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Research article

RESEARCH ON THE NITRATES AND NITRITES CONTENT IN SOME VEGETABLE SPECIES, CLUJ COUNTY, ROMANIA AND ANTWERPEN, BELGIUM

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ABSTRACT

The study presents the monitoring of nitrate and nitrite levels from some vegetable samples (lettuce - *Lactuca sativa*, carrot - *Daucus carota*, parsley root - *Petroselinum hortense*, kohlrabi - *Brasica oleracea* variety *gongyloyides*, spinach - *Spinacea oleracea*, celery - *Apium graveolens*, cabbage - *Brasica oleracea* and beetroot - *Beta vulgaris*) purchased from a local market in Cluj-Napoca, Romania, and a supermarket in Antwerp, Belgium. The analyses were performed using a laboratory molecular adsorption spectrophotometer. Based on the t-test for method evaluation, it was determined that the method is not affected by systematic errors. The nitrite concentrations recorded ranged from 8.6 µg/g for kohlrabi from Romania to 557.3 µg/g for red beet samples from Belgium.

Comparing the results from all vegetable samples, the main conclusion was that nitrates were below the maximum level, while nitrites were significantly above the maximum permitted limit according to European legislation (for lettuce and beetroot).

1.Introduction

The authors argue that the defining characteristic of this study lies in its contextual relevance to Romania's integration into the Schengen area. This process is anticipated to reduce, and ultimately eliminate, the disparities between Romania and other European Union member states across diverse spheres of activity and daily life. In this context, Romanian researchers are encouraged to prioritize comparative investigations that evaluate the extent to which conditions for vegetable and fruit cultivation align with European legislative standards, which are considerably more stringent—particularly with respect to permissible concentrations of nitrates and nitrites (Serra et al., 2024). Accordingly, the present study aims to assess and compare the nitrate and nitrite content of selected vegetables available on the markets of Cluj (Romania) and Antwerp (Belgium).

Nitrogen is the most abundant chemical element, about 80% of the Earth's atmosphere is made up of nitrogen. Nitrogen is a key element for some essential biomolecules: vitamins, amino acids, hormones, enzymes, nucleotides (Pham *et. al*., 2008; Kristin *et. al*., 2010).

Nitrates (NO_3^-) and nitrites (NO_2^-) are natural chemical compounds found in the soil, water, plants and even the human body.

Nitrite and nitrate levels in vegetables are a matter of concern due to their toxicity at high levels and nitrate high accumulation. Moreover, there is a lack of knowledge about their levels in some types of widely consumed vegetables such as lettuce, spinach, or cabbage.

Nitrates, the most oxidized form of nitrogen, have been known for half a millennium BC, before isolating them from the air, for preserving animal foods (E.F.S.A., 2008). Nitrates are natural components and important components in plants due to their potential for accumulation, they form naturally in living or decaying plants and animals, including the human body (Mensinga *et. al*., 2003; Lundberg *et. al*., 2004; Weitzberg *et. al*., 2004; Lundberg *et. al*., 2008; Lundberg *et. al*., 2006).

In Europe there is a tendency for plant nitrate concentrations to be higher in northern latitudes, especially in winter, due to low light intensity, on days with little light (McKnight *et. al*., 1999).

Genetic factors determine the accumulation of nitrates in vegetable matrices, leafy legumes and vegetables in which the consumable part is the root (beets, carrots, radishes) are plant species characterized by the highest content of nitrates. The differences in nitrate accumulation are due to low amounts of reductases (e.g. vegetables from the family *Chenopodiaceae*, family *Cruciferae*, family *Umbeliferae*, family *Compositae*), the deficiency in trace elements involved in the activity of reducing enzymes can lead to large accumulations of nitrate due to inhibition of the reaction discount. Nitrates can also accumulate if the carbohydrate content is low (Camargo *et. al*., 2006; Bryan *et.al*., 2005; Baty *et.al*., 2007).

The type of soil and the mineral content can affect the accumulation of nitrates, nitrates circulate from the soil to the root surface more frequently by convection than by diffusion, so the lack of water in the soil will restrict the intake of nitrates (Greenwood *et. al*., 1986).

Nitrate and nitrite levels in natural farming practices can be influenced by: duration and storage conditions (room temperature, cold, frozen) and food processing (washing, cleaning, bleaching or boiling). From literature data it is know that nitrite concentrations are about one hundred time lower than those of nitrate, while its toxicity is about the same order of magnitude higher (LD_{50} was 85 mg/kg for nitrite, and 3236 mg/kg for nitrate (rat oral)) (Liu et al., 2023; Tsikas, 2023) Nitrate levels in raw vegetables kept at ambient temperature may decrease during storage, but nitrite levels may increase through storage and wilting in close connection with endogenous activity specific to nitro reductase (Van Eysinga, 1984; Weightman *et. al*., 2006; AFSSA, 2007; Kanaan, 1992; Premuzic *et.al*., 2002; Filer *et. al*., 1970; Sánchez-Echaniz *et. al*., 2001; Jakszyn *et.al*., 2004).

After ingestion, the metabolic conversion of nitrate to nitrite will depend on biological factors (bacteria) and chemical factors (pH), which can react with secondary amines to produce nitrosamines (Calderón *et al*., 2025).

Numerous studies have shown that nitrate accumulation in vegetables is not determined just by plant species, but is also affected by several other important factors, such as soil type, texture, pH and organic metters from soil, fertilization regime (especially nitrogen dose, form of nitrogen – nitrates lead to more direct accumulation compared to ammonium or urea, etc), irrigation frequency and amount, climatic conditions (like light intensity, temperature, precipitations and humidity), as well as cultivation methods (e.g., open field vs. greenhouse, conventional vs. organic systems, hydroponic vs. soil, etc.). (Dodocioiu *et al*., 2025; Dezhangah *et al.*, 2022; Dung et al., 2024, Nguyen *et al.*, 2025).

