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Original Paper

Determination of Water-Soluble Vitamins in Palmyrah Sweet Sap and Its Derived Products using RP-HPLC Method

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***Abstract*—Palmyrah sweet sap, obtained from the inflorescence of the *Borassus flabellifer* L. palm tree, is a popular natural drink in Sri Lanka, available seasonally from January to August. It contains a blend of water, sugars, and essential vitamins and minerals. The sweet sap is processed thermally to produce sugary products like crude sugar, jaggery, and treacle. It is crucial to analyze them to ensure their nutritional value, due to the possibility of vitamin decomposition during the production stage. This study aimed to quantify the existing vitamins in sweet sap and its derived products using validated high-performance liquid chromatography (HPLC). The HPLC method validation parameters, such as linearity, accuracy, precision, range, limit of detection (LOD), and limit of quantification (LOQ), were determined. The results showed that the derived products from palmyrah sap had high concentrations of vitamins, with thiamine ranging from 2.81 to 35.16 mg/100g, niacin from 4.35 to 45.03 mg/100g, pyridoxine from 4.44 to 87.16 mg/100g, and ascorbic acid from 5.42 to 52.52 mg/100g. The accuracy of the HPLC method for vitamin analysis was between 98.43 and 100.64%, with a limit of detection ranging from 0.357 to 1.152 ppm and a limit of quantification from 1.081 to 13.573 ppm. The study successfully quantified the existing vitamins in the sap and its derived products, with overall results indicating that the vitamins were retained even after thermal processing.**

Keywords*— *chromatography, crude sugar, jaggery, treacle, validation

I. INTRODUCTION

Palmyra (*Borassus flabellifer* L.) is referred to as the "tree of life" in Tamil, indicating that it yields a wide range of products [1]. The palm tree has various edible parts, including fruits, seed shoots, sap from the inflorescence, and kernels from both young and mature nuts [2]. Palmyrah products contain a variety of compounds, including bioactive components such as tannins, saponins, and phenols, as well as dietary fibers [3]. These products possess antioxidant, anti-diabetic, anti-microbial, anti-obesity, anti-mutagenic, anti-clastogenic, and immunosuppressive properties.

Products made from Palmyrah sweet sap are referred to as Palmyrah sap-based products, such as crude sugar, treacle, and jaggery. The sap is a clear, sweet liquid with a low viscosity and no color, and its pH typically ranges from 7 to 7.4, although this may vary from location to location [4]. The sweet sap contains various vitamins, including ascorbic acid, thiamine, riboflavin, pyridoxine, tocopherol, and niacin [5, 6]. Jaggery is a crystallized solid made from concentrated, unfermented Palmyrah sweet sap and contains vitamins such as riboflavin, thiamine, niacin, ascorbic acid, and cobalamin. It has several medicinal properties, including being antitoxic and anti-carcinogenic and having a cooling effect that may reduce the risk of lung cancer [7]. Treacle is made by heating the Palmyrah sweet sap to one-sixth of its original volume, resulting in a thick, dark syrup [8]. Palm sugar is less processed and unrefined compared to sugarcane sugar and has a low glycemic index. When heated, the sap undergoes the Maillard reaction and caramelization [9,10]. Sugar, jaggery, and treacle are used as food additives [11] instead of table sugar, and Palmyrah sap-based products are enriched with vitamins. However, there are limited studies that quantitatively analyze these vitamins.

Vitamins are complex organic compounds that are crucial to normal metabolism, health, reproduction, and growth. The daily requirement of vitamins is low, ranging from micrograms to milligrams [12, 13]. Vitamins can be classified into two groups based on their solubility: water-soluble vitamins (vitamins B and C), and fat-soluble vitamins (vitamins A, D, E, and K) [14]. Fat-soluble vitamins are found in fatty foods and are transported and absorbed along with dietary lipids [12]. Thiamine was the first discovered vitamin and cannot be synthesized by any animal species. It is stable at high temperatures above 100°C and is absorbed in the small intestine, where it is mostly found in muscle tissue in the human body [14, 15]. Thiamine has a slightly bitter taste and a sulfurous odor, and is hygroscopic [16]. Lean pork, offal, kidney, liver, egg yolk, grains, and cereals are good sources of thiamine, with muscle containing 50% of the total thiamine in humans [17]. Niacin is a simple vitamin that is a white,

crystalline, odorless solid, soluble in alcohol and water. It is more stable in alkaline medium and is resistant to heat, light, and air [18]. Nicotinic acid and nicotinamide are the two forms of niacin, which are absorbed in the stomach and small intestine and excreted in urine in humans [12]. Vitamin B6 is a group of six compounds, including pyridoxal, pyridoxine, pyridoxamine, pyridoxal 5'-phosphate, pyridoxine 5'-phosphate, and pyridoxamine 5'-phosphate. Good food sources of vitamin B6 include liver, offal, beef, poultry, pork, fish, bananas, and potatoes [19, 20]. Pyridoxal phosphate is the main form of vitamin B6 found in foods, although some forms are destroyed by cooking temperatures [21]. Vitamin C is available in two forms: reduced ascorbic acid and oxidized dehydroascorbic acid. Ascorbic acid, the main form of vitamin C, is a strong reducing agent and is a powerful antioxidant that scavenges free radicals and reactive oxygen species [22]. It is widely used in the food industry as an antioxidant, particularly in the canning of fruits and meat. Ascorbic acid is more stable in an acidic medium than an alkaline medium and is destroyed by high temperatures [23].

