ALCHEMY Jurnal Penelitian Kimia

Official Website: https://jurnal.uns.ac.id/alchemy

ISSN 1412-4092
EISSN 2443-4183

Suppressed Ion Chromatographic Analysis of Anionic Macroelements in Nutmeg (*Myristica fragrans*) Diversity Specific to North Maluku

Muhammad Amin[a,b*], Deasy Liestianty[a], Abu Rahmat Ibrahim[c], Nahdiah Amin[d], Budhi Oktavia[e]

[a]*Department of Chemistry Education, Faculty of Teacher Training and Education, Universitas Khairun, Jalan Bandara Babullah, Akehuda, Ternate ,97723, North Maluku, Indonesia*
[b]*Technical Implementation Unit for Basic and Integrated Laboratory, Universitas Khairun, Jalan Jusuf Abdulrahman, Gambesi, Ternate, 97719, North Maluku, Indonesia*
[c]*Department of Agricultural Product Technology, Faculty of Agriculture, Universitas Khairun, Jalan Jusuf Abdulrahman, Gambesi, Ternate, 97719, North Maluku, Indonesia*
[d]*M.Sc Student, Life Science for Food Division, Department of Life Science and Chemistry, Faculty of Applied Biological Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[e]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, Jalan Prof. Dr. Hamka, Air Tawar, Padang, 25131, West Sumatera, Indonesia*

[*]*Corresponding author: muh_amin@unkhair.ac.id*

DOI: 10.20961/alchemy.20.1.80543.151-161

Received 19 November 2023, Revised 13 February 2024, Accepted 8 March 2024, Published 31 March 2024

Keywords:

anionic macroelements; electrolyte potential; ion chromatography; nutmeg (*Myristica fragrans*); suppressed system

ABSTRACT. This study proposed an ion chromatography method for analyzing anionic macroelements (fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate), and applied it to nutmeg (*Myristica fragrans)* diversity to explore the potential of nutmeg as an electrolyte. The analysis method was based on a suppressed conductivity system that simultaneously analyzed the anionic macroelements on Metrohm Supp A 250/4.0 column using 8.0 mM sodium carbonate (Na_2CO_3) and 0.25 mM sodium bicarbonate ($NaHCO_3$) as a mixture eluent. Excellent peak resolution and completed separation were achieved within 18 min when the flow rate of the detector was 0.8 mL/min. The peak height with calibration curves at 2.5 – 30.0 mg/L concentration ranges was obtained for all anionic macroelements. All collected nutmeg samples were specific to North Maluku. Based on the standard samples used, the analysis results revealed that only four anionic macroelements (chloride, nitrate, sulfate, and phosphate) were found in the nutmeg samples, and the concentration of each anion (e.g., chloride ion, and so) was different for each type of nutmeg. The highest total of anionic macroelements concentration was found in the *Myristica succedanea* (1605.05 mg/kg) nutmeg sample, while *Myristica specioga* had the lowest (661.76 mg/kg). The method was validated regarding the detection limit (LOD) and reproducibility.

INTRODUCTION

Nutmeg is a spice derived from the nut of the evergreen tree *Myristica fragrans*. This nutmeg fruit consists of four parts: *pericarpium*, *pulpam*, *fuli*, and seeds. Each piece of nutmeg has a unique flavor and aroma that can be used for various purposes. Among the best-known in the market are *fuli* and seeds as spices, and nutmeg oil is used for medicine. The *pericarpium*, the outermost layer of the nutmeg fruit, is rarely used in cooking or as a spice. However, it contains essential oils that are sometimes also used in perfumes and fragrances. The *pulpam*, the fleshy part of the nutmeg fruit, is not commonly used as a spice. However, it can be made into a refreshing health drink by juicing the fruit. The fleshy part of nutmeg, also known as the mace, is a lesser-known source of many essential elements, such as cationic and anionic macroelements (Rosmalia and Minarni, 2022; Naeem *et al.*, 2016).

Anionic macroelements are minerals that have negatively charged anions and are essential for the body as they maintain various processes that allow it to function correctly. Examples of anionic macroelements include fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate. These elements play an important role in regulating the pH balance in the body, maintaining the electrolyte balance, and aiding in the absorption of nutrients. Adequate intake of anionic macroelements through a balanced diet or supplements can help prevent various health problems such as dehydration, muscle cramps, and high blood pressure (Adeeb and Ali, 2023; Radwińska and Żarczyńska, 2015).

Cite this as: Amin, M., Liestianty, D., Ibrahim, A. R., Amin, N., & Oktavia B., 2024. Suppressed Ion Chromatographic Analysis of Anionic Macroelements in Nutmeg (*Myristica fragrans*) Diversity Specific to North Maluku. *ALCHEMY Jurnal Penelitian Kimia*, 20(1), 151-161. https://dx.doi.org/10.20961/alchemy.20.1.80543.151-161.

The chemical composition of nutmeg is complex, and it contains different compounds, including carbohydrates, proteins, structural fats, lipids, cationic minerals (such as potassium, magnesium, etc.), and anionic macroelements (such as fluoride, chloride, nitrite, nitrate, phosphate, sulfate, etc.) (Rahardiyan, Poluakan and Mauren, 2020). Fluoride and nitrite are important micronutrients that are required in small concentrations. However, if these elements are consumed in excessive quantities, they become toxic and may cause serious health problems (Alharbi and El-Sorogy, 2023).

Other macroelements are also needed in the human body, but they have health impacts if they are excessive. Chloride ions serve as vital electrolytes in the body, regulating pH and facilitating ion transport. However, when they are excessive in the body, they can cause dehydration and imbalance of electrolytes. Phosphate ions act as electrolytes, ensuring muscle and nerve function, fluid balance, and energy production; however, excess phosphorus can harm kidney health and blood vessels. Several other anionic macroelements, such as nitrate and bromide, especially nitrate ions, do not function as electrolytes in the human body. Excess nitrate can pose health risks and be associated with certain diseases. Therefore, it is essential to control nitrate intake from food and drinking.

A recent trend in the health nutritional market is the consumption of electrolyte drinks. These drinks help replenish the body's fluids and electrolytes lost during physical activity or exercise (Muñoz-urtubia *et al.*, 2023); (Ruiz and García, 2022). They are popular among health enthusiasts, athletes, and health-conscious individuals who want to maintain optimal hydration levels and improve performance (Volterman and Moore, 2014).