The European Commission has established the maximum permitted doses for nitrates in lettuce and spinach; these maximum limits being

permanently verified. The effects of nitrates on the food safety of other vegetables are uncertain. The presence of significant amounts of nitrates in potatoes has often been found in arugula, while potatoes often contain insignificant levels of nitrates, but they are consumed more frequently and thus potatoes can be more dangerous (Pate, 1973; Andrews, 1986; Wallace, 1986).

The symbiotic fixation reaction of atmospheric molecular nitrogen is performed by bacteria of the genus *Rhizobium*, which form symbioses with the roots of leguminous plants (Phillips, 1968; Ezeagu *et. al*., 1995; Ezeagu, 1996; Chung *et. al*., 2004; Sánchez-Echaniz *et.al.*, 2001; Phillips, 1968).

The main sources of nitrogen used by plants are minerals, nitrates and ammonium can be adsorbed passively and actively (Schuster *et. al*., 1987; Bucur *et. al*., 2010; Lupea *et. al*., 2001; Cumpătă *et. al*., 2005).

Naturally, a balance is established between nitrates and nitrites in soil, water and plants, which can be broken by the intensive use in agriculture of natural organic fertilizers and especially synthetic nitrogen fertilizers. Their degradation products enrich the soil and can accumulate in cultivated plants to levels harmful to consumers (Ceaușescu, 1978; Mănescu *et.al*., 1994; Bibicu, 1994; Calancea, 2002; Banu *et. al*., 1982).

Nitrate is preferentially adsorbed by plants, the process being selective in relation to the species, metabolic processes, age, environmental factors. Nitrate adsorbed by plant root is reduced in the presence of the enzyme nitrate reductase and NADPH-H $^{+}$ to nitrites, which in turn are reduced to ammoniacal nitrogen in the presence of nitrite reductase and reduced ferredoxin. Nitrogen oxide is also enzymatically transformed into hydroxylamine, amides and amino acids (Hura, 2005).

The maximum quantity of nitrates accumulates in those parts of plants closer to the root, the nitrate content of leafy vegetables species is much higher in stems and petiole, as these organs conduct nitrate ion to the leaf tissues where the process of reducing nitric nitrogen, nitrates being transformed into organic compounds with nitrogen in the process of photosynthesis.

According to literature, vegetables contribute only 2 to 6% to the daily ratio of nitrite exposure, this proportion being exceeded by the endogenous transformation of nitrates from vegetables consumed into nitrites (Cumpătă *et. al*., 2005);

In conditions of refrigeration and freezing of vegetables, insignificant changes were found in terms of nitrate and nitrite content (Banu *et. al*., 1982).

Human exposure to nitrates is mainly exogenous through the consumption of vegetables, water from groundwater sources (Choudhary, 2025) and other foods, they are formed in a small endogenous proportion, exposure to nitrites is instead endogenous through the metabolism of nitrates. The dominant source of dietary nitrate is vegetables, as they contribute to 60–80 % of the total nitrate intake. (Vogiatzi, 2024). Some of the nitrites are consumed as a consequence of their use as food preservatives and to a lesser extent by their presence in vegetables. Nitrates and nitrites are added as preservatives to prevent the growth of bacteria *Clostridium botulinum* or as promoters of some food colors. (Parvizishad et al., 2017). Nitrate as such is non-toxic, but its metabolites remove nitrates from the category of regulated compounds as beneficial because of their potential to have undesirable effects on the body (Filer *et.al*., 1970; Sánchez-Echaniz *et. al*., 2001; Jakszyn *et. al*., 2004).

High concentrations of nitrates in plants, especially vegetables (up to 10 g in a single dose) pose a danger to the human and animal body for two reasons: the possibility of methemoglobin in children by converting nitrates to nitrites in saliva and the formation of carcinogenic nitrosamines under the influence intestinal microflora in the intestinal tract, through its reactions with amines or amides (Chung *et. al*., 2004; Sánchez-Echaniz *et. al*., 2001).

On the other hand, nitrite is a versatile chemical agent which has found numerous

applications ranging from dye manufacture to food preservation, so, the chanse of reaching human body is very high.

2. Materials and Methods

2.1. Sampling of Biological Material

The biological material analyzed in this study consisted of vegetables cultivated both in greenhouses and in open fields. Samples were sourced from specialized agricultural units with produce available for sale in local markets in Cluj (Romania) and from a store in Antwerp (Belgium), covering a range of origins and cultivation methods.

The vegetables collected from local markets included:

- Lettuce (*Lactuca sativa*), L
- Beetroot (*Beta vulgaris*), B
- Carrot (*Daucus carota*), C
- Parsley root (*Petroselinum hortense*), P
- Kohlrabi (*Brassica oleracea var. gongylodes*), K
- Spinach (*Spinacia oleracea*), S
- Celery (*Apium graveolens*), Ce
- Cabbage (*Brassica oleracea*), Cab

The Romanian samples were primarily obtained from private producers located in the vicinity of Cluj-Napoca, while the Belgian samples were collected from a retail outlet in Antwerp.

Upon collection, the vegetable samples were thoroughly cleaned to remove soil residues and stored at low temperatures appropriate for preserving vegetable freshness. 10 grams of each sample were weighed and homogenized using a mortar and pestle. The homogenized material was transferred to a 50 mL volumetric flask. For stabilization and preparation, 25 mL of saturated borax solution was added to each flask containing the ground plant sample. The saturated solution was prepared by dissolving 50 g of $Na_2B_4O_7 \cdot 10H_2O$ in 500 mL of hot distilled water, adding the solid until no further dissolution occurred. The flasks with the added solution were then placed in a boiling water bath and heated for 15 minutes. After cooling, 1 mL of potassium ferrocyanide solution and zinc acetate was added to each sample. The mixtures were then left to stand for 30 minutes. Finally, the samples were brought to volume with distilled water, labeled accordingly, and filtered for further analysis.

