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Comparison of Nitrogen Species Composition in Indonesian Water Spinach and Land Spinach Samples Using Ion Chromatography

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Abstract

This paper proposes an ion chromatographic method for determining cationic nitrogen species (ammonium-NH_4 and methylammonium-$MeNH_4$) and anionic (nitrite-NO_2 and nitrate-NO_3) in raw spinach (*Ipomoea aquatica*) samples. The system was based on non-suppressed and suppressed conductivity for determining cationic and anionic nitrogen species. The cationic species was determined on Metrohm C150/4.0 column using a 2.5 mM nitric acid as the eluent. In contrast, the anionic species was determined on Metrohm Supp A 250/4.0 column using 6.0 mM sodium carbonate and 0.5 mM sodium hydrogen carbonate as a mixture eluent. Excellent peak resolution and completed each (cationic or anionic) determination in 10 min. Limits of detection (signal-to-noise ratio: S/N=3) were estimated at 0.068, 0.091, 0.011, and 0.016 mg/L for ammonium, methylammonium, nitrite, and nitrate, respectively. The RSD (relative standard deviation) were less than 3% for retention time, peak area, and peak height. The coefficient correlation with peak heights versus the concentration range is linear, with $r^2 \geq 0.999$. The method has been applied to the Indonesian water spinach (*Ipomoea Aquatica Forsk*) and land spinach (*Ipomoea Reptans Poir*) samples collected and purchased in the local traditional market, fresh food supermarket, and spinach plantation in Ternate City, North Maluku. As observed, the composition of nitrogen species concentration differed in each spinach leaf and stem from collected samples. However, the nitrate concentration was higher in the spinach leaves than in the stems from all samples, while the ammonium concentration was slightly higher in the spinach stems than in the leaves. As for methylammonium and nitrite, their concentrations were under the limit of detection in all samples studied.

Keywords: Ion chromatography; nitrogen species; water spinach; land spinach

1. Introduction

Indonesian cuisine is renowned for its utilization of flavourful and nutrient-rich ingredients, including two types of spinach: water spinach (*Ipomoea aquatica Forsk*) and land spinach (*Ipomoea reptans Poir*). Water spinach, in particular, is a widely consumed leafy green packed with essential vitamins, minerals, and fiber [1, 2]. However, beyond its macro and micronutrient content, it's important to consider the nitrogen levels present in water spinach when assessing its overall quality and safety as a food source.

According to the World Health Organization (WHO) guidelines promoting a healthy lifestyle, individuals are advised to consume 400 grams of vegetables and fruits daily, comprising 250 and 150 grams per person [3]. However, National data from Statistics of Food Consumption (2023)—BPS, Indonesia, reveals a shortfall in this recommendation, with the average combined consumption of fruits and vegetables at only 209.89 grams per capita daily. Delving into vegetable consumption trends, spinach is the predominant choice nationwide, with an average daily intake of just 10.85 grams per capita [4].

Spinach is a rich source of nitrogen, which can exist in various forms, including cationic nitrogen species (ammonium and methylammonium) and anionic nitrogen species (nitrite and nitrate). These nitrogen species can significantly impact spinach consumption's nutritional quality and safety. Ammonium and methylammonium, readily absorbed by plants, are commonly found in spinach. Conversely, nitrite and nitrate are less readily absorbed and are typically present in smaller quantities. While essential for plant growth, nitrite and nitrate can pose food safety risks due to their potential conversion into carcinogenic nitrosamines. Thus, it is necessary to prevent spinach contamination by nitrite compounds to ensure its safety [5, 6].

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With the global population growth and evolving consumption patterns, vegetable production, including water spinach and land spinach, is increasing across regions, including Indonesia. However, factors like urbanization, environmental pollution, and synthetic fertilizer use in agriculture can elevate specific nitrogen compound levels in plants, notably nitrate, which poses significant health risks when contaminated with nitrite compounds. This increase can lead to significant health risks when contaminated with nitrite compounds. Hence, monitoring spinach's nitrogen content, particularly harmful nitrogen species, is crucial to maintaining its safety and quality. Spinach can continue to serve as a nutritious and safe food source through proper fertilization practices and water management [7].

Nitrogen-based fertilizers, widely used in agriculture, have been linked to increased ammonia levels in plants. This environmental impact can be significant, leading to the formation of hazardous compounds like ammonium nitrate, which contributes to water pollution [8, 9]. Additionally, organic matter decomposition can generate methylammonium, another organic nitrogen compound affecting plant nutritional quality. Thus, it is imperative to understand the effects of nitrogen-based fertilizers and adopt sustainable alternatives or reduce their use.