Nutmeg fruits and seeds contain cationic and anionic macroelements, which have the potential to refresh electrolytes in a healthy drink. However, only a few studies have revealed the nutmeg's content of anionic macroelements such as fluoride, chloride, nitrite, nitrate, sulfate, and phosphate. Therefore, it is important to conduct further research to analyze the anionic macromineral content in nutmeg and its ionic compositions in each species of nutmeg to understand its nutritional properties and potential electrolytes.

There are several methods to analyze anionic macroelements in water samples, including spectrometry UV-Vis for water quality analysis (Guo *et al*., 2020; Mussa *et al*., 2009) and electrochemical method for detecting nitrate (Khodari, 2020; Remes *et al*., 2009). Meanwhile, some classical methods are available, such as colorimetry, which is a technique for analyzing fluoride ions in drinking groundwater (Amereih *et al*., 2013), a gravimetric method to determine sulfate (Ismail *et al*., 2023), and potentiometry methods for determining chloride (Berger, 2012). However, these classic wet methods require intensive labor and intervention and are often difficult to automate. Moreover, it can only be used to determine individual anionic macroelements.

Since its introduction in 1975 by Small, ion chromatography (IC) has proven to be a reliable scientific analysis tool (Weiss, 1995). This tool is commonly employed in analyzing the concentration of cationic and anionic macroelements in various liquid samples (Amin *et al*., 2023; Michalski, 2006; Amin *et al.*, 2007; Amin *et al*., 2008). IC has several advantages over other chromatographic techniques, including high sensitivity, selectivity, and efficiency. It uses an anion exchanger as the stationary phase to determine the anionic macroelements. The eluant used for this purpose is usually a dilute solution of sodium carbonate and sodium bicarbonate. In the suppressed IC, the anionic macroelements are converted to highly conductive acid forms, while in the carbonate bicarbonate eluant, the anions are converted to weakly conductive carbonic acid. The separated acid forms are then measured by conductivity and identified based on their retention time compared to their standards (Jenke, 2011). Capillary electrophoresis (CE) is a method that can also analyze anionic macroelements in water samples, such as coconut water (Richter *et al.*, 2005). In the study, it could analyze chloride, sulfate, and phosphate. However, the weakness of this method is that it had a high detection limit, so it is not very effective in analyzing the macroelements if their concentration is low in the actual samples.

The study aims to report a suppressed IC method for analyzing seven anionic macroelements such as fluoride, chloride, bromide, nitrite, nitrate, sulfate, and phosphate in fruits and seeds nutmeg species, involving minimum pre-treatment sample preparation and processing. This anionic macroelement is important to explore because it has natural electrolyte potential and, at the same time, identifies the nutritional value of each nutmeg, especially the specific nutmeg that grows in North Maluku.

RESEARCH METHODS

The IC equipment used in the experiment was the Eco IC by Metrohm, a compact and versatile system. The equipment consisted of an IC Pump, an IC Separation Center, an MSM Press with a Pump unit, and a conductivity detector controlled by Metrohm magIC Net 3.2 software designed explicitly for analyzing anionic macroelements.

The separation of anionic macroelements was carried out in suppressor mode using a highly efficient Metrosep A Supp 17-250/4 analytical column (250 mm × 4 mm i.d.) connected in series with a Metrosep A Supp 17 Guard/4.0 column (5 mm × 4.0 mm i.d.) that provided additional protection to the analytical column. This setup allowed for accurate and reliable detection of anionic macroelements in the samples.

Standard Solution and Eluent Preparations

The standard solutions were prepared from 1000 mg/L IC-certified multi-elements (fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate) stocks (Merck). The dilution process was carried out to create a calibration curve with five concentrations with wide variations for all anionic macroelements. Details of each anionic macroelement concentration ranged from 2.5 – 7.5 mg /L for fluoride, 3.25 – 15 mg/L for chloride, 5 – 15 mg/L for nitrite, 6.25 – 20 mg/L for three anions (bromide, nitrate, and sulfate), and 10 – 30 mg/L for phosphate, by preparing approximately 10 µL aliquot injections. Before analyzing the samples, a 0.22 µm pore size nylon-mesh disposable syringe filter (Merck) was used to remove particulates that could interfere with the analytes. This step was performed for all samples to ensure consistent and reliable results. New standards and eluents were prepared daily to avoid any chromatogram inconsistencies and ghost peaks that sometimes appeared in the chromatograms, which could result in inaccurate readings. These steps ensured that our results were as precise and accurate as possible.

The eluent was prepared from guaranteed-reagent-grade chemicals (Merck) using deionized water. The eluent carried the sample through the chromatography column and was prepared using a mixture of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$). However, in this study, Na_2CO_3 was set at a fixed concentration of 8 mM, while the concentration of $NaHCO_3$ varied at 0.25, 0.5, and 0.75 mM to examine the effects of variation in $NaHCO_3$ concentration on Na_2CO_3. The eluent flowed through the chromatography column at a 0.8 mL/min rate under the standard operating conditions for Metrosep A Supp 17-250/4 columns that utilize chemical suppression. These conditions offered the optimum separation and elution times for anionic macroelements. A 0.5 M sulfuric acid (H_2SO_4) regenerated the suppressor. Furthermore, the injection volume loop was set at 10 L, indicating the sample volume introduced into the chromatography system.

Nutmeg sampling

This research procured nutmeg samples from small-scale farmers in Ternate City and Tidore Island - two regions renowned for their high-quality nutmeg, as shown in Figure 1. The procurement process involved selecting only the ripest and most aromatic nuts, ensuring that the final sample was of the utmost quality. Once collected, all nutmeg samples were washed with deionized water. This step was crucial to avoid contamination and keep the natural aroma and flavor of the nutmeg. To maintain the quality of the samples, they were then carefully stored in sealed polyethylene bags to prevent any exposure to the external environment. It ensured that the nutmeg used in the study was of the highest quality and free from contaminants.