2.2. Determination of Nitrate and Nitrite Ion Content in Vegetables

The concentrations of nitrate and nitrite ions in the prepared vegetable samples were determined using molecular absorption spectrometry. The analysis was performed at a wavelength of 520 nm using the Griess reagent for nitrites and the Cd-Griess method for nitrates, in accordance with the Romanian standard STAS 11581-83.

A SHIMADZU UV-Visible spectrophotometer (Model No: UV-2550) equipped with 1 cm path length quartz cuvettes was employed for absorbance measurements.

All analyses were carried out between March and November 2024.

2.3. Analyses

2.3.1. FTIR

Fourier Transform Infrared (FTIR) spectroscopy is a valuable analytical technique for characterizing the chemical composition of vegetables. It identifies molecular vibrations, providing insights into compounds like polysaccharides, proteins, lipids, and secondary metabolites.

FTIR results can be complemented by microscopy methods, which can visualize structural aspects of leafy vegetables contributing to nitrate accumulation. Through this technique, various species of vegetables can be evaluated and, eventually, the presence of nitrate groups accumulated in the plant cells of the samples can be identified.

The FT-IR absorption spectra were recorded with a Jasco 6000 spectrometer, at room temperature, in the range 400 ‑ 4000 cm^{-1}, with a spectral resolution of 4 cm^{-1} and using the well-known KBr pellet technique.

2.3.2 Microscopy analyses

Optical microscopy techniques are invaluable for analyzing the structural and compositional aspects of vegetables.

In general, optical microscopy methods enhance the researcher's abilities to analyze and ensure the quality and safety of vegetables in both research and industry settings.

Optical microscope Motic BA310Pol was used to obtain images of analysed vegetables surfaces.

Optical Microscopy in vegetables analysis is utilized to identify histological elements such as epidermis with stomata and trichomes, thin cuticles, large vacuolated cells, cortex rich in chromoplasts (carotenoid or chlorophyl pigments, for example), parenchyma cells containing essential oils, etc.

It provides parameters to assess inadequate conditions and practices during food production.

2.4. Statistical parameters used to evaluate the analytical performance of the nitrite analysis method, ANOVA (one-way) test

For an analysis method to be used for trace level quantification, it must present a series of analytical performance characteristics, among which the absence of systematic errors, accuracy, precision, LOD and LOQ, the response range occupies a central place.

Even if the analysis method has already been validated, the analytical performance characteristics must be checked because they may differ depending on the conditions (apparatus, reagents) in the laboratory where the experiments are carried out.

The verification is carried out using synthetic samples of known rice concentration (as close as possible to the matrix characteristics of the real samples), or certified reference materials and are expressed by means of some statistical variables (Thoma *et. al.,* 2012; Habib, 2011, Hsu, *et. al*., 2009; Gemperline, 2006; International Standard ISO 6635-1984).

The test consists of the following: prepare at least 10 samples of analytical same concentration, measure a property of the analyte or one of these compounds, determine the concentration based on the calibration curve.

The average of the determinations (where n is number of determination), absolute error (e) and standard deviation ($s_{\bar{C}}$) of the selection mean were calculated with the following relations:

$$\bar{C} = \frac{\sum_{1}^{10} C_i}{10} \quad (1)$$

$$e = \left| C_A - \bar{C} \right| \quad (2)$$

$$s_{\bar{C}} = \sqrt{\frac{\sum_{1}^{n} \left(\bar{C} - C_i \right)^2}{n(n-1)}} \quad (3)$$

The value of the variable t is calculated with the relation *(4)* (Gemperline, 2006):

$$t = \frac{\left| C_A - \bar{C} \right|}{s_{\bar{C}}} \quad (4)$$

If the calculated t is less than t tabulated for the same number of degrees of freedom (k = n-1) and a chosen probability, it is concluded that the method is not affected by systematic errors, it is accurate. The accuracy of the method reflects the deviation of the average of the determined values from the value considered true and is expressed by error (module of the difference between the value considered true and the average of the determinations) or the relative error expressed by the relationship *(5)*:

$$e\% = \frac{\left| C_A - \bar{C} \right|}{C_A} \times 100 \quad (5)$$

Precision is a characteristic of performance that reflects the extent to which the method provides repeatable results.

From a statistical point of view, the precision is expressed by the standard selection deviation (s), the standard deviation of the selection mean, $s_{\bar{C}}$, or the relative standard deviation (RSD). For the calculation of variables, either the results of measurements made on the same day

(repeatability) or the results of measurements carried out in successive days (reproducibility) can be used. The relative standard deviation is expressed by the relation (Gemperline, 2006):

$$RSD = \frac{s_1}{\bar{C}} \times 100 \quad (6)$$

LOD and LOQ are two parameters of analytical performance that express the detection and determination characteristics of an analysis method. For the evaluation of LOD and LOQ, the literature describes several variants of which most often the variant of repeated measurements on blank samples is used. In the case of spectrophotometric methods, the minimum signal is calculated using the absorbances measured for blank samples with the following relation (Gemperline, 2006):

$$A_{LOD} = \bar{A}_{blanc} + 3 \times s \quad (7)$$

$$A_{LOQ} = \bar{A}_{blanc} + 10 \times s \quad (8)$$

where:

$\bar{A}_{blanc}$ - the average of the absorbances A_i measured for the blank samples;

s - standard deviation selection of the measured absorbances for the blank samples.

