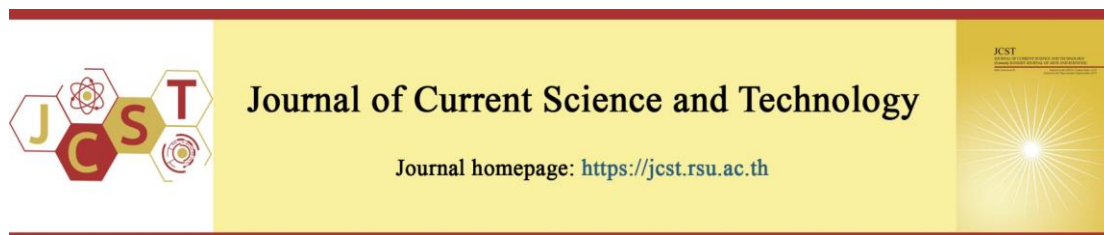


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Simple coating of zinc powder on foam tape based on a downscaling procedure for nitrate determination in vegetable samples

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Abstract

Zinc-Coated Foam Tape (ZCFT) was combined with the Griess reagent for nitrate determination. A portable chip of zinc powder coating was applied to double-sided PU foam tape with side dimensions of 1x1 cm and approximately 7.7 ± 0.4 mg of zinc. The linear range of the proposed method was 2-10 mg L⁻¹, and the obtained percent of nitrate reduction was 23-25%. The real sample application was studied in the determination of nitrate in vegetable samples. The results showed good agreement with the reference method (the NitraVer®5 nitrate reagent powder pillows). Simple detection can be set up using a digital microscope with the ImageJ program for image processing and evaluation. The proposed method, which offers the advantages of simple operation and low cost, can be used as rapid test kit to monitor the nitrate residue in vegetables.

Keywords: downscaling; Griess reaction; nitrate determination; nitrate reduction; zinc powder.

1. Introduction

Today, the consumption of vegetables is widespread among healthy people. The amount of nitrate residue in vegetables depends on the biological properties of the plant, daylight intensity, the type of soil, temperature, humidity, seeding density, plant maturity, vegetation period, harvesting time, size of the vegetable unit, storage time, and the source of nitrogen (Colla, Kim, Kyriacou, & Roupael, 2018). The content of inorganic nitrate (NO₃⁻) in certain vegetables can provide a physiological substrate for the reduction of nitrite (NO₂⁻), nitric oxide, and other metabolic products (NO_x) that cause vasodilation, decrease blood pressure, and support cardiovascular function (Ma, Hu, Feng, & Wang, 2018)

Many methods have been developed for nitrite measurement. The most widely used methods are colorimetry and spectrophotometry, which are based on the Griess reaction (Murray et al., 2017; Pai, Su, Lu, Chou, & Ho, 2021). Other methods have also been reported, such as ion chromatography (Guo et al., 2017), capillary electrophoresis (Martínková, Křžek, & Coufal, 2014), electrochemistry (Li et al., 2020), and spectrofluorometry (Ma, Li, Hu, Cai, & Dou, 2020). These traditional methods provide the highest accuracy and precision, are suitable for laboratory use, and are inexpensive. However, the colorimetric method is necessary for field screening tests. Moreover, the colorimetric method is less complicated and requires no need for power.

The primary process of nitrate reduction partly uses cadmium (Cortas, & Wakid, 1990), which is a toxic metal. An alternative process involves using nitrate reductase (NaR), a natural reducing agent (Campbell, 1999). This reduction is not available for many reasons, including the required resources, stability, and consistency of activity. However, nitrate reductase (NaR) has been described for an automated system where a commercial corn leaf NaR was employed (Patton, Fischer, Campbell, & Campbell, 2002).

