compounds in plant-derived foods.

Although many studies report low nitrite levels in fresh vegetables (0.1–5 mg/kg) [5], recent literature indicates that, under certain conditions, elevated concentrations exceeding 50 mg/kg may be detected, particularly in fresh-cut products. For example, a recent study of nitrite romanian vegetable content determination developed by Burca and Indolean showed the same trend of increased values of nitrites (Carrots Romania (*Daucus carota*) - 40.3 ppm; Parsley root Romania (*Petroselinum crispum*) – 83.8 ppm; Beetroot Romania (*Beta vulgaris*) - 542.8 ppm; Lettuce Romania (*Lactuca sativa*) grown greenhouse middle leaves - 543.9 ppm and Spinach Romania (*Spinacia oleracea*) - 453 ppm) [17]. Berardi et al. [4] demonstrated that these increased levels are primarily associated with microbiological contamination, especially by bacteria from the Enterobacteriaceae family, which promote the reduction of nitrates to nitrites during storage. So, in our study the moderate nitrite concentrations recorded for spinach (17.88 ppm) and lettuce (17.74 ppm) commonly reported as major nitrate accumulators are explained by the May harvest date. Nitrate reductase activity decreases during winter due to reduced light intensity, leading to greater nitrate accumulation [3]. Conversely, the long photoperiod and high light intensity of mid-May favour active nitrate reductase expression and more complete in planta nitrate assimilation, resulting in lower residual nitrite at harvest. Unlike nitrates, for which the European legislative framework establishes maximum levels in certain leafy vegetables through Regulation (EU) 2023/915 [18], no specific limits have yet been defined for nitrites in this category of products. In this context, the presence of elevated nitrite concentrations becomes particularly relevant from a food safety perspective and supports the need for careful monitoring of these compounds, especially in processed plant products.

4. Conclusion

The study demonstrated that the spectrophotometric method based on the Griess reaction is suitable for the determination of nitrites in plant-derived products, exhibiting good linearity ($R^2 = 0.9969$) and sensitivity, as indicated by the low values of the limit of detection (LOD = 0.0056 μg/mL) and the limit of quantification (LOQ = 0.0188 μg/mL).

The results revealed a significant variability in nitrite concentrations, ranging from approximately 5.9 to 80.5 ppm, with the highest values recorded in parsley, red beetroot and carrot. Statistical analysis indicated a non-uniform and asymmetric distribution of the data, characterized by the predominance of low values and the occurrence of isolated high concentrations.

The elevated values may be associated with nitrate reduction under microbial activity and/or favorable post-harvest conditions. In the context where European legislation establishes limits only for nitrates (Regulation (EU) 2023/915), the absence of specific limits for nitrites highlights the need for their monitoring from a food safety perspective.

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Conflicts of interest / Competing interests: No conflict of interest

Ethics approval: No ethic situation.

Availability of data and material: The data have been reviewed depending on the literature.

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