The Anova test (one-way) is usually used to compare the averages of more than two sets of values. If n is the number of data series and m is the total number of values, the test consists of the following: it is calculated: variance of each data series (SSW – sum of squares within the group), variance between data series (SSB- sum of squares between the groups) variance of all values in the considered series (TSS- total sum of squares).

$$R_1 = \frac{SSB}{n-1} \quad (9) \qquad R_2 = \frac{SSW}{m-n} \quad (10)$$

$$F_{calc.(n-1;m-n)} = \frac{R_1}{R_2} \quad (11)$$

F_{calc} is compared. with F_{tab}. (n-1, m-n), $p<0.05$. If $F_{calc}<F_{tab}$ the null hypothesis is admitted, i.e. between the averages of the data series there is no statistically significant difference.

The research carried out aimed to establish the accumulation of nitrate ions and nitrite ions in the organs of greenhouse and field vegetables species in Romania and Belgium, comparing the determined content with the limits imposed by the Romanian legislation in force.

3. Results and discussion

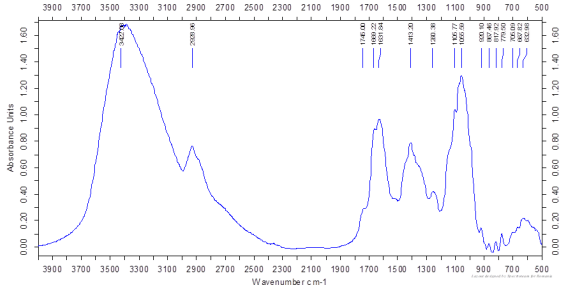
3.1. Fourier Transform Infrared (FTIR) spectroscopy is a useful tool for analyzing nitrate (NO_3^-) and nitrite (NO_2^-) contents in vegetables.

An FTIR study was performed on seven of the eight Romanian plant samples (see Figure 1a-g) in order to identify the functional groups present in plant cell structures that may be involved in the accumulation of nitrates and nitrites. The beet sample was excluded, as its intense red pigmentation interfered with the acquisition of a reliable spectrum.

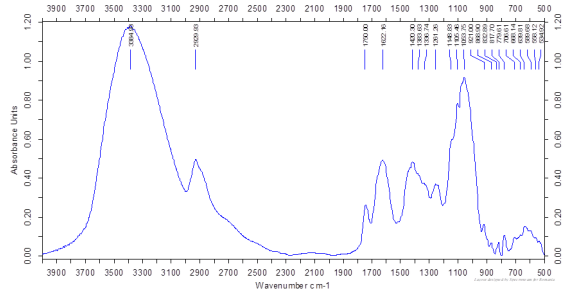
The scientific literature shows that the characteristic absorption bands for nitrate group is one strong absorption around 1380 – 1410 cm^{-1} (asymmetric stretching of NO_3^-) and for nitrite groups (NO_2^-) are characteristic the peaks around 1260–1320 cm^{-1} (asymmetric stretching) and 820–860 cm^{-1} (bending vibration) (Shao et al., 2017, Ma et al, 2021)

Figure 1(a–g) presents the FTIR images of the vegetable surfaces for lettuce (a), carrot (b), parsley root (c), kohlrabi (d), spinach (e), celery (f), and cabbage (g).

In the case of present work, the characteristic nitrate absorption, observed in the range of 1500–1200 cm^{-1}, is evident in all the samples, with prominent peaks appearing around 1401 cm^{-1} and 1350 cm^{-1}.



(a)



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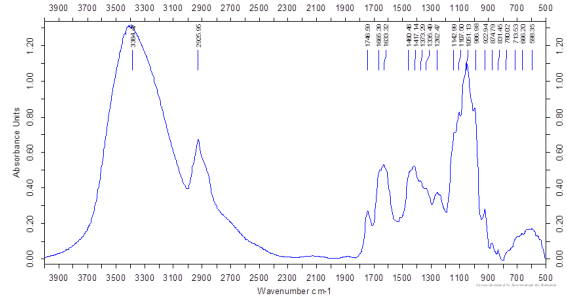

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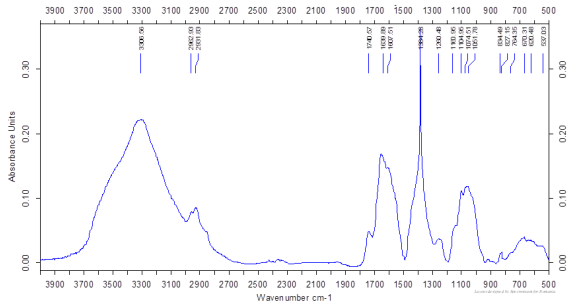

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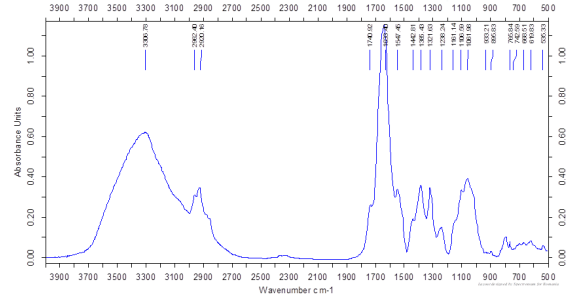

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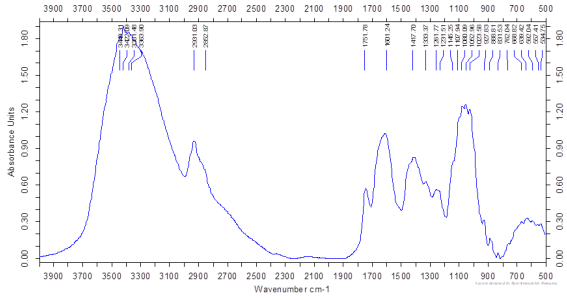