Zinc powder, a less toxic agent, is a potential choice for nitrate reduction. Some reports have attempted to employ this metal in a mixed powder with zinc-manganese (Mir, 2007), zinc powder (Murray et al., 2017; Jaikang, Wangkarn, Paengnakorn, & Grudpan, 2019) and zinc granules in a micro column of flow injection analysis (Ellis, Shabani, Gentle, & Mckelvie, 2011). Recently, researchers have reported coatings on a filter paper (Ferreira, Mesquita, & Rangel, 2020) and zinc-powder-doped tapioca-starch thin film (Choodum, Tiengtum, Taweekarna, & Wongniramaikul, 2020). However, in previous reports, employing zinc powder reduction has required complicated processes of coating, weighing, or transferring. These can affect the consistency of the trace amounts of zinc and the reaction rate, especially in an equilibrium.

The Griess reaction is a technique that involves employing a sulfanilamide in an acid solution of nitrite with diazotization, resulting in the formation of a reactive diazonium salt. Upon the addition of an electron-rich coupling agent (e.g., N-(1-naphthyl)ethylenediamine (NED)), this diazonium salt reacts to produce a highly colored diazo dye (Váradi et al, 2019).

In this study, we proposed a method for nitrate analysis with a simple coating, consistency, and rapid reduction using a zinc powder instead of a cadmium particle or a nitrate reductase enzyme to reduce nitrate to nitrite. The proposed method was developed using a simple coating of zinc powder and double-sided foam tape as a coating supporter of the trace amount zinc powder for nitrate reduction with a Griess reagent. Then, the color development was measured via digital microscope and image processing. The vegetable sample preparation involved hot water extraction and interference illuminating with a syringe filter. This method demonstrated rapid consistency, simple coating and use, downscaling, and uncomplicated

processes with low cost for nitrate monitoring in the vegetable sample.

2. Objectives

The objective of this study was to study the efficiency of the zinc powder coating on double-sided foam tape for nitrate reduction.

3. Materials and methods

3.1 Chemicals

All chemicals used within this work were of analytical-grade purity. The Griess reagent was prepared with N-(1-Naphthyl) ethylenediamine dihydrochloride (NED). Hydrochloric acid 37 %w/w was purchased from Fluka (Swiss, Switzerland). The sulfanilamide powder was prepared from the commercial Thailand drug that contained 99.3 %w/w. The nitrate and nitrite standard solution (1000 mg L⁻¹) was precisely prepared from sodium nitrite and sodium nitrate, which were also purchased from Fluka (Swiss, Switzerland). Zinc powder with a maximum particle size of 150 microns and a 99.9 % purity was purchased from Sigma-Aldrich (Gillingham, UK). NitraVer® 5 Nitrate Reagent Powder Pillows (Colorado, USA) was used to determine the MR and HR nitrate in the actual sample. The foam tape was made of high-quality PU plastic, making it more durable than the general foam imported from the USA. It was coated with acrylic adhesive, allowing it to stick with high holding power.

3.2 Solution and reagent preparation

The A solution (1% w/v sulfanilamide) was prepared by dissolving 1 g of sulfanilamide in 10 mL of 37 %w/w hydrochloric acid and 60 ml with MilliQ water, for a total solution of 100 ml. The B solution (0.1% w/v NED) was prepared by dissolving 0.1 g of N-(1-Naphthyl) ethylenediamine dihydrochloride in 100 mL of MilliQ water. The Griess (NED) reagent solutions were prepared by mixing A and B solutions in a 1:1 ratio.

3.3 Sample preparation

First, 5 g of vegetable samples were blended and extracted with 5 ml of hot water in the transport tube. Then, the syringe filter with nylon material was employed to eliminate interference in the sample. Next, 1 mL of the sample solution was mixed with 1 mL of the NED reagent and added to one piece of the zinc-coated foam tape (ZCFT) for 90 seconds.

3.4 Instrumentations

The Agilent Cary 60 UV-Visible spectrophotometer (Agilent, CA, USA) was implemented with 10 mm quartz cells to record the absorption spectra and measure absorbance. The Hach DR 4000 U Spectrophotometer UV/VIS (HACH, Loveland, USA) was employed for the performance test. DANIU HD 2.0MP 1000X3 in 1 USB, 1920x1080 pixel resolution (China) was used for the microscopic imaging. The Bal-CX220 (Hong Kong) analytical balance was chosen with the exact weighing of 0.1 mg (0.0001g) readability.

