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Comparison of Sample Pretreatment and Analytical Method for Nitrate Determination in Vegetables

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Abstract

Different sample pretreatment extraction techniques and analytical methods are often used for nitrate determination in vegetables; however, the effects of these variables have not been properly examined. Therefore, sample pretreatment (oven-dried and fresh extraction) and nitrate determination methods (colorimetric method (salicylic method (SM)) and high-performance liquid chromatography (HPLC) were compared in Chinese kale (K) and morning glory (M) in this study. Oven-dried and fresh samples (K and M) were extracted by maceration and determined for nitrate content by the SM. As a result, the nitrate contents of both the oven-dried samples were significantly lower than the fresh samples ($p < 0.01$). A high correlation between the SM and HPLC was observed in 21 oven-dried samples of K and M. The nitrate contents using SM were significantly lower than the HPLC ($p < 0.01$). Both methods could be used for nitrate analysis in vegetables with an awareness of impurities in the SM.

Keywords: Colorimetric by Salicylic acid, HPLC, Nitrate, Vegetables

Introduction

Accumulation of nitrate in plants is a significant problem worldwide and is also a threat to human health, especially for infants [1-3]. Nitrates are almost totally absorbed in the blood and some are converted into nitrite. These nitrites then react with the blood to create methemoglobin that can lead to the occurrence of methemoglobinemia [4]. This blood disorder interferes with the oxygen transport system of the body. Nitrite is also involved in the synthesis of nitrosamines that can cause stomach cancer [5]. Thus, nitrate concentration is restricted in food (naturally or as an additive) and drinking water. Sample pretreatments and different extraction methods and analysis techniques have been studied to assess the concentration of nitrate in food or plants. The toxicity of nitrate can affect animals due to the contaminated feed [6], and also have an influence on plant nutrition [7]; therefore, a precise nitrate analysis method would be important. The intake estimates for nitrate and nitrite in Europe and the United States (US) from food are 31 - 185 mg/day in Europe and 40 - 100 mg/day in the US. Nitrate intake from sources other than vegetables, such as, drinking water and cured meat products has been estimated to be about 35 - 44 mg/person/day for a human weighing 60 kg [8].

Sample pretreatment methods for nitrate analysis consist of reducing the sample size by chopping fresh produce or crushing dried material. Many extraction methods and techniques have also been reported for nitrate analysis in plants and vegetables. Various extraction solutions were used, such as cold or hot distilled water [9-16], solvents as alkaline solutions [17], or methanol [18]. Furthermore, numerous extraction methods including maceration, shaking, immersion heating, or microwave technology were involved. Nitrate determination using the colorimetric method has been widely used to estimate the nitrate content. This has been on diazo-coupling reactions leading to azo dye formation (Cd-Griess method [19]). However, the drawbacks of this procedure involve pH dependence, diazotization temperature, and coupling time. The Cd-Greiss method also requires a large sample volume of carcinogenic reagents and lacks high sensitivity due to sample matrix interference. Thus, simpler, faster, more sensitive, and selective methods have now been recommended, such ascolorimetric analysis using salicylic acid (SM) as a rapid procedure without interference from other ions present in plant tissue [9]. Moreover, HPLC [20,21], ion [22] and gas chromatography [23,24], capillary electrophoresis (CE) [25] and polarographic methods [26] are also sensitive, accurate, and reliable methods. In addition, several reports have assessed extraction solutions, extraction methods, and different analytical techniques to optimize nitrate determination efficiency in plants-[10,27-30]. For best results, samples should be analyzed as soon as possible after collection; however, this is impractical for collection in remote locations or for large sample batches, necessitating sample storage before analysis. Freezing, freeze-drying, or oven-drying were the common procedures described in the literature for vegetable storage or sample pretreatment before nitrate analyses with no information about their respective reliabilities. Thus, a comprehensive comparison of different sample pretreatment and analytical techniques would enable the selection of the optimal procedure for selective nitrate quantification requirements. Therefore, the objective of this study was to compare the pretreatment samples (oven-dried and fresh) and 2 different nitrate measurement methods (colorimetric (SM) and HPLC) in vegetables.

Materials and methods

Sample collection

Two fresh vegetables, 35 K and 36 M samples were purchased from local markets in several provinces comprising Bangkok, Nakhon Pathom, and Nonthaburi between April and July 2019. Each fresh sample weighed approximately 500 g.