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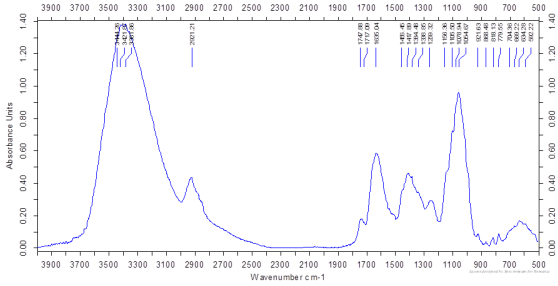


(g)

Figure 1(a–g). The FTIR images of the vegetable surfaces for lettuce (a), carrot (b), parsley root (c), kohlrabi (d), spinach (e), celery (f), and cabbage (g).

These absorption bands correspond to the asymmetric stretching mode of the N–O bond (ν_3), which results from the splitting of the ν_3 mode into two bands, designated as ν_3-high and ν_3-low (Shaviv et al, 2003).

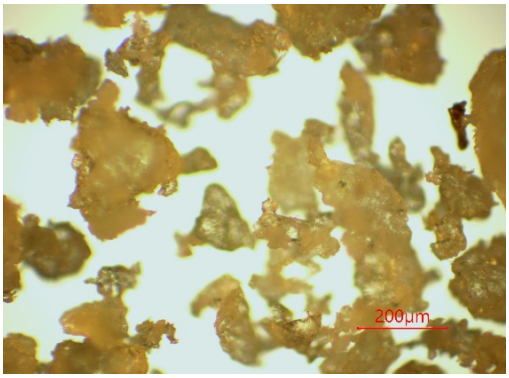
Additionally, a peak at 1245 cm^{-1} is associated with nitrite (NO_2^-) absorption.

The peak near 1460 cm^{-1} corresponds to N=O vibrations, while the peaks at 1375 cm^{-1} and 1363 cm^{-1} are attributed to N=O and N–O vibrations, respectively. A band at approximately 1300 cm^{-1} is also assigned to N–O stretching and are in good agreement with literature data (Shao et al, 2017, Shaviv et al., 2003).

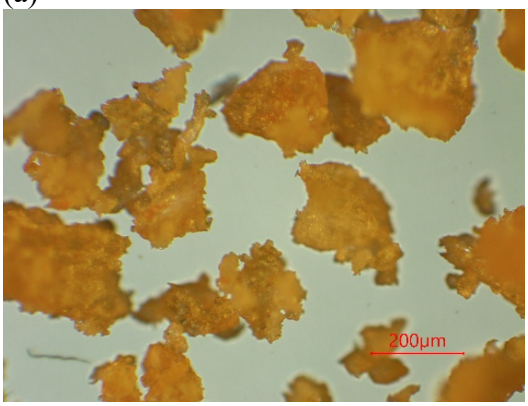
3.2. Optical Microscopy Investigations

For the same seven plant samples (out of the eight Romanian ones), an optical microscopy study was also carried out to visualize their structural features. And, in this case, the beet sample was excluded from the optical microscopy study because the intense red pigmentation of the biological material completely covered the surface of the vegetable, thus preventing image acquisition through microscopy.

Figure 2a-g shows that the vegetable tissues samples have roughness structures, with large vacuoles, where nitrates can be easily stored.



(a)



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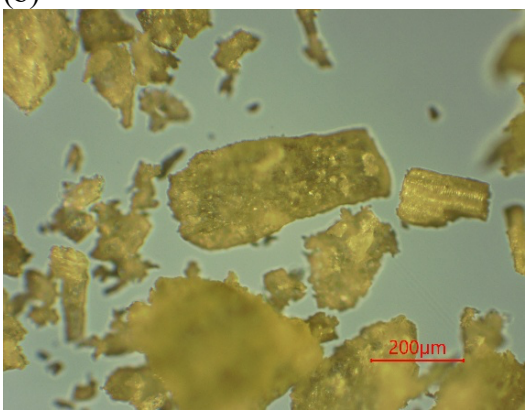

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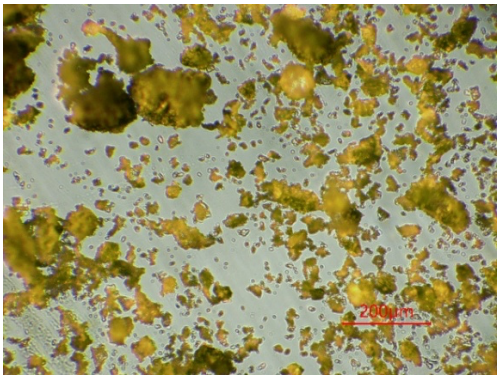

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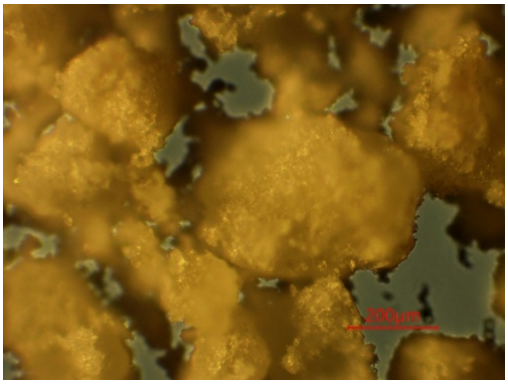