3.5 Zinc-coated foam tape (ZCFT) chip preparation

The chip of zinc-coated foam tape (ZCFT) was prepared using double-sided foam tape which was made of high-quality PU plastic and coated with acrylic adhesive sticking (thickness 1.6 mm), as shown in Figure 1. The foam tape supporter was precisely cut with side dimensions of 1 x 1 cm and coated with zinc dust by mixing and shaking. Then, the homogeneous coating of each ZCFT chip was checked by employing microscopic imaging and the image processing program (Figure 2).

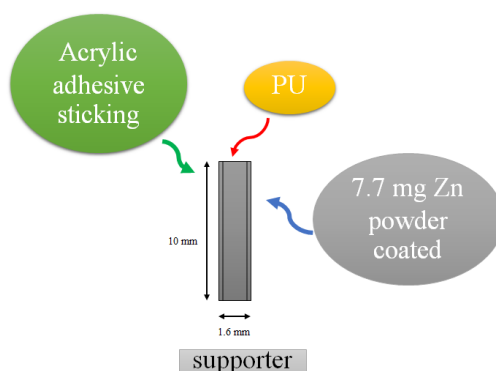


Figure 1 A side view of the zinc coated foam tape (ZCFT)

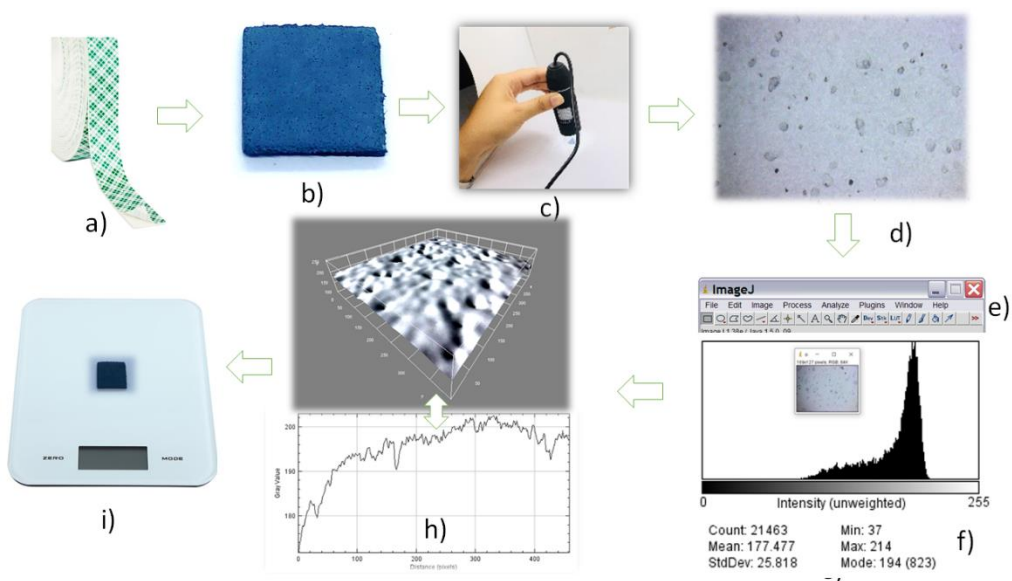


Figure 2 Testing method for the homogeneous zinc-coated foam tape preparation: a) foam tape, b) zinc-coated foam tape, c) digital microscope photograph, d) image of 1000X, e) image J program, f) 3D plot, g) histogram, h) plot profile, and i) weighing of the zinc-coated foam.

The combination of the sample solution, Griess reagent, and zinc powder was established by mixing the sample solution and Griess reagent reaction (Murray et al., 2017; Pai et al., 2021), the ZCFT chip for one piece/sample was added, and the solution was shaken by inverting the tube (2-3 times) and waiting for 90 seconds, as shown in Figure 3. The samples were transferred into a cuvette and the color was detected with a spectrophotometer at an absorbance of 540 nm or color chart. The reaction mechanisms for the reduction of nitrate to nitrite using zinc, and the subsequent detection of nitrite employing the Griess

assay, are highlighted in Eq. (1) and shown in Figure 3.