The primary sources of cationic (ammonium and methylammonium) and anionic (nitrite and nitrate) nitrogen species intake are food, especially vegetables, and drinking water. A study suggests that vegetables contribute significantly to our daily nutritional intake of these compounds, especially nitrite and nitrate, providing approximately 43% and 85% of nitrite and nitrate intakes, respectively [10]. The Food and Agriculture Organization and the WHO have determined that the maximum amount of nitrate a person should consume daily is 3.7 mg/kg of their body weight, meaning that a 60-kg adult can consume up to 222 mg of nitrate daily. Similarly, the maximum daily nitrite intake for a 60-kg adult is 0.07 mg/kg of body weight, or 4.2 mg of nitrite per day [11]. In Indonesia, drinking water quality requirements are regulated by the Regulation of the Indonesian Ministry of Health No. 492 of 2010, specifying a maximum nitrate level of 50 mg/L and a maximum nitrite level of 3 mg/L [12]. A study reported by Uddin *et al*., found detectable levels of nitrates in all fruits and vegetables tested, with significant variations in nitrate concentration observed among root and tuber vegetables and fruits. Root and tuber vegetables exhibited the highest nitrate content at722.80 mg/kg,significantly surpassing fruit vegetables at 141.75 mg/kg.Conversely, fruit samples showed the lowest nitrate levels at 62.74 m/kg compared to vegetables [13].

Several techniques have been developed to detect cationic and anionic nitrogen species in various samples. Electrochemistry-based methods encompass potentiometry [14], conductometry [15], amperometry [16], voltammetry [17], and capillary electrophoresis [18]. In conjunction, spectroscopy-based methods have also been utilized. These include UV-Vis spectrophotometry [19] and flow injection analysis [20]. However, both electrochemistry and spectrometry-based methods have limitations in detecting methylammonium in samples. These methods are typically unable to measure the concentration of methylammonium directly when present in the sample.

Ammonium and methylammonium are nitrogen compounds commonly used in agriculture to prepare fertilizer. These compounds are the source of nitrogen, which is important for plant growth. However, excessive fertilizer use can cause environmental problems such as water pollution and soil degradation. Plants, including spinach lettuce, can absorb these nitrogen species from the soil and water around them through their roots, which have been given fertilizers. Therefore, it is also important to determine plant such as spinach samples' methylammonium levels to ensure they are within safe limits [21].

An advanced and sensitive analytical method, ion chromatography (IC), is a suitable tool for determining and understanding the compositions of these nitrogen species in various types of samples, including vegetables [22- 27].This analysis can offer insights into the nitrogen species content of widely consumed vegetables and provide a deeper understanding of potential health risks and environmental impacts associated with exposure to specific nitrogen compounds [28].

This study aims to find the optimization of chromatographic conditions that can precisely determine the cationic nitrogen species (ammonium and methylammonium) and anionic (nitrite and nitrate) concentrations in leaves and stems of water spinach and land spinach. It can also be applied to understand the distribution and accumulation of nitrogen species in spinach, which are significant for food safety and nutrition. This comprehensive analysis offers an understanding of the nutritional content of widely consumed vegetables and sheds light on potential health risks and environmental impacts associated with specific nitrogen compounds. Ultimately, these findings contribute to ensuring the quality and safety of regional foods, such as those in North Maluku, while providing valuable information about the variations in the nutritional composition of spinach.

2. Experimental

2.1. IC system

A suitable IC system is an Eco IC (Metrohm, Switzerland) comprising a micro membrane Suppressor Module (MSM), a Metrosep C4-150/4.0 (150 mm x 4.0 mm I.D.) sample column set with a Metrosep *C4 Guard*/4.0 guard column for determining ammonium and methylammonium ions, a Metrosep A Supp 17-250/4.0 (250 mm x 4.0 mm I.D.) sample column set with a Metrosep A Supp 17 Guard/4.0 guard column for determining nitrite and nitrate ions, 10-µl sample loop, and a Metrohm MagIC Net 3.2 software.

The detection system for determining cationic nitrogen species (ammonium and methylammonium) and anionic (nitrite and nitrate) were non-suppressed and suppressed conductivity, respectively. The adjusted eluent flow rate of the analytical pump was at 1.0 and 0.8 mL/min for determining cationic and anionic nitrogen species, respectively.

2.2. Preparation of reagents and solutions

All prepared nitrogen species solutions were diluted 1000 mg/L IC certified multi-elements (Merck) of ammonium, methylammonium, nitrite, and nitrate stocks to prepare 10, 20, 30, 40, and 50 mg/L for calibration purposes. The prepared standard calibration solutions diluted the stock appropriately using Milli-Q purified water just before use. The membrane syringe filter's 0.22 µm pore diameter was used for sample particulate removal. Standards and eluents were prepared daily to avoid unknown peaks that sometimes appeared in the chromatograms.

2.3. Preparation of eluents and suppressor regenerate

The eluent (2.5 mM HNO_3) was prepared for determining ammonium and methylammonium by dissolving 1.4 mL concentrated HNO_3 in a 1.0 L volumetric flask with Milli-Q purified water. As well as the mixed compounds (6.0 mM Na_2CO_3 and 0.5 mM $NaHCO_3$) were prepared for determining nitrite and nitrate by weighing 0.636+/-0.001 g of anhydrous sodium carbonate and 0.042+/-0.001 g of sodium hydrogen carbonate, and then in Milli-Q purified water and transfer to a 1 L volumetric flask and then dilute to volume with Milli-Q purified water.