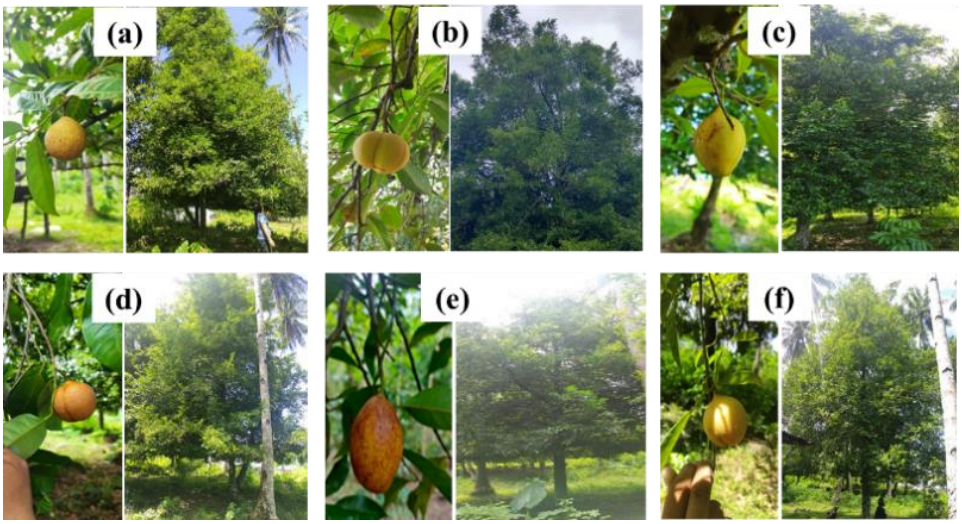

Figure 1. The nutmeg plantation area in Jambula (Ternate) and Tidore Island, North Maluku, has a variety of nutmeg species labeled: (a) *Myristica malabarica*, (b) *Myristica succedanea*, (c) *Myristica specioga*, (d) *Myristica facttua*, (e) *Myristica argentea*, and (f) *Myristica fragnans.*

Extract Nutmeg Preparation

The process of preparing the nutmeg extract involved several steps. Initially, the bulk nutmeg was separated into fruit and seeds. The separation process ensured that the two parts were kept separate to avoid contamination. After separation, individual nutmeg fruit and seed samples were obtained and cut into pieces measuring 0.5 – 1.0 cm. The size of the pieces was chosen to facilitate the extraction process. To extract the nutmeg compounds, 20 g of each sample was poured into separate 100 mL polypropylene centrifuge tubes. Then, 10 mL of deionized water was added to each tube. Deionized water was used to ensure that the water used did not contain any contaminants that could interfere with the extraction process. After adding the water, each sample was homogenized using a regular household mixer to ensure the nutmeg pieces were well mixed. The resulting slurry of samples was then squeezed and filtered through filter papers. This step was necessary to remove solid materials that were not soluble in water. After filtering, the tubes were covered and shaken for 5 min to ensure thorough mixing of the samples. The next step involved centrifuging the tubes at 2,500 rpm for 5 min. Centrifugation was necessary to separate the liquid from the solid materials. After centrifugation, the liquid portion of the samples was filtered again through a 0.22 µm pore size nylon-mesh disposable syringe filter (Merck). This filter aimed to remove any residues that might have formed during extraction. Once the samples were filtered, they were ready for injection into the IC system. The photographs of the nutmeg extract in different species are shown in Figure 2. The figure visually represents nutmeg extracts and their differences obtained from various places.

The difference in color between nutmeg fruit and seed extracts when dissolved in distilled water may be due to different pigment content and chemical compositions. Nutmeg seeds tend to be darker because they contain higher amounts of pigments. Meanwhile, nutmeg fruits contain less pigment, which causes a lighter color.

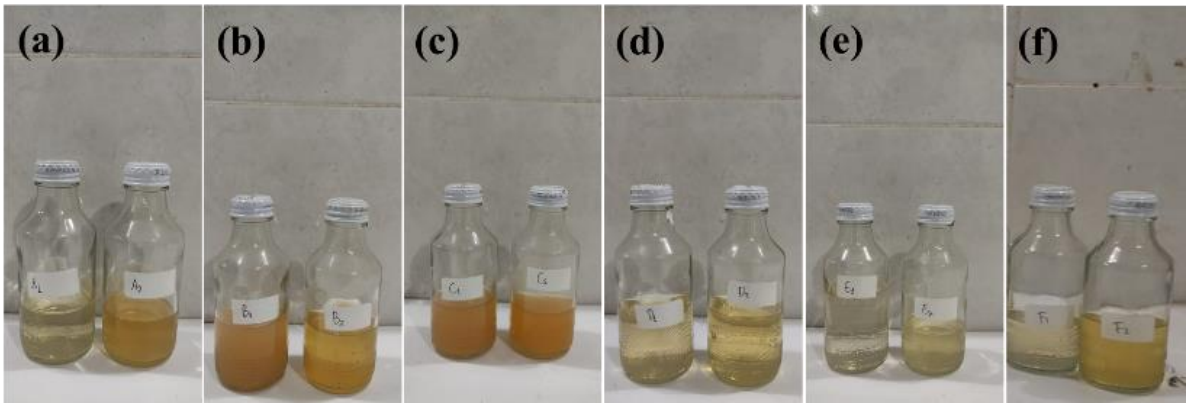

Figure 2. Extracts of nutmeg fruits and seeds of six diverse nutmeg were found in North Maluku. Samples type: nutmeg fruits (A_1, B_1, C_1, D_1, E_1, and F_1), and nutmeg seeds (A_2, B_2, C_2, D_2, E_2, and F_2). Nutmeg species: (a). *Myristica malabarica*, (b). *Myristica succedanea*, (c). *Myristica specioga*, (d). *Myristica fattua* €. *Myristica argentea*, and (f). *Myristica fragrans*.

RESULTS AND DISCUSSION

The Effects of Differences Concentrations in Combined Eluent

The chromatograms of seven anionic macroelements at different eluent concentrations have been presented in Figure 3. The graph shows that as the eluent concentration increased, the retention time values for fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate decreased, indicating that the anions eluted faster. The results demonstrate that the eluent concentration is important in separating anionic macroelements. Currently, the concentration of Na_2CO_3 was set to be fixed, while the concentration of $NaHCO_3$ was varied. Interestingly, when the combination eluent concentration was 8.0 mM Na_2CO_3 and 0.75 mM $NaHCO_3$, this resulted in a longer analysis time, although it provided good separation of the anionic macroelements. The longer analysis time could lead to a delay in the analysis, which is not desirable. Therefore, finding a combination of eluent that provides good separation of the anionic macroelements in a shorter analysis time is important.