The proposed formula evaluated the final concentration:

$$\text{Final concentration (mg NO}_3\text{-N/Kg)} = [\text{mg L}^{-1} \times \text{DF} \times \text{FV}] / \text{WT},$$

mg L⁻¹ obtained from the calibration; DF = dilution factor (2 folds); FV = final volume (5 mL); WT = Weight (5 g)

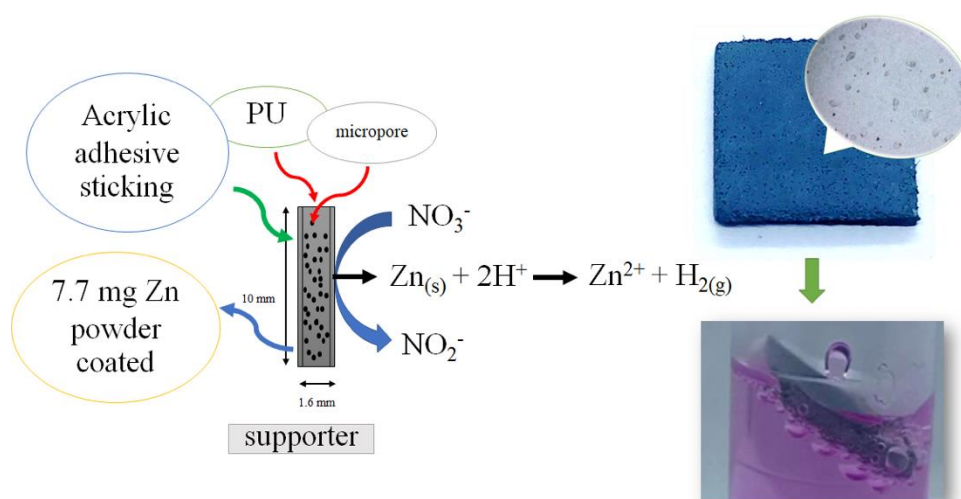


Figure 3 Nitrate reduction with ZCFT for 90 seconds

4. Results and discussion

4.1 Optimization of zinc reduction conditions

4.1.1 Testing of homogenous zinc coating

The zinc powder coating was specifically coated by admixing the foam tape chip into the zinc powder and shaking five times using an up-down motion. The ZCFT was knocked for the homogenous coating into a paper box (using an up-down motion 10 times). The homogenous zinc coating was studied using an available digital microscope for the smartphone and image processing, as shown in Figure 2. The results showed a trending pattern of the plot profile of the random chip of ZCFT (n=5) in Figure 4 (A). The criteria of the homogenous coating were the lines of the plot profile trends and the standard deviation of the histogram for the result of weighing using the evaluation of zinc-coated foam tape (10 replicates), which was 7.7(±0.4) mg of zinc.

4.1.2 Optimization of the zinc amount and size for nitrate reduction

In the optimization of the zinc amount and coating size, we tested ZCFT of sizes 1.0 x 1.0, 1.0 x 0.5 and 0.5 x 0.5 cm (~7.7, ~4.0, and ~2.0 mg of Zn, respectively). All sizes of ZCFT were used for the reducing test in 2 mg L⁻¹ and 10 mg L⁻¹ of nitrate solution with three replicates (n=3). The absorbance at 540 nm was measured for 90 seconds, and the results are shown in Figure 4 (B). The highest and steadiest state of absorbance was observed at 1.0 x 1.0 cm, which was sufficient to reduce the analytical concentration range and showed lower sensitivity and consistency. In contrast, a higher quantity of zinc was likely to cause the overreduction to ammonia or other nitrogen compounds (Choodum, Boonsamran, NicDaaid, & Wongniramaikul, 2015), leading to an

increased turbidity and reduced absorbance (increased background). The ZCFT of 1 x 1 cm or ~7.7 (± 0.4) mg was the optimal condition for the nitrate determination, which showed less absorbance than the previous report (Mir, 2007; Murray et al., 2017; Jaikang et al., 2019; Choodum et al., 2020).