It is crucial to ensure the presence of vitamins in sweet sap-based products after their production, which involves thermal processing. High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for the separation and quantification of vitamins in complex samples. HPLC separates the components of a sample based on differences in their physical and chemical properties, allowing for accurate and precise quantification of specific vitamins. The choice of stationary and mobile phase, as well as the type of HPLC column used, will depend on the specific vitamins being analyzed and their chemical properties. There are various applications of HPLC in vitamin analysis, including the determination of vitamins in food, supplements, and biological fluids. The HPLC method should be validated to ensure the reliability and accuracy of the results, and the method should be proven to be suitable for its intended purpose [24]. This validation process involves checking the linearity, accuracy, precision, robustness, range, limit of detection (LOD), limit of quantification (LOQ), selectivity, and ruggedness of the method [25]. These parameters play a critical role in the evaluation of all quantitative analyses.

II. Materials And Methods

Chemicals and raw materials

The vitamin standards, thiamine (B1), niacin (B3), pyridoxine (B6), and ascorbic acid, were procured from Sigma-Aldrich. The mobile phases used in the study, including HPLC-grade methanol, HPLC-grade water, potassium dihydrogen phosphate, and orthophosphoric acid, were also sourced from Sigma-Aldrich. The sweet sap, treacle, jaggery, and sugar used in the study were randomly collected from Chavakacheri Palm-Cooperative societies in Jaffna District, Sri Lanka.

Instrumentation

The determination of water-soluble vitamins was carried out using a validated high-performance liquid chromatography (HPLC) method with slight modifications, as described in the study by Sami et al. [26]. The analysis was performed using the Ultimate 3000 system from Thermo Scientific equipped with an RS wavelength detector. Chromatographic separations were carried out on a reversed-phase HPLC column with C18 stationary phase (Hypersil Gold) with dimensions of 250 x 4.6 mm and a particle size of 5 µm. An isocratic delivery mobile phase consisting of 5% methanol and 95% 0.1 M KH_2PO_4 buffer with pH 3.5 was used at a flow rate of 1 ml/min, with a sample injection volume of 10 µL. The ultraviolet absorbance was measured at 270 nm at 25°C.

Standard and sample preparation

The standard stock solutions of thiamine, niacin, pyridoxine, and ascorbic acid (each 100 ppm) were prepared by dissolving 10 mg of each vitamin in 100 ml of 0.1 M potassium dihydrogen phosphate buffer solution with pH 3.5. The identification of vitamins was confirmed by injecting the standards separately and using their respective retention times. The calibration curves of the working standards were made by serial dilution of the stock standards (different sets of standard dilutions) with buffer solution. The calibration curves were constructed from chromatograms as peak area vs. concentration of standard.

An amount of 2.5 g of sweet sap sample and 1.25 g of each sample such as jaggery, sugar, and treacle were separately dissolved in 25 ml of 0.1 M potassium dihydrogen phosphate buffer solution with a pH of 3.5. The samples were homogenized using a vortex mixer and centrifuged at 4000 RPM for 15 minutes. The supernatant solution was collected and stored in the refrigerator at freezing temperature until analysis. The sample extracts were filtered through a 0.45 µm pore-sized nylon syringe filter paper before injection. A 10 µL aliquot of the filtrate was subjected to HPLC analysis.

Vitamin identification and quantification

The vitamins were identified by comparing them with their respective vitamin standards using the retention time. The concentration of each vitamin was then quantified by creating calibration curves, which were constructed by plotting the peak area versus the concentration of the standards.

HPLC Method validation

Analytical method validation was performed in terms of linearity, range, accuracy, limit of detection, and limit of quantification for water-soluble vitamin analysis.

Limit of Detection (LOD): The lowest amount of an analyte that can be detected by the signal is called the limit of detection [27]. It is not a quantitative measurement. LOD depends on the sensitivity of the instrumental setup. It is valid for a single analyte according to the particular analytical method [28]. This can be calculated using Equation 1.

$$LOD = 3.3\ \sigma/S \quad \text{(Equation 1)}$$

Where, σ = standard deviation of the response, &S = slope of the curve.

Limit of Quantitation (LOQ): The lowest amount of analyte that can be quantitatively measured with precision and accuracy [29]. LOQ is calculated by Equation 2.

$$LOQ = 10\ \sigma/S \quad \text{(Equation 2)}$$

Where, σ = standard deviation of the response, & S = slope of the curve. LOQ is equal to 3× LOD.

Linearity: It is one of the important method validation parameters that can obtain results of variable data that are proportional to the concentration of the analyte. Linearity is determined by correlation coefficient (r). The r value close to 1 is considered as perfect linear calibration.