Sample pretreatment

The edible parts of the fresh vegetable samples were washed, cleaned, and chopped into small pieces. For the sample pretreatment comparison method, 25 samples each of K and M were divided into fresh samples for fresh extraction (coded as Fresh-K and Fresh-M for K and M, respectively) and dried samples using a hot air oven (coded Oven-dried-K and Oven-dried-M for K and M, respectively) for the oven-dried extraction. The oven was heated to 65 °C for 24 h [31]. The dried samples were ground and kept in a desiccator until analysis. For the comparison of the method, 21 samples comprising 10 samples of K and 11 samples of M were prepared as oven-dried following the sample pretreatment comparison method.

Nitrate extraction

Extraction using oven_dried samples (oven-dried extraction): The method of extraction was modified from Cataldo *et al.* [9]. Two hundred mg of the samples were extracted by maceration with 10 mL of water for 12 h after extraction, the samples were centrifuged at 2,500 rpm for 10 min and then filtrated through a 0.45-micron membrane filter.

Extraction using fresh samples (fresh extraction): The method of extraction was modified from Yu *et al.* [32]. In brief, 20 g of each finely chopped vegetable was homogenized with 75 mL of deionized water and then diluted to a final volume of 100 mL with deionized water. The sample was macerated at 4 °C for 12 h. The mixture was then transferred to a centrifuge tube and centrifuged for 10 min at 2,500 rpm. The supernatant was filtrated through a 0.45-micron membrane filter.

Nitrate analysis

Analysis by the colorimetric method (SM): This method was adapted from Cataldo *et al.* [9]. An aliquot of 200 µL of 5 % of salicylic acid in concentrated sulfuric acid (H_2SO_4) was added to 200 µL of the extraction sample. After 20 min at room temperature, 5 mL of 2 M of sodium hydroxide (NaOH) was added and the mixture was left to stand for 15 min. The reaction was measured for absorbance at 410 nm with a spectrophotometer. Nitrate concentration was reported in units of nitrate (mg NO_3/kg FW).

Analysis by the HPLC method: This analysis was modified from Chou *et al.* [12]. In brief, an aliquot of 10 µL of the sample was subjected to the HPLC system (Shimadzu LC10) equipped with an autosampler. The analysis was performed at 30 °C with a PDA-detector set at 213 nm. Nitrate was eluted from the Inertsil ODS-3 C18 HPLC column (4.6×250 mm^2. I.D, 5 µm) with 0.01 M of octylammonium orthophosphate in 30 % (v/v) of methanol pH 7.0 at a flow rate of 0.8 mL/min.

All extractions and nitrate analyses were done in triplicate. Sample pretreatment comparisons were performed on the same day. All nitrate contents of the oven-dried samples were expressed on a fresh weight basis after correction for the dry matter content.

Statistical analysis

Descriptive statistics including the mean and standard deviation were conducted for all the results. A paired t-test was also used to compare the results, and a linear regression relationship was established to facilitate the data conversion between the 2 analyses. All statistics were performed using MS Excel 2010 or SPSS 14 operating on the Windows 10 system.

Results and discussion

Sample pretreatment comparisons

The results of fresh-K and oven-dried-K for the determination of nitrate using the SM method are shown in **Figure 1** and **Table 1**. The highest and lowest nitrate contents were found in fresh-K at 5,166 mg/kg FW and oven-dried-M at 211 mg/kg FW, respectively. Samples of oven-dried-K had a significantly lower nitrate content than fresh-K (t-test $p < 0.01$; less than 0.01 - 0.70 times). Likewise, when using M, the results showed that oven-dried-M had significantly lower nitrate contents than fresh-M (t-test $p < 0.01$) (**Figure 2**). The highest nitrate content was found in fresh-M (5,158 mg/kg FW), and the lowest value was determined in oven-dried-M (44 mg/kg FW).