4.1.3 Kinetic studies

The evaluation of kinetics for reducing time, including time for measuring a dynamic of nitrate to nitrite, is necessary for specifying the detection time of work. To establish the difference of kinetic profiles, the difference in nitrate concentration was evaluated for the solutions with concentrations of 2-10 mg L⁻¹. Figure 4 (C) shows that increases of the linearity and slope were

achieved within 90 seconds and decreased at 120 seconds. The reaction time could be further reduced using other nitrogen compounds (Choodum et al., 2015). A reaction time of 90 seconds was selected as the optimal condition. Some previous studies have recommended nitrate redetection with metallic zinc and strict control of the standing time to avoid reducing nitrogen to a lower oxidation state (Ellis et al., 2011). The physical properties of the foam tape contained the air inside a microporous structure, which may have helped hydrogen gas in the reducing reaction (see Figure 3). Besides, the effect of the zinc particle surface influence on the kinetic of the reducing time showed a more rapid reduction in previous reports (Mir, 2007; Murray et al., 2017; Jaikang et al., 2019; Choodum et al., 2020).

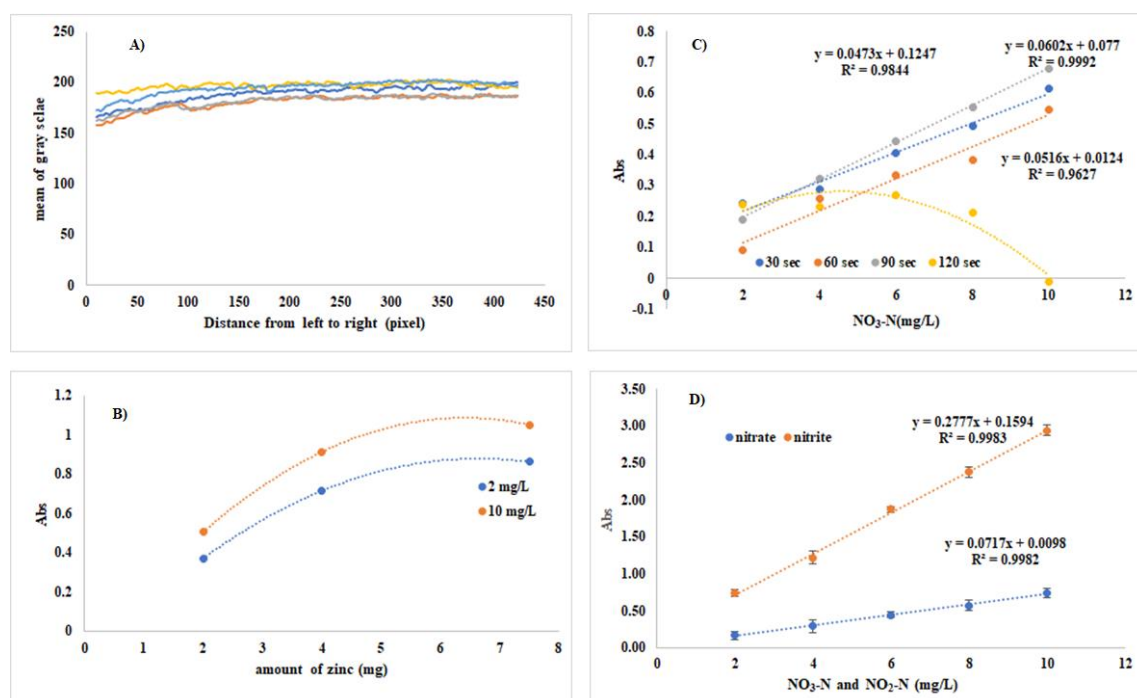


Figure 4 A) Trending pattern of plot profile of random ship of ZCFT B) Zinc amount and size optimum for reducing C) Reducing time D) Calibration curve of NO₃-N and NO₂-N

4.1.4 Efficiency evaluation of ZCFT

The influence of interference ions was tested by applying various ions to nitrate at a concentration of ~10,000 mg L⁻¹ (except Cr³⁺, which was applied at a concentration of 5000 mg L⁻¹) (Choodum et al, 2020). We observed no color products from any of the ions in nitrate tests (shown in Figure 5) that indicated high specificity of the

method. Then, each ion was added to standard solutions of nitrate to investigate the tolerance ratios. The absorbance at 540 nm from the colorimetric products, which was obtained in the presence of percent relative error interfering ions at 10,000 mg L⁻¹ (except Cr³⁺, 5000 mg L⁻¹), varied from +3.5 to -4.5%. The results showed that nitrate can tolerate interfering ions even at high concentrations.