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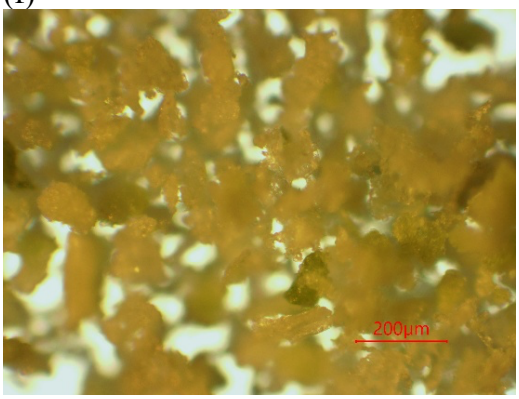

(g)

Figure 2. Optical microscope images of the vegeatables surfaces of lettuce (*Lactuca sativa*) (a), carrot (*Daucus carota*) (b), parsley root (*Petroselinum hortense*) (c), kohlrabi (*Brasica oleracea* variety *gongyloyides*) (d), spinach (*Spinacea oleracea*) (e), celery (*Apium graveolens*) (f), and cabbage (*Brasica oleracea*) (g)

As is well known, nitrates are primarily stored in the vacuoles of parenchyma cells, particularly within the leaves and roots of plants. Although optical microscopy does not allow for

the direct detection of nitrate or nitrite ions, it remains a valuable tool for examining the anatomical compartments where these ions are most likely to accumulate.

Certain vegetables are especially prone to nitrate accumulation due to their physiology and cultivation conditions.

Lettuce *(Lactuca sativa)* is well known for its high nitrate content, especially under conditions of low light intensity or excessive nitrogen fertilization.

Parsley root *(Petroselinum hortense)* may also accumulate significant nitrate levels, depending on fertilization practices and soil characteristics.

In the case of spinach *(Spinacia oleracea)*, nitrate accumulation is often extremely high, particularly within the spongy mesophyll parenchyma. In some instances, nitrate concentrations in spinach can exceed 2,500 mg/kg fresh weight.

3.1. Evaluation of Optimal Quantification Conditions and Performance Characteristics of the Nitrite Analysis Method

The maximum sensitivity of the spectrophotometric method is achieved when measurements are carried out at the optimal wavelength corresponding to the highest molar absorptivity. Under these conditions, the extinction coefficient reaches its maximum value, and the slope of the calibration curve is maximized, ensuring improved analytical sensitivity.

Based on the absorption spectrum obtained using the Griess reagent, the optimal wavelength for nitrite determination was established at 525 nm.

A calibration curve was constructed in the concentration range of 0–0.3 mg/L, using standard nitrite solutions. The spectrophotometer software was used to automatically plot the calibration curve through its "Quantitative" option, facilitating subsequent concentration determinations.

The resulting calibration line is presented in Figure 3, and its linearity confirms the reliability of the method within this range.

The equation of the calibration line is:

$$A=0.91C+0.1 \qquad (12)$$

The calibration curve demonstrates a linear relationship between absorbance and nitrite concentration within the investigated range (0–0.3 mg/L).

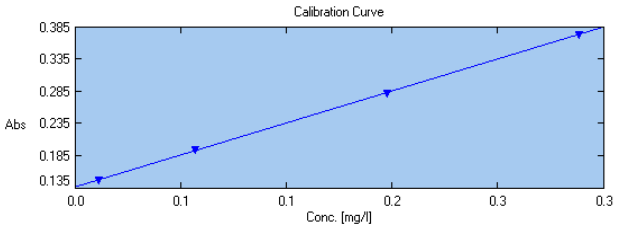

Figure 3. Calibration curve for the determination of nitrite ion concentration using the Griess spectrophotometric method (λ = 525 nm, concentration range: 0–0.3 mg/L).

The coefficient of determination (R^2 = 1.000) indicates perfect linearity, confirming that 100% of the variation in absorbance is attributable to changes in concentration.

This result also suggests a high degree of precision and the absence of systematic errors in the measurement process.

A summary of the statistical analysis of the calibration data is presented in Table 1.

Since the calculated value of the *t*-statistic is lower than the critical (tabulated) value at the chosen confidence level and number of replicates, it can be concluded that the method is not affected by systematic errors and is therefore accurate.

The accuracy of the method, evaluated in terms of relative error, was found to be 2%, which demonstrates that the measured values are in close agreement with the reference value and confirms the reliability of the procedure for real sample analysis.

The standard deviation was calculated as $s = 7.03\times10^{-3}$, and the corresponding relative standard deviation (RSD) of 3.44% reflects a satisfactory level of precision, indicating that replicate measurements are consistent and free

from significant variability. Taken together, these findings confirm that the method not only produces accurate results but also ensures reproducibility within the generally accepted limits for spectrophotometric quantification at this concentration level. This combination of accuracy and precision highlights the robustness of the method and supports its suitability for routine analytical applications

The limits of detection (LOD) and quantification (LOQ) were evaluated, and the results are summarized in Table 2.

The determined LOD and LOQ values are well below the expected nitrite concentrations in real vegetable samples, thereby confirming the method's suitability for the analysis of nitrite ions in actual biological matrices.

Table 1. Data obtained in the determination of variable t.

C_i (µg NO_2^- / mL)	$\bar{C}$ (µg NO_2^- / mL)	C_A (µg NO_2^- / mL)	$s_{\bar{C}}$	e	$t_{calc(9.99\%)}$	$t_{tab(9.99\%)}$
0.208						
0.201						
0.195						
0.208						
0.197	0.204	0.200	2.22×10^{-3}	4×10^{-3}	1.80	2.82
0.206						
0.210						
0.208						
0.208						
0.201						

Table 2. Results obtained in the determination of LOD and LOQ.

A_i	$\bar{A}$	s	A_{LOD}	A_{LOQ}	LOD (µg NO_2^- / mL)	LOQ (µg NO_2^- / mL)
0.108						
0.106						
0.101						
0.101						
0.098	0.101	3.57×10^{-3}	0.112	0.137	0.011	0.039
0.099						
0.101						
0.099						
0.101						
0.097						

Table 3. Results obtained when determining nitrite ion concentration in Romanian cabbage.