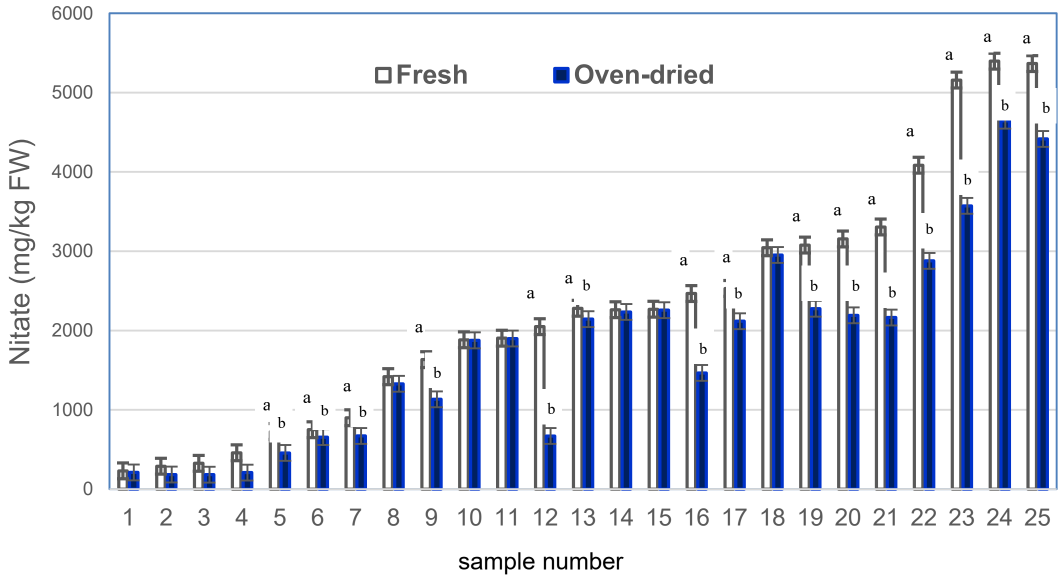

Figure 1 Comparison of mean ($n = 9$) nitrate contents in fresh and oven-dried-K. For each comparison of pretreatment between oven-dried and fresh sample, bars with the different letters are significant ($p < 0.01$).

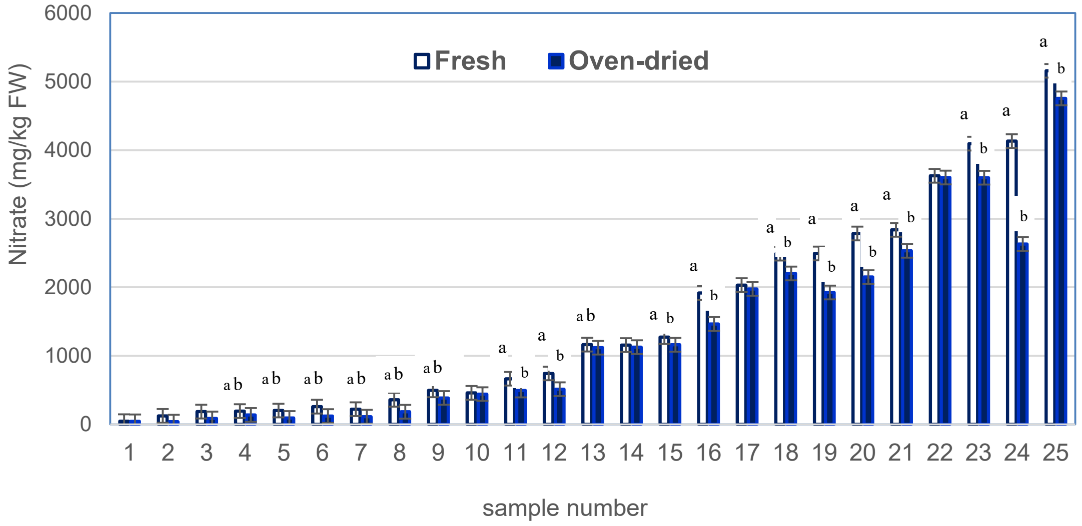

Figure 2 Comparison of mean ($n = 9$) nitrate contents in fresh and oven-dried-M. For each comparison of pretreatment between oven-dried and fresh samples, bars with the different letters are significant ($p < 0.01$).

The drying process reduced the nitrate content since the effect of heat on the vegetables resulted in the nitrate decomposing to other compounds. Furthermore, this process could lead to loss of plant quality that would affect the extract, such as changes in color and loss in nutrients (nitrate) or quality [33,34]. The drying process in the vegetables also reduced the water content. Nitrates are soluble in water and may exudate from the vegetables and be retained in the oven-dried container resulting in lower nitrate content. The results of the present study concurred in part with Pinto *et al.* [30] who compared oven-dried and fresh vegetables, including spinach and lettuce samples for nitrate analysis by HPLC. They found that the nitrate contents of oven-dried spinach were lower than the nitrate contents of fresh produce, whereas oven-dried lettuce showed a higher nitrate value than the fresh sample.