The suppressor regenerate (0.05 M, H_2SO_4) was prepared by transferring 2.8 mL of concentrated Sulfuric acid (S.G = 1.84) into a 1 L volumetric flask containing Milli-Q purified water and then diluting to volume with Milli-Q purified water.

All the solutions, including eluents and suppressor regenerant, were then filtered using a pore size of 0.22 µm membrane filter. In optimizing the performance of solutions and avoiding the appearance of unknown peaks on the chromatograms, they were then prepared daily.

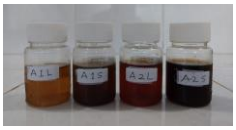
2.4. Collection and preparation of extract water spinach and land spinach samples

Both raw water spinach and land spinach were collected and purchased in 3 sample locations: a local traditional market, a fresh food supermarket, and a spinach plantation area in Ternate City, North Maluku. Two samples were collected at each of the 3 sampling locations: water spinach and land spinach, for a total of 6 samples. The raw collected spinach is shown in Figure 1. The collected spinach was washed thoroughly to remove surface contaminants and dried using paper towels. The leaves and stems were then separated for each sample, so each sample had 6 samples of spinach leaves and 6 samples of spinach stems.

Figure 1: Indonesian spinach (*Ipomoea aquatica*), (A) land spinach, (B) water spinach.

Bulk spinach was then separated between leaves and stems. All the samples were then placed in an oven and set to a temperature of 105 °C to remove any residual water content for 30 min. The leaves and stems were then finely chopped or ground to ensure sample homogeneity. A 5 g portion of the finely chopped spinach sample was transferred to a beaker, adding 250 mL Milli-Q purified water and then stirring the mixture to create a homogenous suspension. The spinach suspension was filtered through filter paper to remove any solid particles. The liquid portion of the samples was finally filtered again using a nylon syringe filter 0.22 µm pore size (Merck) to remove possibly formed precipitated before injection into the IC instrument.

Table 1: Extracts of spinach samples of three sampling locations

Sampling location	Extracts of spinach samples	Remarks of samples
A. Local traditional market		A1L= Water spinach leaves
		A1S= Water spinach stems
		A2L= Land spinach leaves
		A2S= Land spinach stems
B. Fresh food supermarket		B1L= Water spinach leaves
		B1S= Water spinach stems
		B2L= Land spinach leaves
		B2S= Land spinach stems
C. Spinach plantation area		C1L= Water spinach leaves
		C1S= Water spinach stems
		C2L= Land spinach leaves
		C2S= Land spinach stems

3. Results and Discussion

3.1. Determination of Cationic and Anionic Nitrogen Species with Standard samples

In Figures 2 and 3 chromatograms, the cationic nitrogen species (ammonium and methylammonium) and the anionic nitrogen species (nitrite and nitrate) were entirely separated and eluted in 10 min for each chromatogram. Every peak was meticulously assessed, resulting in well-balanced, especially the anionic nitrogen, symmetrical peaks, and excellent separation, demonstrating a high level of resolution in the chromatographic analysis.

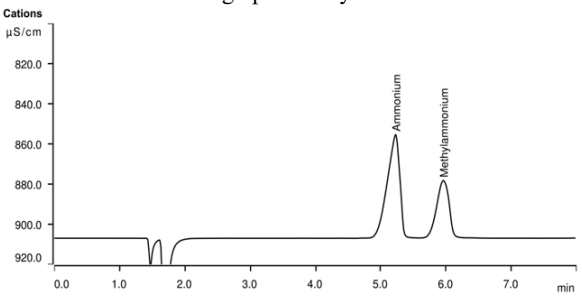


Figure 2: Ion chromatogram of cationic nitrogen species on retention time with a standard sample. Eluent concentration: 2.5 mM HNO_3. Analytical column: Metrosep C4-150/4.0 (150 mm x 4.0 mm I.D.). Flowrate of eluent: 1.0 mL/min. The volume of the sample is 10 µL. Cationic nitrogen species (conc., in mg/L): ammonium (50) and methylammonium (50).

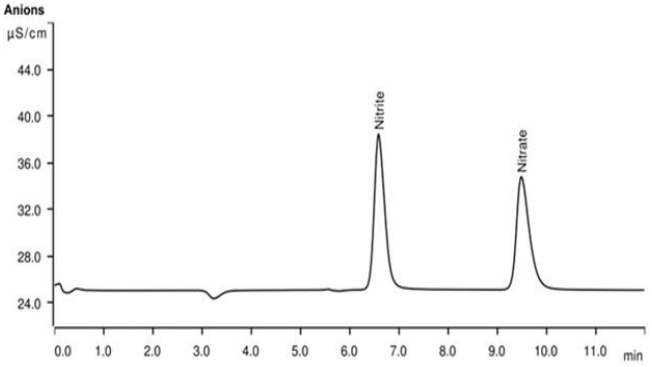


Figure 3: Ion chromatogram of anionic nitrogen species on retention time with a standard sample. Mixed eluent concentration: 6.0 mM Na_2CO_3 and 0.5 mM $NaHCO_3$. Analytical column: Metrosep A Supp 17-250/4 (250 mm x 4.0 mm I.D.). Flowrate of eluent: 0.8 mL/min. The volume of the sample: 10 µL. Anionic nitrogen species (conc., in mg/L): nitrite (50) and nitrate (50). Suppressor regenerate: 0.1 M H_2SO_4, complete with auto-step with fill.