Furthermore, when the combination eluent was 8.0 mM Na_2CO_3 and 0.5 mM $NaHCO_3$, the sulfate (as divalent anionic macroelements) tended to overlap with phosphate (as trivalent), making it difficult to distinguish between the two peaks. The overlap of two peaks makes it impossible to analyze the exact concentration of each ion. Therefore, choosing the right combination of eluents is crucial to avoid peak overlap. Based on the results in

Figure 3, a combination eluent concentration of 8.0 mM Na_2CO_3 and 0.25 mM $NaHCO_3$ provided good separation of the anionic macroelements in a shorter analysis time.

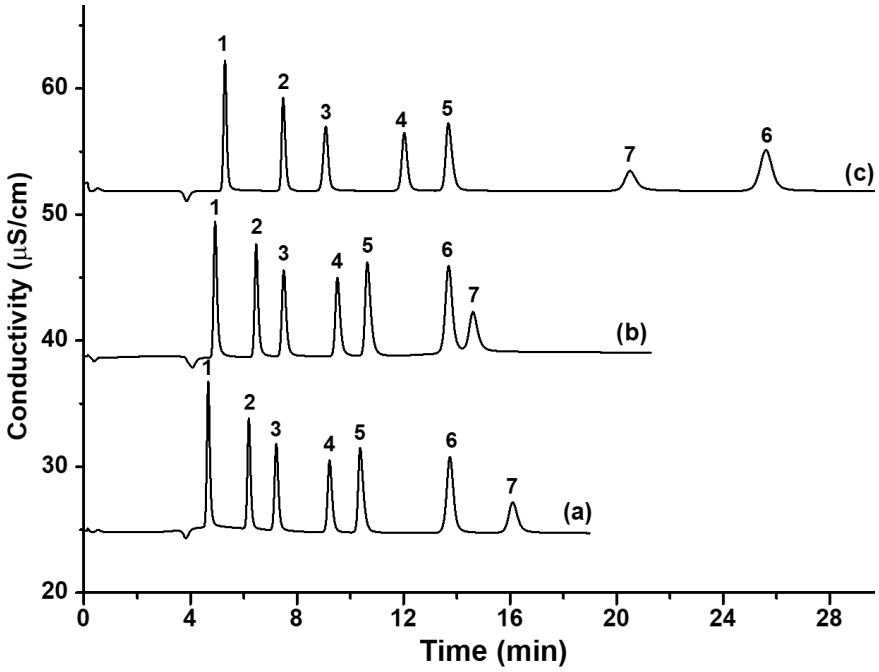

Figure 3. Different combinations of eluent concentrations were used to obtain an ion chromatography profile for anionic macroelements at the retention time. Eluent concentrations: (a) 8.0 mM Na_2CO_3 + 0.25 mM $NaHCO_3$, (b) 8.0 mM Na_2CO_3 + 0.5 mM $NaHCO_3$, and (c) 8.0 mM Na_2CO_3 + 0.75 mM $NaHCO_3$. Separation of the column: Metrosep A Supp 17-250/4. Eluent flow rate: 0.8 mL/min. Injection loop volume: 10 µl. Column temperature: 35 ℃. Anionic macroelements (concentration in mg/L): 1=fluoride (7.5), 2 = chloride (10), 3 = nitrite (15), 4 = bromide (20), 5 = nitrate (20), 6 = sulfate (20), and 7 = phosphate (30).

Analysis of Anionic Macroelements using Standard Sample

The method described in Figure 3, especially 3a, successfully separated the seven anionic macroelements, fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate, within 18 min. The difference in the elution order of sulfate and phosphates anionic macroelements in Figure 3(a-c) can be attributed to the differing chemical properties of the two anionic macroelements and the influence of eluent concentration. Sulfate and phosphate ions have distinct chemical characteristics affecting their interactions with the stationary phase and relative migration rates in the chromatographic systems. The interactions between ions and the stationary phase can also be influenced by eluent concentration, which can alter the strength of interactions and, consequently, the elution order of anionic macroelements in IC. Therefore, as in Figure 3(a), the eluent combination was the optimal combination of eluent concentrations to achieve satisfactory separation for all anionic macroelements and was selected for further experiments. This combination of eluent provided good separation of the anionic macroelements in a shorter analysis time.

Validation of the Method

In order to ensure the accuracy and reliability of the analytical results, several validation methods were employed. These methods included determining detection limits (LOD), selectivity, calibration curve, and the relative standard deviation (RSD).

Detection limits (LOD)

In the present study, the detection limits (LOD) were determined by injecting a 10-L volume of the standard solution and were calculated at the signal-to-noise ratio (S/N) of 3. The basis of the S/N = 3 approach is defining the LOD as that analyte concentration large enough to produce a signal (peak) more significant than the noise, with a ratio of 3:1. The S/N = 3 method is commonly used in determining LOD in chromatographic / voltametric

/ spectroscopic methods (Desimoni and Brunetti, 2015). The method used in the study was found to be effective in analyzing anionic macroelements, and the results are shown in Table 1. The detection limit for all the anionic macroelements ranged between 2.52 – 48.51 µg/L.

Table 1. Summarized data on detection limit (LOD), regression equation, and retention time (t_R) of anionic macroelements.

Anionic macroelements	LOD (µg/L)		Regression equation	Retention times, t_R (min)
	Present study	Previous study[a]		
Fluoride	2.52	-	y = 1.4064x+0.7720	4.67
Chloride	4.46	90	y = 0.7874x+0.3125	6.19
Nitrite	8.51	-	y = 0.4624x+0.0061	7.22
Bromide	13.72	-	y = 0.2919x+0.1213	9.21
Nitrate	11.64	-	y = 0.3390x–0.0963	10.37
Sulfate	12.94	120	y = 0.3247x–0.4432	13.74
Phosphate	48.51	500	y = 0.0844x–0.1561	16.09

[a]Richter *et al.*, 2005

Selectivity

The retention of the seven focused anionic macroelements using Na_2CO_3 and $NaHCO_3$ as the combination of eluent followed the order of fluoride > chloride > nitrite > bromide > nitrate > sulfate > phosphate, analysis and separation were obtained within 18 min. The selectivity of the anion-exchanger column used in this study, which has a quaternary ammonium functional group, was evaluated by injecting an individual element standard solution into the IC system. Also, this retention order is based on their ionic charge and relative size. The single ionic charge and small size will appear earlier in the eluate because they have a lower affinity for the resin. Meanwhile, ions with a double charge and larger size will appear later in the eluate because they have a higher affinity for the resin. The retention sequence of these anionic macroelements has been mentioned in Table 1.