Table 1 Regression equations between nitrate contents (mg/kg FW) estimated by oven-dried and fresh vegetables.

Sample	Equation	R^2	Coefficient of correlation (r)
K ($n = 25$)	Oven-dried-K = 0.7688×Fresh-K + 39.428	0.8869	0.942**
M ($n = 25$)	Oven-dried-M = 0.8635×Fresh-M – 33.727	0.9563	0.978**

**r values significant at $p < 0.01$

The linear regression relationship between oven dried and fresh vegetables of K and M are shown in **Table 1**. The slope values of K and M were 0.768 and 0.863, respectively. These indicated the consistency between the results from the 2 methods and confirmed that the oven-dried nitrate content was less for fresh vegetables. The correlation coefficients (r) of K and M were 0.942 and 0.978, respectively.

Comparison of the methods for nitrate analysis

A comparison of the nitrate analysis methods between the colorimetric method (SM) and HPLC was studied in 21 samples consisting of 10 oven-dried-K samples and 11 oven-dried-M samples. The results showed that the NO_3-N content values analyzed by the HPLC ranged from 0.001 to 1.487 %, while the nitrate content values using the SM ranged from 0.019 to 0.776 % (**Table 2**). There were significant differences between the 2 methods, (t-test, $p < 0.01$). The SM demonstrated a lower nitrate content than the HPLC in almost all the dried vegetable samples (18 samples, 86 %). This was possibly due to slight errors from the interference of impurities in the samples. The nitrate content using the SM was lower than the HPLC approach by approximately 60 %. The linear regression relationship between the 2 methods is shown in **Figure 3**.

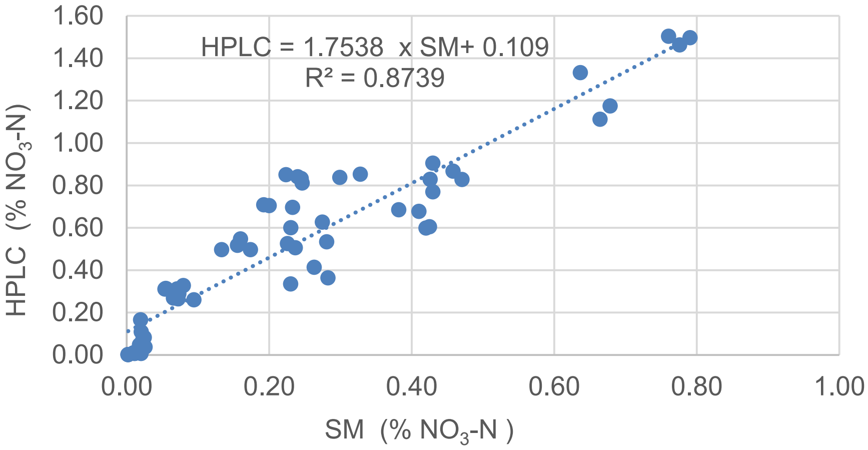

Figure 3 Correlation between the mean of nitrate levels in K and M measured by the HPLC (Y-axis) and the colorimetric (salicylic method; SM, X-axis) with a coefficient of correlation (r) of 0.935, ($p < 0.01$).

Table 2 Comparison of nitrate content (% NO_3-N DW) between SM and HPLC methods in oven-dried-K and oven-dried-M.

K*	SM Mean ± SD	HPLC Mean ± SD	% less than HPLC	M*	SM Mean ± SD	HPLC Mean ± SD	% less than HPLC
1	0.019 ± 0.001	0.040 ± 0.006	53	1	0.002 ± 0.000	0.001 ± 0.000	–56
2	0.055 ± 0.001	0.312 ± 0.002	82	2	0.011 ± 0.002	0.010 ± 0.001	–16
3	0.023 ± 0.003	0.051 ± 0.017	55	3	0.014 ± 0.005	0.007 ± 0.000	–121
4	0.070 ± 0.004	0.273 ± 0.013	74	4	0.022 ± 0.003	0.118 ± 0.042	82
5	0.156 ± 0.020	0.513 ± 0.029	70	5	0.082 ± 0.012	0.299 ± 0.035	73
6	0.208 ± 0.021	0.703 ± 0.006	70	6	0.259 ± 0.026	0.370 ± 0.040	30
7	0.244 ± 0.003	0.827 ± 0.015	71	7	0.414 ± 0.028	0.786 ± 0.111	47
8	0.248 ± 0.029	0.521 ± 0.014	52	8	0.418 ± 0.008	0.627 ± 0.044	33
9	0.220 ± 0.060	0.581 ± 0.057	62	9	0.452 ± 0.023	0.841 ± 0.022	46
10	0.284 ± 0.054	0.846 ± 0.008	67	10	0.660 ± 0.021	1.206 ± 0.113	45
				11	0.776 ± 0.015	1.487 ± 0.022	48