3.2. Validation of the method

3.2.1. Accuracy

Table 2 shows the information regarding the consistency of signals, encompassing retention times, peak areas, and peak heights. The repeatability results indicate that the variation in retention time was less than 2.88%, while for peak area, it was less than 2.62%, and for peak height, it was less than 2.93%.

Table 2: Relative standard deviation (RSD) of nitrogen species under the ion chromatographic conditions in Figures 2 and 3

Nitrogen Species	RSD (%), n=5		
	Retention time	Peak area	Peak height
Cationic:	0.78	0.34	1.89
Ammonium			
Methyl ammonium	0.93	1.46	1.92
Anionic:	2.23	1.83	1.98
Nitrite			
Nitrate	2.88	2.62	2.93

These findings affirm that the measurements were remarkably consistent and exhibited minimal variability, thereby confirming the dependability of the analytical method within the specified conditions.

3.2.2. Calibration curve

For quantitation purposes, calibration curves were constructed by plotting the peak heights against the concentrations of specific cationic and anionic nitrogen species found in the standard solutions, as depicted in Figure 4. These curves demonstrated quadratic patterns that are indicative of conductometric detection methods, both without and with chemical suppression for the cationic and anionic nitrogen species, respectively. In this analysis, a sensitivity diagram was employed using a synthetic mixture of cationic nitrogen species, which included ammonium and methylammonium, as well as anionic nitrogen species comprising nitrite and nitrate at various concentrations. The diagram, as shown in Figure 4, served a dual purpose: it was used for calibrating the instruments and for identifying the nitrogen species present in the water spinach and land spinach samples that were being examined, with the details of these findings presented in Table 1.

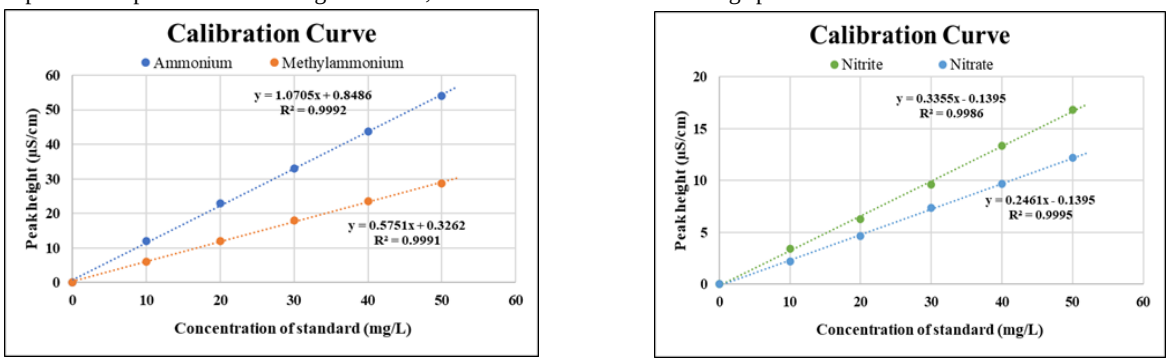


Figure 4: Calibration plots in the linear range of 10 to 50 mg/L to determine the cationic (ammonium and methylammonium) and anionic (nitrite and nitrate) nitrogen species (n=5 for each concentration).

3.2.3. Limit of detection (LOD) and limit of quantitation (LOQ)

To assess sensitivity, we determined the limit of detection and limit of quantitation (LOD and LOQ, respectively) for the studied cationic and anionic nitrogen species. LOD was calculated as three times the standard deviation (SD) of the results from five replicates, and LOQ was determined as three times the LOD value. The synthetic mixtures analysed contained minimum analyte concentrations (10 mg/L for each ionic nitrogen species) for the LOD and LOQ assessments.

Table 3: Data on limit of detection (LOD), limit of quantitation (LOQ), and retention time (t_R) of ionic nitrogen species

Nitrogen Species	LOD (mg/L)	LOQ (mg/L)	Retention times, t_R (min)
Cationic:			
Ammonium	0.068	0.225	5.14
Methyl ammonium	0.091	0.312	5.93
Anionic:			
Nitrite	0.011	0.037	8.31
Nitrate	0.016	0.053	10.42

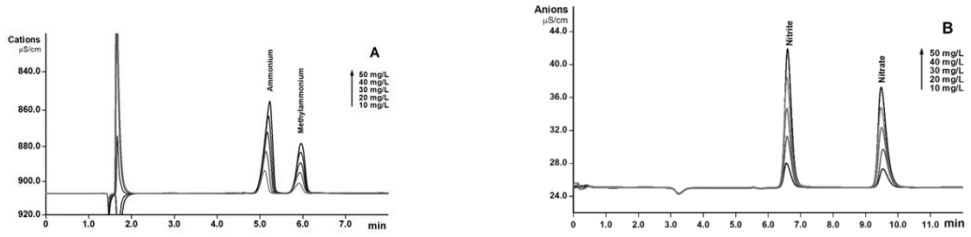


Figure 5: Sensitivity diagrams of (A) cationic and (B) anionic nitrogen species. Range of standard concentrations: 10, 20, 30, 40, and 50 mg/L. Other chromatographic conditions, as in Figure 2 for (A) and Figure 3 for (B).