Calibration curve

The data presented in Table 1 demonstrate that the regression equation for all anionic macroelements is linear, meaning that the response of the analytical method is proportional to the concentration of all elements. Figure 4 and Table 2 illustrate the variation of standard sample concentrations, including the sensitivity diagram for all anionic macroelements. The regression coefficient (r) values ranging from 0.9991 to 0.9997 indicate a strong correlation between the anionic macroelement's concentration and the analytical method's response, as shown in Figure 5. It means that the proposed method is highly accurate and reliable for measuring the concentration of anionic macroelements.

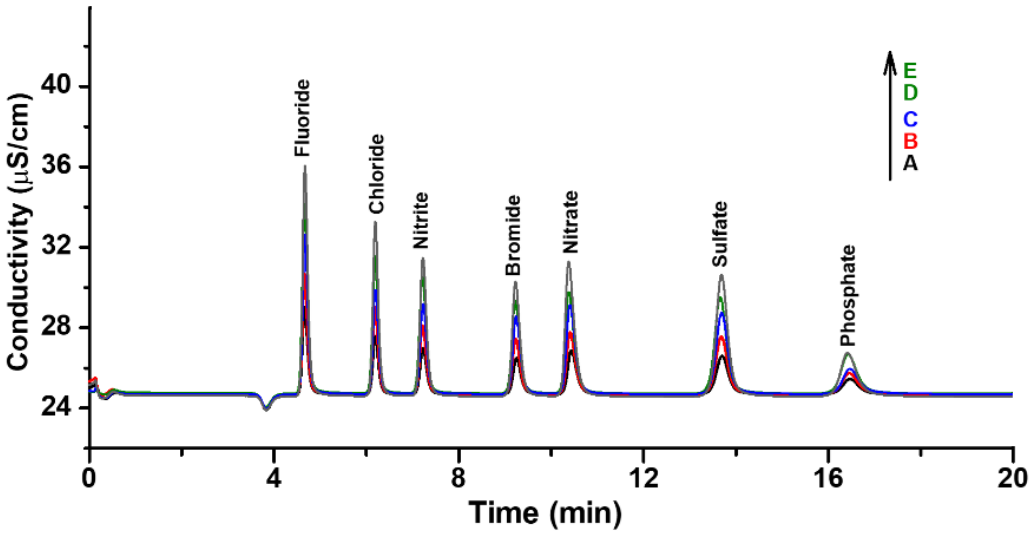

Figure 4. The sensitivity diagrams of anionic macroelements. The other operating conditions, as in Figure 3a.

Table 2. Grouping of anionic macroelement standard concentrations range

Anionic macroelements	Range of standard concentration (mg/L)				
	Group A	Group B	Group C	Group D	Group E
Fluoride	2.50	3.75	5.00	6.25	7.5
Chloride	3.25	5.00	6.25	8.25	10.0
Nitrite	5.00	7.50	10.00	12.50	15.0
Bromide	6.50	10.00	13.25	16.50	20.0
Nitrate	6.50	10.00	13.25	16.50	20.0
Sulfate	6.50	10.00	13.25	16.50	20.0
Phosphate	10.00	15.00	20.00	25.00	30.0

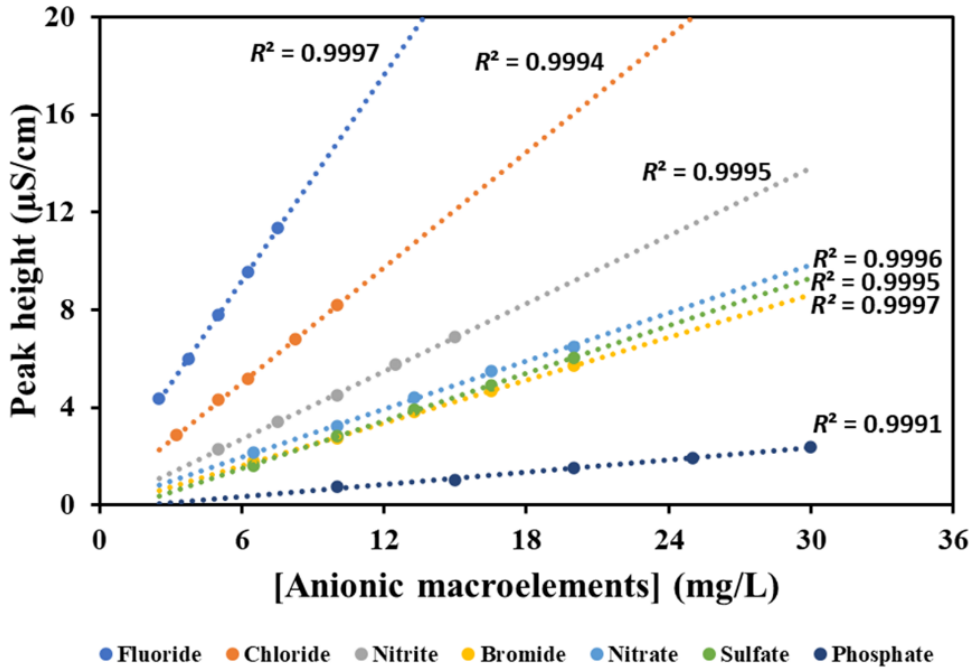

Figure 5. The calibration plots of anionic macroelements.

Relative standard deviation (RSD)

The information presented in Table 3 provides detailed data on the retention time and repeatability of analyte signals over five consecutive analyses carried out under the specific conditions outlined in Figure 3(a). The analysis focuses explicitly on anionic macroelements, with the detector responses for retention time, peak height, and peak area showing a relative standard deviation (RSD) range of 0.48 – 1.76%, 0.64 – 1.85%, and 0.83 – 1.96%, respectively. This data was to be used to evaluate the degree of precision and accuracy of the analytical method used to analyze anionic macroelements. The RSD value can be calculated using the formula RSD = $(s/\overline{x})$.100%, where the *s* value is the data's standard deviation and $\overline{x}$ is the measurement data's average. Repeated measurements were carried out in this study with standard samples five times.