*K = Chinese kale, M = morning glory

The 2 methods showed a high correlation coefficient (*r*) of 0.935 suggesting that the model could be used as a predictor to convert the colorimetric results into HPLC data. The equation of the 2 methods is shown below:

$$\text{HPLC} = 1.7538\times\text{SM} + 0.109 \tag{1}$$

The slope was almost 2 indicating good consistency between the results from the 2 methods, in which the SM content had less nitrate than the HPLC. These differences may be due to interference from contaminants present in the samples. The HPLC was recognized as being more accurate and sensitive than the SM; thus, it could be presumed that the low nitrate level contained in that sample required a high sensitivity detector. Moreover, impurities could interfere with the accuracy of nitrate absorbance using the SM, while the HPLC would separate nitrate from various impurities in the chromatographic column. The working principle of the SM was based on ultraviolet-visible spectroscopy (UV Vis) following the Beer-Lambert law [35] stating that the absorbance value was the reciprocal of transmittance. The linear relationship displayed a small range (0.3 - 0.7) between the absorbance value and concentration of solution [36]. Therefore, the linearity range of SM was narrower than the HPLC. Thus, impurities in the sample that decreased the absorbance of the SM fell outside the linear range of UV Vis. These factors resulted in the inability of the SM to accurately measure the nitrate concentration.

The results of the present study showed good agreement with previous reports of colorimetric analysis methods, such as the Cd-Griess method compared to the HPLC. Schuster and Lee [37] compared the nitrate analysis between the colorimetric (Cd-Griess method) and HPLC in carrots. They found that the Cd-Griess method gave a 63 % lower result of the nitrate contents than the HPLC. Furthermore, the accuracy and recovery of the Cd-Griess method were less than the HPLC. Sanderson *et al.* [38] also found that amounts of nitrate in fresh meat and roast meat using the HPLC were 70 and 60 % higher than the Cd-Griess method, respectively. Nevertheless, analysis using colorimetric methods (Cd-Griess method) may not be that different when compared with the HPLC method. Lyons *et al.* [39] also found that the amount of nitrate using the modified Cd-Griess method was not different from the HPLC for the analysis of nitrate in beetroot, celery, and carrots. However, the results did not concur with a report by Yu *et al.* [32]. They compared nitrate analysis between the HPLC and spectrophotometer methods for fresh vegetable samples of Cantonese cabbage, cabbage, celery, Chinese cabbage, kale, lettuce, turnip greens, and spinach. Their results showed significant differences between the 2 methods and that both methods were highly correlated with an R square value equal to 0.969. The nitrate obtained from the spectrometer method was higher than the HPLC but still had similar values with the most relative errors within ± 10 %. These contrasting results may relate to the sample preparation methods that they used. They prepared samples by extraction with water and then used ion-exchange solid-phase extraction (SPE) cartridges to remove interference from the samples before measuring the absorbance with a spectrophotometer. In this case, ion-exchange SPE cartridges would only remove ions; thus, other interferences would not be removed and may be able to absorb the analyzed light, which would result in higher nitrate values than those obtained using the HPLC.

Conclusions

The nitrate concentration of samples pretreated as oven-dried extraction was much lower than that of fresh extraction, based on the nitrate tests in vegetables in this study. Furthermore, the amount of nitrate was affected by the analysis procedures. The colorimetric method (SM) showed lower nitrate contents than the HPLC possibly due to impurity interference. However, the SM and HPLC showed a significant correlation. Therefore, both methods could be used for nitrate analysis in vegetables with the awareness of impurities in the SM. The results of this study suggest that interference removal occurs by adsorbent; such asactivated carbon or ion-exchange SPE cartridges. These modifications to the colorimetric method (SM) may meet the criteria and give similar results to the HPLC.