3.3. Determination of cationic nitrogen species

The current research investigated the composition of cationic nitrogen species (ammonium and methylammonium) in leaves and stems of Indonesian water spinach (*Ipomoea Aquatica Forsk*) and land spinach (*Ipomoea Reptans Poir*) samples.

Samples were collected and processed following the methods outlined in collecting and preparing raw water spinach and land spinach section. Subsequently, the analysis was conducted after passing the samples through a 0.22-µm membrane filter.

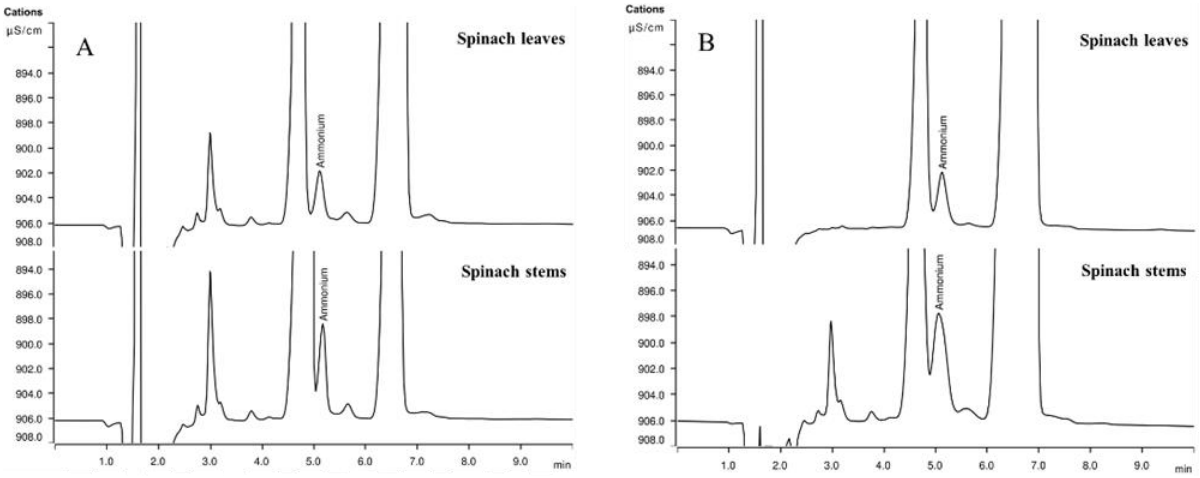


Figure 6: Ion chromatograms of cationic nitrogen species in water spinach (A) and land spinach (B) samples obtained from local traditional market. Other chromatographic conditions, as in Figure 2.

As indicated in Figure 6, the results show ammonium was found in all samples, both in the leaves and stems of water spinach and land spinach. Their identification was based on comparisons with a standard cationic nitrogen mixture (Figure 2).

Chromatogram displaying similar patterns were observed for the collected spinach. And as for methylammonium, the concentration is below the instrument's detection limit. Nevertheless, there were significant differences in the relative concentrations and compositions of the ammonium cationic among the two types of spinach from the three spinach sampling locations. Table 4 shows the summarized results of cationic nitrogen determination in 6 leaf extracts and 6 stem extracts of Indonesian water spinach (*Ipomoea Aquatica Forsk*) and land spinach (*Ipomoea Reptans Poir*). After determining and calculating the samples, the ammonium range in the water spinach leaves samples was found to be 24-52 mg/kg; in the water spinach stems, it was 59-79 mg/kg. The ammonium range in the land spinach leaves was 39-45 mg/kg; in the land spinach stems, it was 52-85 mg/kg.

The ammonium content in the water spinach leaves purchased from the local traditional market and the stems were 39 and 79 mg/kg, respectively. In contrast, in the land spinach, spinach leaves and spinach stem samples were 41 and 85 mg/kg, respectively. The ammonium content in the water spinach leaves purchased from the fresh food supermarket and the stems were 24 and 59 mg/kg, respectively.

As in the land spinach, spinach leaves and stem samples were 45 and 77 mg/kg, respectively. Similarly, in samples obtained from the spinach plantation area in Ternate City, the ammonium content in the water spinach leaves and the stems were 52 and 61 mg/kg, respectively. In the land spinach leaves and spinach stem, samples were 39 and 52 mg/kg, respectively.

A comparison of the ammonium range in leaves and stems of water spinach and leaves and stems of land spinach is presented in Figure 7. In the water spinach samples, the highest ammonium content in leaves was found in the sample from Ternate's spinach plantation area (52 mg/kg), followed by the local traditional market (39 mg/kg), and the least was from the fresh food supermarket (24 mg/kg). For stems, the highest was from the local traditional market (79 mg/kg), followed by the spinach plantation area (61 mg/kg), and lastly, the fresh food supermarket (59 mg/kg.