Sample type	Sample weight (g)	Concetration (µg NO_2^-/g)	$\bar{C} \pm t\times s_{\bar{C}}$, (µg NO_2^-/g)	$t_{(3.99\%)}$
(a)	10.8721	2.03		
	10.5585	1.98	**1.99±0.06**	
	10.5645	1.97		
	10.8791	2.01		
(b)	10.3988	1.95		
	10.1689	1.92		

	10.1893	1.89	**1.91±0.06**	4.54
	10.3241	1.91		
(c)	10.5035	1.83		
	10.1486	1.84	**1.86±0.06**	
	10.4123	1.89		
	10.1233	1.86		

Table 4 ANOVA test results (nitrite ion concentration in Romanian cabbage).

SSW	SSB	TSS	$F_{tab.(3.44)}$,p<0.05	$F_{calc.(3.44)}$,p<0.05
0.0097	0.0375	0.0472	4.26	17.39

Table 5. Results obtained when determining nitrite ion concentration in Belgium cabbage.

Sample type	Sample weight (g)	Concetration (µg NO_2^-/g)	$\bar{C} \pm t \times s_{\bar{C}}$, (µg NO_2^-/g)	$t_{(3.99\%)}$
(a)	10.7721	1.83		
	10.5535	1.78	**1.81±0.09**	
	10.5145	1.77		
	10.8791	1.85		
(b)	10.6221	1.82		
	10.5689	1.81	**1.80±0.05**	
	10.6893	1.77		4.54
	10.3471	1.79		
(c)	10.4129	1.76		
	10.0842	1.75	**1.78±0.07**	
	10.5123	1.78		
	10.5439	1.82		

Table 6. ANOVA test results (nitrite ion concentration in Belgium cabbage).

SSW	SSB	TSS	$F_{tab.(3.44)}$,p<0,05	$F_{calc.(3.44)}$,p<0,05
0.0089	0.0020	0.0109	4.26	1.01

3.2. Determination of Nitrite Ion Concentration in Cabbage Samples

The nitrite ion concentrations measured in Romanian cabbage are summarized in Table 3.

To assess the statistical significance of the differences among mean values, a one-way ANOVA test was performed. The results of the ANOVA analysis are presented in Table 4.

Since the calculated F-value exceeds the critical (tabulated) value, it can be concluded that the differences between the means are statistically significant. This suggests that removal of the outer leaves before consumption may lead to a reduction in nitrite intake, potentially improving the safety of the vegetable for human consumption.

The results for cabbage samples obtained from a Belgian source (Antwerp) are presented in Table 5, and the corresponding ANOVA outcomes are summarized in Table 6. In this case, the calculated F-value is lower than the critical value, indicating no statistically significant differences between the means.

These findings imply that the nitrite ion concentrations in cabbage from the Belgian and Romanian producers are comparable, and no significant variation is observed between different parts of the cabbage from the Belgian source.

On the other hand, the result of the ANOVA test for the Belgian sample suggests that, for this source, the outer leaf layer should not be

removed prior to preparation, as its removal does not significantly influence the nitrite ion concentration.

3.3. Determination of Nitrate and Nitrite Content in Root and Leafy Vegetables

Samples of carrots (C), parsley (P), beetroot (B), celery (Ce), kohlrabi (K), lettuce (L), spinach (S) and cabbage (Cab) purchased from markets in Romania and Belgium were analyzed.

Each vegetable was cut into pieces, weighed (approximately 10 g), and ground according to the procedure described in the experimental section.

As shown in Table 7, the nitrite concentrations obtained after analysis were generally within the maximum permitted limit of 200 µg/g, as specified in Order No. 84/91/2002 issued by the Ministry of Health and Family (MSF) and the Ministry of Agriculture, Food and Forestry (MAPM). However, the nitrite concentrations recorded for beetroot and spinach samples exceeded the maximum allowed limit by approximately eight times (see Table 7 and Figure 4).

As shown in Table 7, carrot samples from Belgium contain higher levels of nitrates 88.09 µg NO_3-/g comparred to 68.5 µg NO_3-/g (samples from Romania) whereas the nitrite content is nearly identical across samples (42.0 µg NO_2-/g, in the case of Belgian samples and 40.3 µg NO_2-/g, for Romanian samples).

Regarding the Belgian samples of parsley roots, the nitrate content value is 28.3 µg NO_3-/g, compared with 26.4 µg NO_3-/g for Romanian parsley. The nitrite content is also quite close (83.8 µg NO_2-/g, for Romanian samples compared to 81.2 µg NO_2-/g).

The Romanian beetroot samples accumulates smaller amount of nitrates than the samples of Belgian beet (137.4 µg NO_3-/g comparred to 187.5 µg NO_3-/g, Table 7).

The root of Romanian celery shows a slightly higher amount of nitrates than samples of Belgian celery. (17.6 µg NO_3-/g comparred to 17.0 µg NO_3-/g) just like the nitrite content (49.8 µg NO_3-/g at Romanian samples comparred to 47.9 µg NO_3-/g for Belgian samples).

Romanian Kohlrabi accumulates a larger amount of nitrates compared to the Belgian one (38.2 µg NO_3-/g comparred to 23.8 µg NO_3-/g) , as is the case with the nitrite content in the Kohlrabi root.

Similar observations can be made regarding the nitrate content in lettuce, spinach, and cabbage, both for the Romanian and Belgian vegetable samples (see Table 7).

Spinach leaves are known to accumulate high levels of nitrates, which is why spinach receives particular regulatory attention from the European Community.