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References

[1] L Knobeloch, B Salna, A Hogan, J Postle and H Anderson. Blue babies and nitrate-contaminated well water. *Environ. Health Perspect.* 2000; **108**, 675-8.

[2] G Walton. Survey of literature relating to infant methemoglobinemia due to nitrate-contaminated water. *Am. J. Public Health Nations Health* 1951; **41**, 986-96.

[3] J Sanchez-Echaniz, J Benito-Fernandez and S Mintegui-Raso. Methemoglobinemia and consumption of vegetables in infants. *Pediatrics* 2001; **107**, 1024-8.

[4] RC Darling and FJW Roughton. The effect of methemoglobin on the equilibrium between oxygen and hemoglobin. *Am. J. Physiol.* 1942; **137**, 56-68.

[5] M Rezaei, A Fani, AL Moini, P Mirzajani, AA Malekirad and M Rafiei. Determining nitrate and nitrite content in beverages, fruits, vegetables, and stews marketed in Arak, Iran. *Int. Sch. Res. Notices* 2014; **2014**, 439702.

[6] CT MacKown and JC Weik. Comparison of laboratory and quick-test methods for forage nitrate. *Crop Sci.* 2004; **44**, 218-26.

[7] M Errebhi, CJ Rosen and DE Birong. Calibration of a petiole sap nitrate test for irrigated ‘russet Burbank’ potato. *Commun. Soil Sci. Plant Anal.* 1998; **29**, 23-35.

[8] NG Hord, Y Tang and NS Bryan. Food sources of nitrates and nitrites: The physiologic context for potential health benefits. *Am. J. Clin. Nutr.* 2009; **90**, 1-10.

[9] DA Cataldo, M Maroon, LE Schrader and VL Youngs. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun. Soil Sci. Plant Anal.* 1975; **6**, 71-80.

[10] CL Bedwell, DW Hamar, ML Hoesterey, JP Sonderman and KG Odde. Comparison of four methods for forage nitrate analysis. *J. Vet. Diagn. Invest.* 1995; **7**, 527-30.

[11] C Cruz and MAM Loucao. Comparison of methodologies for nitrate determination in plants and soils. *J. Plant Nutr.* 2002; **25**, 1185-211.

[12] SS Chou, JC Chung and DF Hwang. A high performance liquid chromatography method for determining nitrate and nitrite levels in vegetables. *J. Food Drug Anal.* 2003; **11**, 233-8.

[13] HB Novaes, DS Vaitsman, PB Dutra and DV Perez. Determination of nitrate in lettuce by ion chromatography after microwave water extraction. *Quim. Nova* 2009; **32**, 1647-50.

[14] MPN Gent. Composition of hydroponic lettuce: Effect of time of day, plant size, and season. *J. Sci. Food Agric.* 2012; **92**, 542-50.

[15] V Kmecl and D Znidarcic. Accreditation of the analytical method used for nitrate determination in vegetables. *Arch. Biol. Sci.* 2015; **67**, 295-302.

[16] P Alexander, P Handawa and UT Charles. Determination of nitrate and nitrite contents of some edible vegetables in guyuk local government area of Adamawa State, Nigeria. *Am. Chem. Sci. J.* 2016; **13**, 1-7.

[17] UI Gaya and S Alimi. Spectrophotometric determination of nitrate in vegetables using phenol. *J. Appl. Sci. Environ. Manage.* 2006; **10**, 79-82**.**

[18] A Najdenkoska. Development of hplc method for analysis of nitrite and nitrate in vegetable. *J. Agric. Food Env. Sci.* 2016; **67**, 33-9.

[19] HT Nguyen, KTK Vo, LTT Bui, HH Hua and GE Oko. Gries - Ilosvay spectrophotometry for determination of nitrite in water and vegetables in Vietnam. *Asian J. Chem. Sci.* 2018; **5**, 1-9.

[20] M Sabolova and L Kourimska. Vitamin C and nitrates contents in fruit and vegetables from farmers' markets and supermarkets. *Potr. S. J. Food Sci.* 2020; **14**, 1124-30.

[21] R Uddin, MU Thakur, MZ Uddin and GMR Islam. Study of nitrate levels in fruits and vegetables to assess the potential health risks in Bangladesh. *Sci. Rep.* 2021; **11**, 4704.