Table 3. Relative standard deviation (RSD) of anionic macroelements under the chromatographic conditions in Figure 3(a).

Anionic macroelements	RSD (%), n=5		
	Retention time	Peak height	Peak area
Fluoride	0.48	0.64	0.83
Chloride	0.62	0.43	0.69
Nitrite	1.77	1.08	1.07
Bromide	1.59	1.11	0.96
Nitrate	1.27	1.73	1.48
Sulfate	1.59	1.78	1.96
Phosphate	1.76	1.85	1.79

Application to the Diverse North Maluku Nutmeg Samples

The main aim of this study was to examine the anionic macromineral of six diverse nutmeg species: (a) *Myristica malabarica*, (b) *Myristica succedanea*, (c) *Myristica specioga*, (d) *Myristica facttua*, (e) *Myristica argentea*, and (f) *Myristica Fragnans*) found in North Maluku. To conduct the analysis, samples were prepared using the abovementioned method, which entailed the extraction of nutmeg extract and filtration through a 0.22 µm pore size nylon-mesh disposable syringe filter.

The results of the analysis revealed the presence of several anionic macroelements in all the nutmeg species studied. These macroelements were identified as fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate. The identification was based on comparisons of a standard anionic macroelements mixture, as shown in Figure 3(a). The chromatograms obtained for all the nutmeg species collected showed a similar pattern, as depicted in Figure 6. However, there were significant differences in the species' relative concentrations and compositions of the anionic macroelements. Table 4 demonstrates this study's results, revealing that chloride, nitrate, sulfate, and phosphate were the most prevalent macroelements in fruits and seeds. Additionally, the research discovered differences in anionic macroelements among various nutmeg species.

The study results showed that the phosphate content in the seed was higher than that of the fruit. Furthermore, the mineral amounts in the fruit of all six nutmeg species showed significant variations for all seven anionic macroelements, including chloride, sulfate, and phosphate. The chloride content ranged from 17.63 to 79.55 mg/kg, sulfate ranged from 51.65 to 165.89 mg/kg, and phosphate ranged from 94.36 to 313.92 mg/kg. Similarly, in all the nutmeg seeds, the content of chloride ranged from 36.75 to 224.12 mg/kg, nitrate ranged from 2.74 to 5.15 mg/kg, sulfate ranged from 145.30 to 327.53 mg/kg, and phosphate ranged from 248.65 to 808.09 mg/kg. No collected nutmeg fruit samples contained fluoride, bromide, or nitrate. On the other hand, fluoride and bromide were not found in any of the nutmeg seed samples collected, but nitrate was found in all types of nutmeg. These findings suggest that nutmeg can be a good source of macro elements for the human body, especially phosphate, chloride, and sulfate. These macro elements are crucial in maintaining bone health, fluid balance, and nerve function. However, more research is needed to understand these element's bioavailability and potential health benefits.

Table 4. Summarized data for anionic macroelements in fruits and seeds nutmeg samples. The chromatographic conditions, as in Figure 3(a).

Nutmeg species	Samples type	Anionic Macroelements Concentration (mg/kg)							Total (mg/kg)
		Fluoride	Chloride	Nitrite	Bromide	Nitrate	Sulfate	Phosphate	
Myristica malabarica	fruit	n.d.	79.55	n.d.	n.d.	n.d.	87.95	313.92	1086.25
	seed	n.d.	73.59	n.d.	n.d.	2.74	157.65	370.85	
Myristica succedanea	fruit	n.d.	76.24	n.d.	n.d.	n.d.	163.05	291.65	1605.05
	seed	n.d.	224.12	n.d.	n.d.	n.d.	327.53	522.45	
Myristica specioga	fruit	n.d.	35.55	n.d.	n.d.	n.d.	51.65	94.36	661.76
	seed	n.d.	59.45	n.d.	n.d.	2.98	169.12	248.65	
Myristica fattua	fruit	n.d.	37.49	n.d.	n.d.	n.d.	100.86	158.15	1021.38
	seed	n.d.	67.80	n.d.	n.d.	3.81	277.12	376.15	
Myristica argentea	fruit	n.d.	17.63	n.d.	n.d.	n.d.	77.69	122.58	1294.25
	seed	n.d.	36.75	n.d.	n.d.	3.15	228.45	808.09	
Myristica fragrans	fruit	n.d.	32.65	n.d.	n.d.	n.d.	165.89	192.71	926.89
	seed	n.d.	80.05	n.d.	n.d.	5.15	145.30	305.14	

n.d.=not detected

The analysis of the fruit sample results revealed that *Myristica malabarica* had the highest chloride concentration at 79.55 mg/kg. In contrast, the sample of *Myristica argentea* had the lowest concentration at 17.63 mg/kg. Sample *Myristica fragrans* showed the highest sulfate concentration at 165.89 mg/kg, while sample *Myristica specioga* had the lowest at 51.65 mg/kg. In the end, the analysis indicated that sample *Myristica malabarica* had the highest phosphate concentration at 313.92 mg/kg, and sample *Myristica specioga* had the lowest concentration at 94.36 mg/kg. The obtained results suggest a significant variation in the levels of these three chemicals across the different samples, indicating that the samples may have come from different sources or experienced different environmental conditions.