In the land spinach samples, the ammonium content in leaves from the local traditional market and the Ternate's spinach plantation area was quite close (41 mg/kg and 39 mg/kg, respectively), with the supermarket having a slightly higher content (45 mg/kg). For stems, the highest content was from the local traditional market (85 mg/kg), followed by the supermarket (77 mg/kg), and the least from Ternate's spinach plantation area (52 mg/kg). The use of ammonium-based fertilizers can influence the ammonium concentration in vegetables, with over-fertilization leading to elevated ammonium levels in the plant tissues [29]. The spinach samples purchased from the traditional market, land spinach, had slightly higher ammonium content in leaves and stems than water spinach. Whereas the samples from the fresh food supermarket, water spinach had lower ammonium content than land spinach for both leaves and stems. In contrast, in the Ternate's spinach plantation area, water spinach leaves had a higher ammonium content than land spinach, but the stems of land spinach had a lower content than water spinach.

Table 4: Summarized results of cationic nitrogen determination in 6 leaf extracts and 6 stem extracts of Indonesian water spinach (*Ipomoea aquatica Forsk*) and land spinach (*Ipomoea reptans Poir*).

Sampling location	Cationic nitrogen species	Concentration (mg/kg) Water spinach		Land spinach	
		Leaves	Stems	Leaves	Stems
Local traditional market	Ammonium	39	79	41	85
	Methylammonium	<LOD	<LOD	<LOD	<LOD
Fresh food supermarket	Ammonium	24	59	45	77
	Methylammonium	<LOD	<LOD	<LOD	<LOD
Spinach plantation area	Ammonium	52	61	39	52
	Methylammonium	<LOD	<LOD	<LOD	<LOD

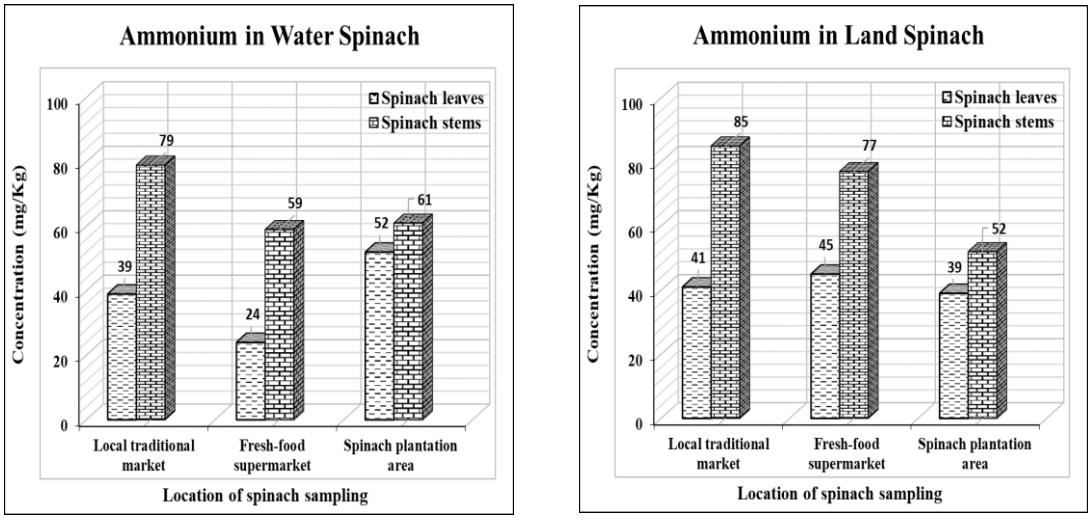


Figure 7. Comparison of ammonium concentration (mg/kg) of the two types in extract spinach from three different sampling locations.

3.4. Determination of anionic nitrogen species

These findings underscore the importance of monitoring nitrogen content in vegetables frequently consumed by humans. The high NO_3 content in spinach indicates a potential health risk if consumed excessively. Therefore, agricultural management and nitrogen fertilizer use require careful consideration to reduce adverse environmental and human health impacts [5][30].

This research revealed significant nitrogen composition differences between water and land spinach from three sampling locations. Land spinach has higher concentrations of NO_3 than water spinach, and NO_3 concentrations of land spinach are found more in the leaves than in the stems, as shown in Figures 8-10 and the summarized concentration in Table 5. The nitrate levels in water spinach leaves from the local traditional market were 787 mg/kg, while the stems had 33 mg/kg. On the other hand, land spinach leaves from the same market had 2230 mg/kg, and the stems had 11 mg/kg.

Water spinach leaves from the fresh food supermarket showed a nitrate concentration of 500 mg/kg, and the stems had 67 mg/kg. In contrast, land spinach from the same supermarket had 2923 mg/kg in the leaves and 3 mg/kg in the stems. Water spinach leaves contained 524 mg/kg of nitrate from the Ternate City plantation area, and the stems had 82 mg/kg. For land spinach from this location, the leaves had a nitrate content of 3327 mg/kg, and the stems had 107 mg/kg.