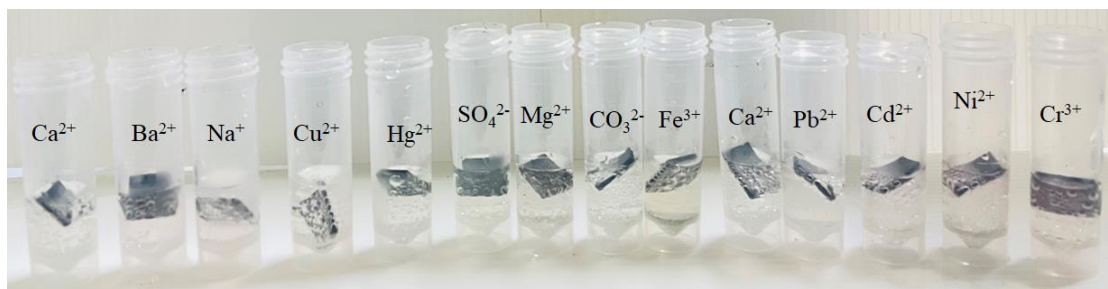


Figure 5 Color product from interference ions in nitrate tests

However, the method only achieved an analytical range up to 10 mg L⁻¹ NO₃-N in the acidic reaction. Nitrite is an intermediate ion that is similar to other forms. The optimal zinc amount and reducing time were determined at the analytical concentration range. The percent reduction of nitrate to nitrite by zinc can be calculated by the ratio of actual absorbance value NO₃-N after the reduction to the absorbance value NO₂-N at the same concentration (Jaikang et al., 2019) in the

range of 2-10 mg L⁻¹ (n = 3). Table 1 shows the percent reduction of the study range at 23-25%. Some reports have recommended a metal reduction of nitrate that can convert a significant portion of nitrate or nitrite to ammonia (exceeding 60 %). However, in this study, only a small portion of nitrate (< 30 %) was reduced to nitrite (unpublished data), and only about 20 % of nitrate was converted to nitrite by the metal reduction (Mir, 2007).

Table 1 Efficiency evaluation of ZCFT

mg L ⁻¹	Abs (NO ₃ -N)	Abs (NO ₂ -N)	%Reduction
2	0.19	0.74	26.0
4	0.32	1.22	26.6
6	0.44	1.87	23.7
8	0.56	2.37	23.4
10	0.68	2.94	23.1

*The % reduction = Abs of NO₃-N after reduction to NO₂-N / Abs of NO₂-N x 100, * Δ Abs = [Abs of NO₃-N - Abs of NO₂-N] where is corrected Abs value of NO₃-N to NO₂-N

4.2 Validation of methods

4.2.1 Analytical characteristics

First, a calibration of nitrite and nitrate was generated using the standard solutions and Griess reagent (n=3), in which the linear range of nitrite was determined between 2 and 10 mg L⁻¹ NO₂-N RSD≤5, with R²=0.998 (n=3). Following this, the nitrate standards were analyzed using the optimized conditions of the zinc-coated reduction, and the nitrate calibration curve was established at 2-10 mg L⁻¹ NO₃-N RSD≤5, with R²=0.998 (n=3), as shown in Figure 4 (D). The limit of detection (LOD) and limit of quantitation (LOQ) of this method, as obtained by the spectrophotometer, were 0.005 mg L⁻¹ and 0.016 mg L⁻¹ NO₃-N (n=10), respectively. For both calibration curves, the difference of slope is illustrated in Figure 4 (D). A higher concentration would increase the difference of the signal. However, the intercept or background of the

nitrate calibration curve was lower than that of the nitrite, indicating higher sensitivity, as shown in the previous report (Mir, 2007; Murray et al., 2017).