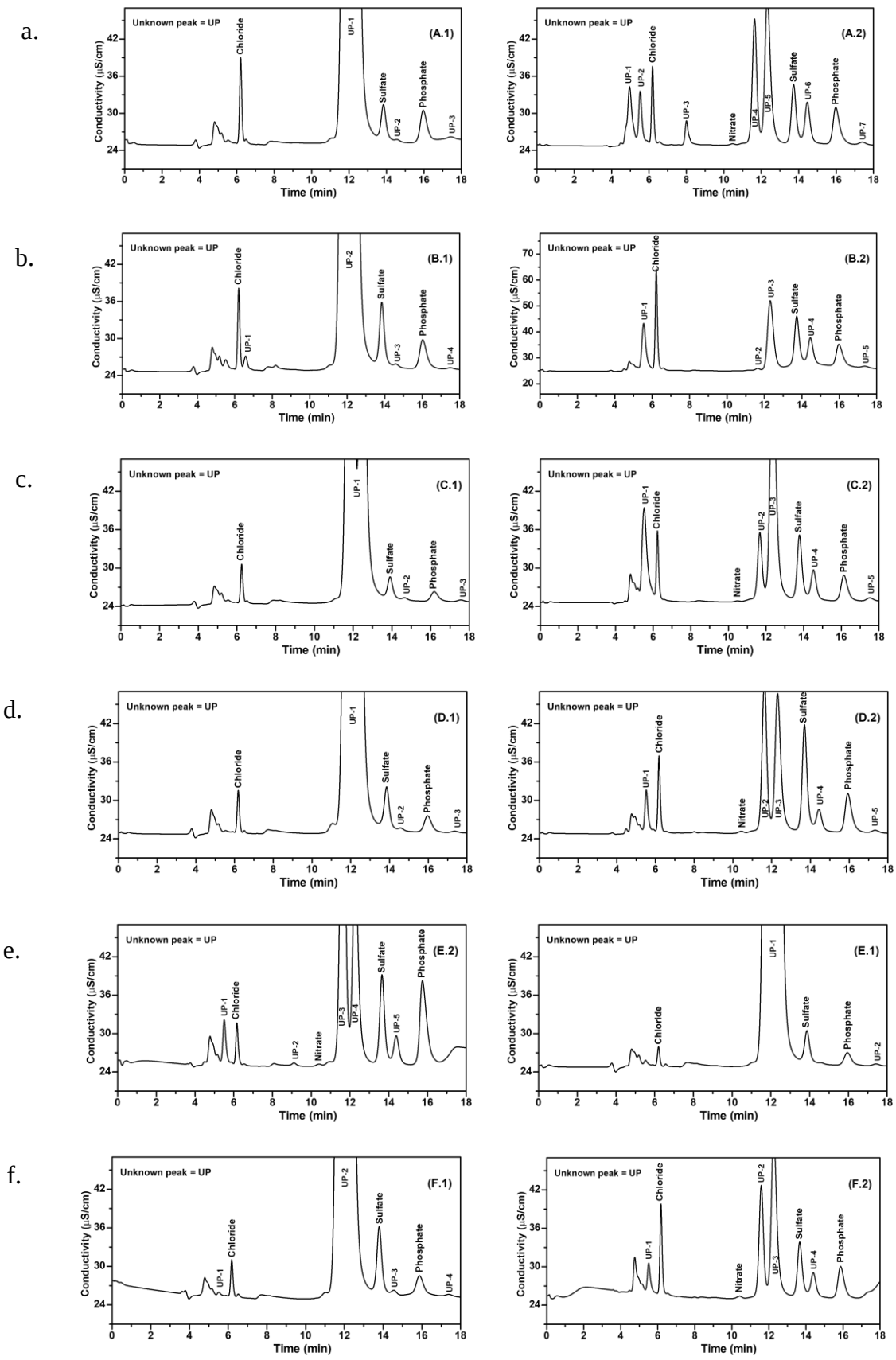

Figure 6. Ion chromatograms of anionic macroelements in (A.1, B.1, C.1, D.1, E.1, and F.1) nutmeg fruits and (A.2, B.2, C.2, D.2, E.2, and F.2) nutmeg seeds samples. The examined nutmeg species: a. *Myristica malabarica*, b. *Myristica succedanea*, c. *Myristica specioga*, d. *Myristica fattua* e. *Myristica argentea*, and f. *Myristica fragrans*. The other chromatographic conditions, as in Figure 3a.

In the seed samples, sample *Myristica succedanea* had the highest chloride concentration among all samples, with a significant amount of 224.12 mg/kg, compared to the lowest concentration of chloride observed in sample *Myristica argentea*, which was only 36.75 mg/kg. Similarly, sample *Myristica fragnans* had the highest nitrate content, with a concentration of 5.15 mg/kg, and the lowest nitrate concentration was observed in sample *Myristica malabarica*, which was only 2.74 mg/kg. Furthermore, sample *Myristica succedanea* had the highest sulfate concentration, 327.53 mg/kg, and sample *Myristica fragnans* had the lowest sulfate concentration, only 145.30 mg/kg. Lastly, sample *Myristica argentea* had the highest phosphate concentration, with a level of 808.09 mg/kg, and sample *Myristica specioga* had the lowest phosphate concentration, with 248.65 mg/kg. These results could be significant for further research in seed analysis and could prove helpful in agriculture and farming practices.

In Figure 6, several unknown peaks appear in the chromatogram. As in all types of nutmeg samples, there are some possibilities for the appearance of additional compounds or ionic elements on the chromatograms when analyzing the anion content. It includes sample contamination, anionic macroelements that were not previously identified, chemical reactions, and contamination in the chromatography column. However, all unknown peaks have been confirmed using the standard anionic macroelements, which are not the target anionic elements in this study.

CONCLUSION

The present study was to develop a practical analytical methodology in IC to analyze anionic macroelements in six diverse nutmegs (*Myristica fragrans)* collected from North Maluku. The study included the targeted anionic macroelements, including fluoride, chloride, nitrite, bromide, nitrate, sulfate, and phosphate to be separated. The method employed 8.0 mM Na_2CO_3 and 0.5 mM $NaHCO_3$ as an eluent combination to enhance sensitivity and allow for the analysis of all anionic macroelements. The obtained detection limits ranged from 2.52 – 48.51 µg/L, significantly lower than another previous method. The study results showed that *Myristica succedanea* had the highest total concentration of macroelements (1605.05 mg/kg) while *Myristica specioga* had the lowest (661.76 mg/kg). It was also found that fluoride, bromide, and nitrite were under the method's detection limit in the nutmeg fruit and seed samples. The presented method has several advantages over other methods. Determining all the anionic elements is possible, and the detection limits are also significantly lower. The results of this study will be helpful for researchers and stakeholders interested in information on the actual composition of anionic microelements in nutmeg, its nutritional properties, and the potential for electrolyte balance in nutmeg species drinks.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTIONS

MA, NA, and BO: Conceptualization, supervision, design of the study, analysis tools, writing original draft preparation. DL, ARI, and NA: reagents/sampling/preparation. DL and NA: statistical analysis and validation. All authors have read, reviewed, and approved the manuscript.