[22] DGD Silva, ALD Carvalho, EDJ Araujo, ME Rocha, BO Moreira and DLS Santos. Development of an analytical method for determination of nitrate in leafy vegetables using ion chromatography. *OALib. J.* 2020; **7**, e6471.

[23] D Coviello, R Pascale, R Ciriello, AM Salvi, A Guerrieri, M Contursi, L Scrano, SA Bufo, TRI Cataldi and G Bianco. Validation of an analytical method for nitrite and nitrate determination in meat foods for infants by ion chromatography with conductivity detection. *Foods* 2020; **9**, 1238.

[24] B Campanella, M Onor and E Pagliano. Rapid determination of nitrate in vegetables by gas chromatography mass spectrometry. *Anal. Chim. Acta* 2017; **980**, 33-40.

[25] FD Betta, L Vitali, R Fett and ACO Costa. Development and validation of a sub-minute capillary zone electrophoresis method for determination of nitrate and nitrite in baby foods. *Talanta* 2014; **122**, 23-9.

[26] MI Ximenes, S Rath and FG Reyes. Polarographic determination of nitrate in vegetables. *Talanta* 2000; **51**, 49-56.

[27] IF Boros, L Sipos, N Kappel, L Csambalik and M Fodor. Quantification of nitrate content with FT-NIR technique in lettuce (*Lactuca sativa L.*) variety types: A statistical approach. *J. Food Sci. Technol.* 2020; **57**, 4084-91.

[28] KA Anderson and TE Case. Evaluation of plant nitrate extraction techniques and effect on commonly used analytical methods of detection. *Commun. Soil Sci. Plant Anal.* 1999; **30**, 1479-95.

[29] C Monton and C Luprasong. Effect of temperature and duration time of maceration on nitrate content of *Vernonia cinerea (L.)* Less.: Circumscribed central composite design and method validation. *Int. J. Food Sci*. 2019; **2019**, 1281635.

[30] E Pinto, C Petisca, LF Amaro, O Pinho and IMPLVO Ferreira. Influence of different extraction conditions and sample pretreatments on quantification of nitrate and nitrite in spinach and lettuce. *J. Liq. Chromatogr. Relat. Technol.* 2010; **33**, 591-602.

[31] OC Lastra. Derivative spectrophotometric determination of nitrate in plant tissue. *J. AOAC Int.* 2003; **86**, 1101-5.

[32] TH Yu, SP Hsieh, CM Su, FJ Huang, CC Hung and LM Yiin. Analysis of leafy vegetable nitrate using a modified spectrometric method. *Int. J. Anal. Chem.* 2018; **2018**, 6285867.

[33] CW Fennell, ME Light, SG Sparg, GI Stafford and JV Staden. Assessing African medicinal plants for efficacy and safety: Agricultural and storage practices. *J. Ethnopharmacol.* 2004; **95**, 113-21.

[34] M Keinanen and R Julkunen-Tiitto. Effect of sample preparation method on birch (Betula pendula Roth) leaf phenolics. *J. Agric. Food Chem.* 1996; **44**, 2724-7.

[35] G Langergraber, N Fleischmann and F Hofstadter. A multivariate calibration procedure for UV/VIS spectrometric quantification of organic matter and nitrate in wastewater. *Water Sci. Technol.* 2003; **47**, 63-71.

[36] P Nagaraja, NGS Al-Tayar, A Shivakumar, AK Shrestha and AK Gowda. A simple and sensitive spectrophotometric method for the determination of trace amounts of nitrite in environmental and biological samples using 4-amino-5-hydroxynaphthalene-2,7-disulphonic acid monosodium salt. *Spectrochim. Acta A Mol. Biomol. Spectrosc*. 2010; **75**, 1411-6.

[37] BE Schuster and K Lee. Nitrate and nitrite methods of analysis and levels in raw carrots, processed carrots and in selected vegetables and grain products. *J. Food Sci.* 1987; **52**, 1632-6.

[38] JE Sanderson, JR Consaul and K Lee. Nitrate analysis in meats; comparison of two methods. *J. Food Sci.* 1991; **56**, 1123-4.

[39] DJ Lyons, LE McCallum, WJ Osborne and PE Nobbs. Assessment of procedures for the determination of nitrate and nitrite in vegetable extracts. *Analyst* 1991; **116**, 153-7.