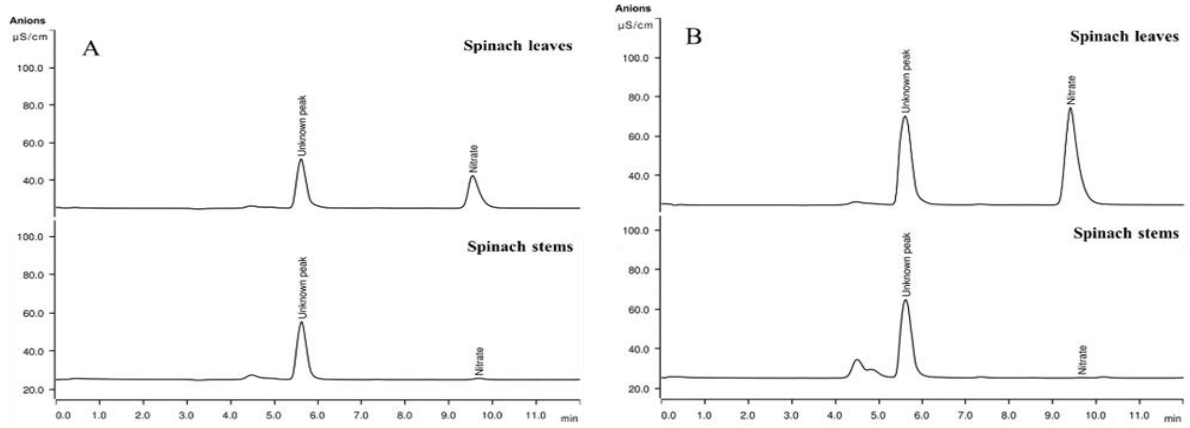


Figure 8. Ion chromatograms of anionic nitrogen species in (A) water spinach and (B) land spinach samples obtained from local traditional market. Other chromatographic conditions, as in Figure 3.

Figure 11 compares nitrate levels in both types of spinach, differentiating between leaves and stems.

A study by Anjana *et al.* reported that nitrate concentrations in fresh leafy vegetables procured from local markets ranged from 71 to 4293, 204 to 4451, 288 to 524, 289 to 769, and 684 to 1071 mg/kg fresh weight of samples for spinach, Chenopodium, fenugreek, coriander, and Sowa, respectively. In their study, especially for the spinach sample, aligns with this present study, where nitrate concentrations vary according to sampling location. However, the results obtained only showed the nitrate concentration in the leaves. Meanwhile, they did not examine the stem part of the studied vegetables [31].

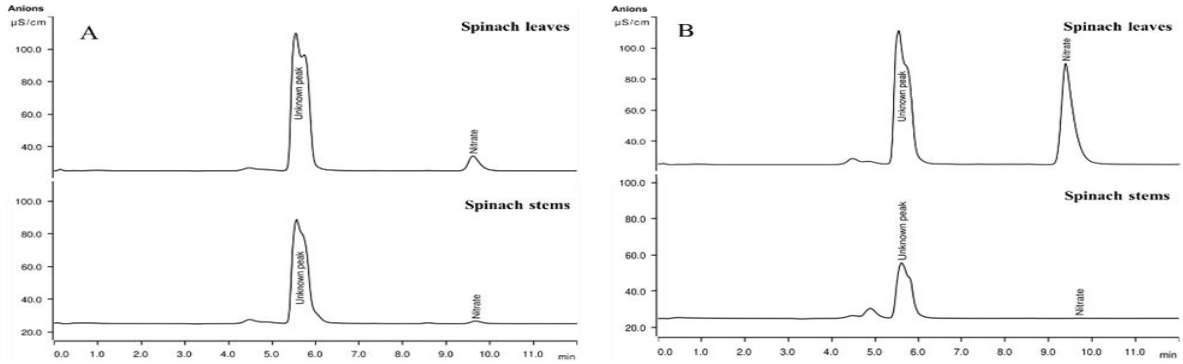


Figure 9: Ion chromatograms of anionic nitrogen species in (A) water spinach and (B) land spinach samples obtained from fresh food supermarket. Other chromatographic conditions, as in figure 3.

Upon examining nitrate levels in spinach from various sources, it's evident that land spinach leaves consistently have higher nitrate content than water spinach leaves, irrespective of their sampling locations. Specifically, land spinach leaves contain 2230 mg/kg at the local traditional market, while water spinach leaves have 787 mg/kg

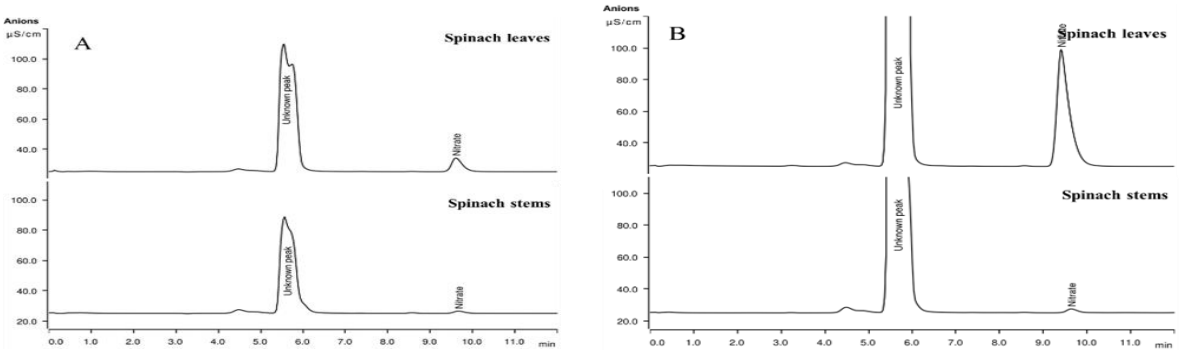


Figure 10: Ion chromatograms of anionic nitrogen species in (A) water spinach and (B) land spinach samples obtained from spinach plantation area in Ternate City. Other chromatographic conditions, as in figure 3.