REFERENCES

Adeeb, A., and Ali, H., 2023. Overview of the Vital Roles of Macro Minerals in the Human Body. *Journal of Trace Elements and Minerals*, 4(June), 100076. https://doi.org/10.1016/j.jtemin.2023.100076.

Alharbi, T., and El-Sorogy, A. S., 2023. Health Risk Assessment of Nitrate and Fluoride in the Groundwater of Central Saudi Arabia. *Water*, 15(12), 1–12. https://doi.org/10.3390/w15122220.

Amereih, S., Barghouthi, Z., and Majjiad, L., 2013. Colorimetric Determination of Fluoride in Drinking Groundwater Using a Polymeric Zirconium Complex of 5-(2-Carboxyphenylazo)-8- Hydroxyquinoline. *Journal of Advances in Chemistry*, 4(2), 460–465.

Amin, M., Liestianty, D., Bahri, S., Ibrahim, A. R., Muliadi, and Amin, N., 2023. Ion Chromatographic Composition of Macromineral Cations in Diversity of North Maluku Nutmeg Species (Myristica spp.) Fruits Potentially as Electrolyte Supplement. *AIP Conference Proceedings*, 2720(040003), 1–10. https://doi.org/10.1063/5.0137059.

Amin, M., Lim, L. W., and Takeuchi, T., 2007. Tunable Separation of Anions and Cations by Column Switching in Ion Chromatography. *Talanta*, 71(4), 1470–1475. https://doi.org/10.1016/j.talanta.2006.07.020.

Amin, M., Lim, L. W., and Takeuchi, T., 2008. Determination of Common Inorganic Anions and Cations by Non-Suppressed Ion Chromatography with Column Switching. *Journal of Chromatography A*, 1182(2), 169–175. https://doi.org/10.1016/j.chroma.2008.01.007.

Berger, M., 2012. Potentiometric Determination of Chloride in Natural Waters: An Extended Analysis. *Journal of Chemical Education*, 89, 812–813. https://doi.org/10.1021/ed2005449.

Desimoni, E., and Brunetti, B., 2015. About Estimating the Limit of Detection by the Signal to Noise Approach. *Pharmaceutica Analytica Acta*, 6(3), 1–4. https://doi.org/10.4172/2153-2435.1000355.

Guo, Y., Liu, C., and Ye, R., 2020. Advances on Water Quality Detection by UV-Vis Spectroscopy. *Applied Sciences*, 10(19), 1–18. https://doi.org/10.3390/app10196874.

Ismail, I. A., Budiman, S., Arkhaan, F. N., Nihati, I., Irvandi, Sepriyanata, and Afifah, N., 2023. Gravimetric Determination of Sulphate. *IJCC: Indonesian Journal of Chemical Connections*, 1(1), 30–35.

Jenke, D., 2011. Application of Ion Chromatography in Pharmaceutical and Drug Analysis. *Journal of Chromatographic Science*, 49(7), 524–539. https://doi.org/10.1093/chrsci/49.7.524.

Khodari, M., 2020. Electrochemical Determination of Some Anions Using Different Models of Electrodes. http://dx.doi.org/10.13140/RG.2.2.13561.29289.

Michalski, R., 2006. Ion Chromatography as a Reference Method for Determination of Inorganic Ions in Water and Wastewater. *Critical Reviews in Analytical Chemistry*, 36(2), 2011. https://doi.org/10.1080/10408340600713678.

Muñoz-urtubia, N., Vega-muñoz, A., Estrada-muñoz, C., Salazar-sep, G., Contreras-barraza, N., and Castillo, D., 2023. Healthy Behavior and Sports Drinks : A Systematic Review. *Nutrients*, 15(2915), 1–14. https://doi.org/10.3390/nu15132915.

Mussa, S. A. Ben., Elferjani, H. S., Haroun, F. A., and Abdelnabi, F. F., 2009. Determination of Available Nitrate, Phosphate, and Sulfate in Soil Samples. *International Journal of PharmTech Research*, 1(3), 598–604.

Naeem, N., Rehman, R., Mushtaq, A., and Ghania, J. Ben., 2016. Nutmeg: A Review on Uses and Biological Properties. *International Journal of Chemical and Biochemical Sciences*, 9, 107–110.

Radwińska, J., and Żarczyńska, K., 2015. Effects of Mineral Deficiency on the Health of Young Ruminants. *Journal of Elementology*, 19(3), 915–928.

Rahardiyan, D., Poluakan, M., and Mauren, E., 2020. Physico-chemical Properties of Nutmeg (Myristica fragrans houtt) of North Sulawesi Nutmeg. *Fullerene Journal Of Chem*, 5(1), 23–31. https://doi.org/10.37033/fjc.v5i1.146.

Remes, A., Manea, F., Sonea, D., and Burtica, G., 2009. Electrochemical Determination of Nitrate from Water Sample Using Ag-doped Zeolite-Modified Expanded Graphite Composite Electrode. *Ovidius University Annals of Chemistry*, 20(1), 61–65.

Richter, E. M., Jesus, D. P. De, Muñoz, R. A. A., and Lago, C. L. 2005. Determination of Anions , Cations , and Sugars in Coconut Water by Capillary Electrophoresis. *Journal of the Brazilian Chemical Society*, 16(6), 1134–1139. https://doi.org/10.1590/S0103-50532005000700008.

Rosmalia, D., Minarni, and Marjoni, M. R., 2022. Effect of Nutmeg (Myristica Fragrans) Methanolic Extract to the Growth of Dental Plaque Bacteria. Denta. *Jurnal Kedokteran Gigi*, 16(2), 74–80. https://doi.org/10.30649/denta.v16i2.3.

Ruiz, Y., and García, M. A., 2022. Isotonic Sports Drinks : Formulation and Physiological Effects of Their Consumption. *QhaliKay. Revista de Ciencias de La Salud*, 6(2), 73–84. https://doi.org/10.33936/qkrcs.v6i2.4534.

Volterman, K. A., and Moore, D. R., 2014. Hydration for Optimal Health and Performance in Athletes. *Agro Food Industry Hi Tech*, 25(2), 4–7.

Weiss J, 1995. Ion chromatography. 2nd ed., VCH, Weinheim. https://doi.org/10.1002/jhrc.1240190411.