4.2.2 Recovery test

Recoveries were calculated for each vegetable sample. The results are presented as a percentage, which was calculated using the equations recommended by Eaton (2005). The nitrate concentration was determined within the non-spiked samples by adding the standard solution of nitrate at 2 mg L⁻¹ and following the zinc-coating method of five samples (n=3) of the results of the recovery calculations were presented as percent values in Table II. According to the WRC, it is recommended that a percent recovery value must be observed within the range of 85-115%, which as seen as suitable in term validation (Jayawardane, Wei, McKelvie, & Kolev, 2014). The percent

recovery obtained for the vegetable samples was within this accreditation range and thus demonstrated reasonable accuracy. The sample

preparation process and zinc-coated reduction are applicable for nitrate determination in vegetable samples.

Table 2. Recovery test

Sample name (n=5)	Added (mg L ⁻¹ NO ₃ -N)	Found (mg L ⁻¹ NO ₃ -N)	Recovery (%)
SV1	0.0	4.0	-
	2.0	6.2	108
SV2	0.0	4.9	-
	2.0	7.1	112
SV3	0.0	3.3	-
	2.0	5.6	115
SV4	0.0	0.9	-
	2.0	2.7	106
SV5	0.0	7.7	-
	2.0	9.6	95

4.3 Real sample and performance test

The application of real sample analysis was necessary to evaluate the concentration of analyte in the sample. The value regulation was compared with the analyzed level to determine the applicable range. This experiment was designed for five vegetable samples (n=5). The concentration was calculated from the abovementioned equation, which was used as a factor to correct the reduced efficiency. NitraVer® 5 Nitrate Reagent Powder Pillows were employed for the determination of nitrate using the cadmium reduction method for a performance test in the same portions. The results of the analysis are shown in Figure 6. The results are in agreement with the

standard reagent method and within the range of the regulation reports (FAO/WHO, 2013). In addition, the results are in agreement with the application of the t-test, as the values of $t_{observed}$ (0.76) are less than $t_{critical}$ (2.76) at the 95 % confidence level. The regulation reports, which recommend the acceptable daily intake (ADI) for nitrate, were produced by the Scientific Committee on Food (SCF) in 2002. The amounts range from 0 to 3.7 mg/kg body weight/day, which is equivalent to an intake of 222 mg nitrate/day for an adult weighing 60 kg. Previous studies have shown that the average adult daily consumes approximately 400 g of various vegetables. Hence, the average intake of nitrate is 157 mg/day (FAO/WHO, 2013).

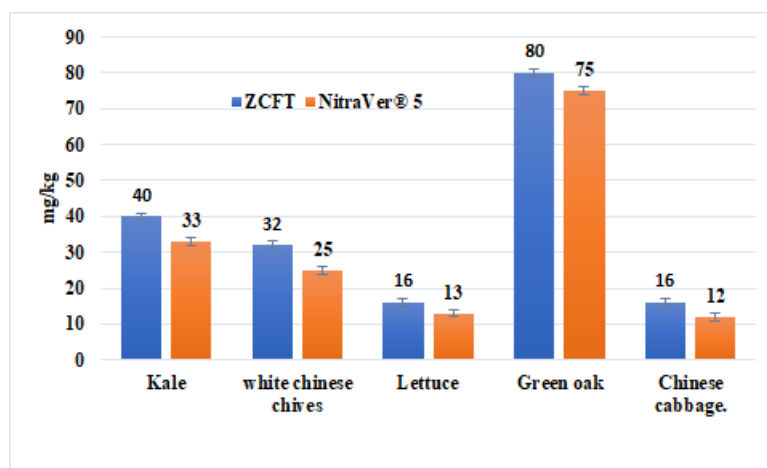


Figure 6 Determination of nitrate content in vegetable samples comparing the proposed method (ZCFT) to reference method (NitraVer® reagent)

5. Conclusion

In this study, we proposed a nitrate determination method based on nitrate reduction using a zinc coating foam tape and a Griess reagent. The developed ZCFT chip allows for simple operation, rapid analysis time, and an uncomplicated process. The proposed method was developed to achieve a portable, rapid, simple to use and prepare, low-budget, and green chemical analysis. Moreover, the proposed method is suitable for monitoring nitrate onsite at farms and in the field.

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