This trend continues in the fresh food supermarket, where land spinach leaves have a notable 2923 mg/kg compared to the water spinach's 500 mg/kg. Similarly, land spinach leaves peak at 3327 mg/kg at the Ternate City plantation area, overshadowing water spinach's 524 mg/kg. Interestingly, water spinach stems generally contain more nitrates than their land spinach counterparts, with the highest difference seen at the fresh food supermarket, where water spinach stems have 67 mg/kg against land spinach was 3 mg/kg. These variances suggest that factors like farming practices, soil quality, and water sources might influence nitrate accumulation. Water spinach from the fresh food supermarket might be optimal for those keen on lower nitrate intake. Still, one should consider other factors like freshness, quality, and potential chemical exposures.

Table 5: Summarized results of anionic nitrogen determination in 6 leaf extracts and 6 stem extracts of Indonesian water pinach (*Ipomoea Aquatica Forsk*) and land spinach (*Ipomoea Reptans Poir*).

Sampling location	Anionic nitrogen species	Concentration (mg/kg)			
		Water spinach		Land spinach	
		Leaves	Stems	Leaves	Stems
Local traditional market	Nitrite	<LOD	<LOD	<LOD	<LOD
	Nitrate	787	33	2230	11
Fresh food supermarket	Nitrite	<LOD	<LOD	<LOD	<LOD
	Nitrate	500	67	2923	3
Spinach plantation area	Nitrite	<LOD	<LOD	<LOD	<LOD
	Nitrate	524	82	3327	107

<LOD=under detection limit

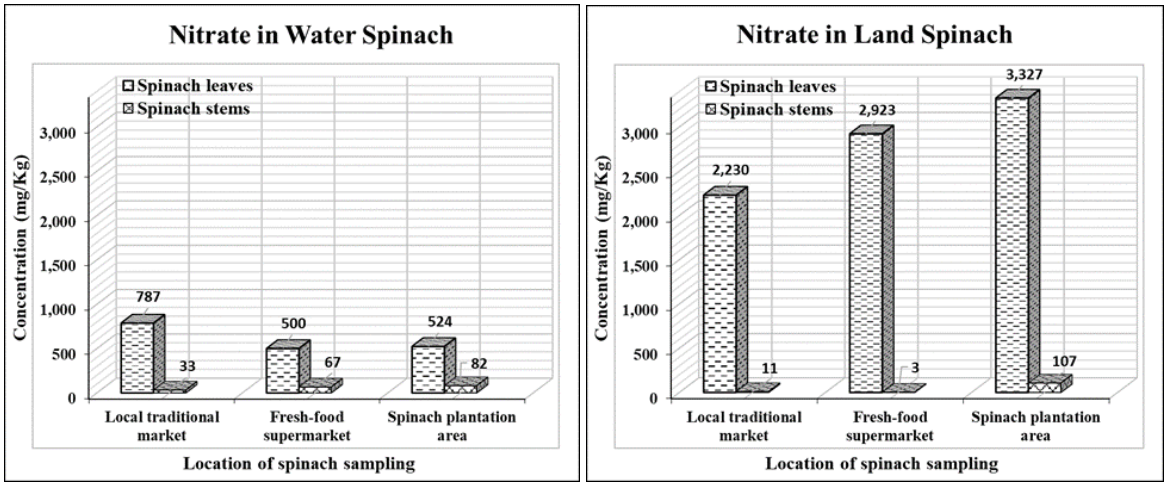


Figure 11: Comparison of nitrate concentration (mg/kg) of the two types in extract spinach from three different sampling locations.

4. Conclusions

Water spinach (*ipomoea aquatica forsk*) and land spinach (*ipomoea reptans poir*), which originate from the local traditional market, fresh food supermarket, and Ternate City spinach plantation area, which is determined using ion chromatography. The local traditional market consistently shows higher ammonium concentrations in both water and land spinach samples, which could indicate unregulated use of ammonium-based fertilizers. On the other hand, samples from the supermarket and Ternate City spinach plantation area show comparatively lower and more consistent ammonium concentrations. The land spinach leaves have a higher nitrate concentration than water spinach leaves, while water spinach stems tend to have a higher nitrate concentration than land spinach stems. Nitrate content varies depending on the
spinach source, which may reflect differences in farming practices, soil quality, and water sources. As for methylammonium and nitrite, their concentrations were under the limit of detection in all samples studied.

5. Conflicts of interest

The authors declare that they have no competing interests.

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