Overall, the nitrite concentrations measured across the analyzed vegetable samples showed considerable variation, with several values exceeding legal limits.

Recorded concentrations ranged from 8.6 µg/g for kohlrabi from Romania to 557.3 µg/g for red beet samples from Belgium.

The differences are, however, quite small, which indicates that the types of soil in which the studied vegetables were grown are similar and probably comparable amounts of nitrogen fertilizers were used in both types of samples (Romanian and Belgian).

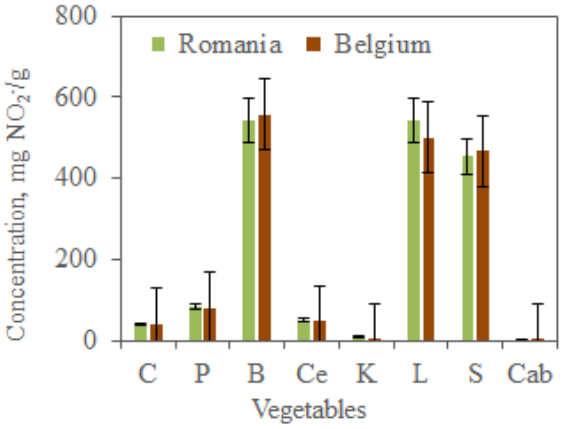

Figure 4. Nitrite content of root vegetables analyzed: carrot, parsley, beet, celery, kohlrabi, lettuce, spinach and cabbage (from Romania and Belgium).

Table 7. Results obtained (average of three determinations) in the nitrate and nitrite vegetable content determination.

Species	The analyzed organ	Nitrates (µg NO_3-/g)	Nitrites (µg NO_2-/g)
Carrots Romania (*Daucus carota*)	central cylinder	68.5	40.3
Carrots Belgium *(Daucus carota*)	central cylinder	88.09	42.0
Parsley root Romania (*Petroselinum crispum*)	central cylinder	26.4	83.8
Parsley root Belgium (*Petroselinum crispum*)	central cylinder	28.3	81.2
Beetroot Romania (*Beta vulgaris*)	pulp	137.4	542.8
Beetroot Belgium (*Beta vulgaris*)	pulp	187.5	557.3
Celery Romania (*Apium graveolens*)	pulp	17.6	49.8
Celery Belgium (*Apium graveolens*)	pulp	17.0	47.9
Kohlrabi Romania (*Brassica oleracea,-gongyloides*)	petiol	38.2	8.60
Kohlrabi Belgium (*Brassica oleracea-gongyloides*)	petiol	23.8	2.56
Lettuce Romania (*Lactuca sativa*) grown greenhouse	middle leaves	98.3	543.9
Lettuce Belgium (*Lactuca sativa*) grown greenhouse	middle leaves	94.9	500.08
Spinach Romania (*Spinacia oleracea*)	leves	198.4	453
Spinach Belgium (*Spinacia oleracea*)	leves	193.4	468
Cabbage Romania (*Brassica oleracea*)	leves	30.5	1.99
CabbageBelgium (*Brassica oleracea*)	leves	29.6	1.81

4. Conclusions

The Regulation (EU) 2023/915 has established maximum levels for nitrates in vegetables, in particular green leafy vegetables such as spinach (2000–3500 mg/kg), lettuce (2000–5000 mg/kg) and rucola (6000–7000 mg/kg). This Regulation has also a limit of 200 mg/kg for nitrates in baby food and processed cereal-based food for infants and young children [Commission Regulation (EU) 2023] (Vogiatzi et al., 2024)

Comparing these data — endorsed by EU-accredited bodies — with the analyses presented in this article shows that nitrate levels in Romanian vegetables, as in Belgian ones, comply with the applicable nitrate/nitrite regulatory limits.

The work aimed to carry out a monitoring study on the concentration levels of nitrates and nitrites in eight vegetable species commonly found in a market in Cluj Napoca, Romania, and four vegetable species purchased from a supermarket in Antwerp, Belgium.

This comparative approach represents a strong point of the current research, with the observation that the chosen analytical methods (FTIR spectroscopy and Optical microscopy) can be enriched with others, such as SEM, high-pressure liquid chromatography (HPLC),

Following the analysis of the experimental data, a relatively high concentration of nitrate ions was found in root vegetables, especially in red beet from Romania with a concentration of 542.8 µg NO_3^-/g and red beet from Belgium, for which a concentration of nitrate ions of 557.3 µg NO_3^-/g was recorded.

The leafy legumes also showed significant nitrate concentrations, so for the Romanian

spinach a concentration of 198.4 µg NO_3^-/g was registered and for the Romanian salad, produced in the greenhouse, a nitrate ion concentration of 98.3 µg NO_3^-/g was determined.

The differences between the concentrations of nitrate ions recorded for the same species, but in different locations - Romania and Belgium - are due to differences in temperature in the growing areas, light intensity, cultivation methods, chemical, physical, and geological characteristics of the soil, as well as varying amounts of soil moisture, etc.

On the other hand, the nitrite content in all samples was low - below the permissible values in the two European states and, therefore, is unlikely to pose any health threat. However, to maximize the health benefits of vegetable consumption, measures must be taken to reduce exposure to nitrates and nitrites, while maintaining the recommended intake of vegetables for the general population.

Our study presents a series of limitations due to the lack of cultivation data, among which the most important is that we did not evaluate with certainty how much seasonality affects nitrate levels in vegetables. Therefore, research could be continued by statistically analyzing summer and winter crops.

In conclusion, in order to increase the health benefits through the consumption of vegetables and fruits, it is imperative to take measures for intake reduction of nitrates and nitrites, maintaining the recommended green vegetables consume, for the good health of modern humans.

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