Accuracy: It indicates the closeness of the test result to the accepted or certified reference value. It highly depends on precision and trueness. Accuracy is usually expressed as recovery percentage by assay. It is obtained using an average of nine determinations over three or above concentration levels [30].

Data Analysis

The one-way ANOVA was performed to determine the difference between samples, and the Tukey paired-wise comparison method was used to identify the differences between sample means. Results were presented as mean values with standard deviation respectively

III. RESULTS AND DISCUSSION

In the development of the HPLC method, we followed a previously reported procedure [26] but made some modifications. The study utilized a C18 column that was 250 mm in length and utilized both rapid separation and the Ultimate 3000 liquid chromatography system to determine the polyphenolic content. To identify the vitamins, four different vitamin standards were used for comparison with the chromatograms generated from Palmyrah sweet sap and its derived products. From Figure 1, it can be observed that a good separation can be achieved within 10 min using the above conditions described. Symmetrical, sharp, and well-resolved peaks were observed for the four vitamin standards. The elution order and the retention times for ascorbic acid, thiamine, niacin, and pyridoxine were 3.5, 3.7, 4.5, and 5.2 min respectively. Method validation parameters were obtained from the analysis of chromatograms for vitamin working standards as shown in Table 1. These values indicate that the developed HPLC method can be used for quantify the vitamins B1, B3, B6 and C.

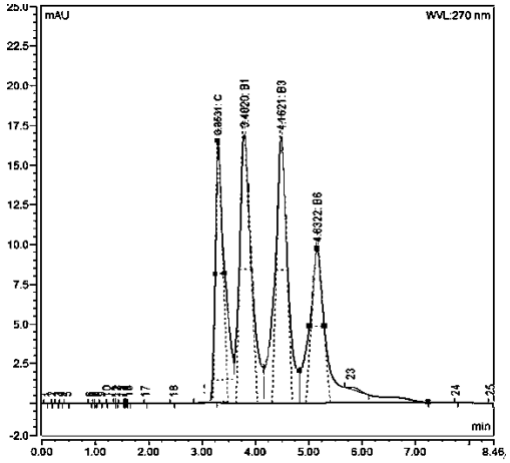

Fig 1. HPLC chromatogram for each vitamin standard

Figure 1 depicts the calibration curve for each vitamin standard. According to Table 1, validation parameters include the recovery mean/accuracy, slope, intercept, linearity range, correlation coefficient, standard error of intercept, standard deviation of intercept, limit of detection (LOD), and limit of quantitation (LOQ). The recovery mean/accuracy indicates the percentage of the actual amount of vitamin recovered compared to the expected amount, and it is expressed as mean ± standard deviation. The recovery mean/accuracy was found to be high for all four vitamins, with Thiamine having the highest value (99.99 ± 0.81%) and Niacin having the lowest value (98.43 ± 5.28%). The slope and intercept values are parameters obtained from the regression analysis and are used to calculate the concentration of vitamins in the samples. The slope values range from 0.1694 to 0.2309, while the intercept values range from -0.059 to 0.2174. The linearity range is the range of concentrations in which the regression analysis is considered to be linear. The linearity ranges for the four vitamins are 2-20 ppm for Thiamine, 4-50 ppm for Niacin and Pyridoxine, and 1-10 ppm for Ascorbic Acid. The correlation coefficient (r) indicates the degree of linearity of the regression analysis.

TABLE 1. HPLC METHOD VALIDATION PARAMETERS FOR EACH VITAMIN

Validation parameter	Thiamine	Niacin	pyridoxine	Ascorbic acid
Recovery Mean/Accuracy (%)	99.99 ± 0.81	98.43 ± 5.28	99.29 ± 3.69	100.64 ± 4.48
Slope	0.2309	0.2094	0.1694	0.2223
Intercept	0.0658	0.2174	-0.059	0.0498
Linearity range (ppm)	2 – 20	4 – 50	4 - 50	1 – 10
Correlation coefficient (r)	0.9999	0.998	0.9995	0.9995
SE of intercept	0.010	0.100	0.057	0.017
SD of intercept	0.025	0.284	0.162	0.039
LOD (ppm)	0.357	4.4792	1.152	0.571
LOQ (ppm)	1.081	13.573	3.550	1.733

All four vitamins had high correlation coefficients, with values ranging from 0.998 to 0.9999. The standard error of intercept (SE of intercept) and the standard deviation of intercept (SD of intercept) indicate the precision of the regression analysis. The SE of intercept values ranged from 0.010 to 0.100, while the SD of intercept values ranged from 0.039 to 0.284. The limit of detection (LOD) is the lowest concentration of a substance that can be accurately detected, while the limit of quantitation (LOQ) is the lowest concentration of a substance that can be accurately quantified. The LOD values for the four vitamins ranged from 0.357 ppm for Thiamine to 4.4792 ppm for Niacin, while the LOQ values ranged from 1.081 ppm for Thiamine to 13.573 ppm for Niacin. The validation parameters indicate that the analysis method used was precise and accurate for determining water-soluble vitamins in samples, with high recovery mean/accuracy, high correlation coefficient, and low limit of detection and limit of quantitation.

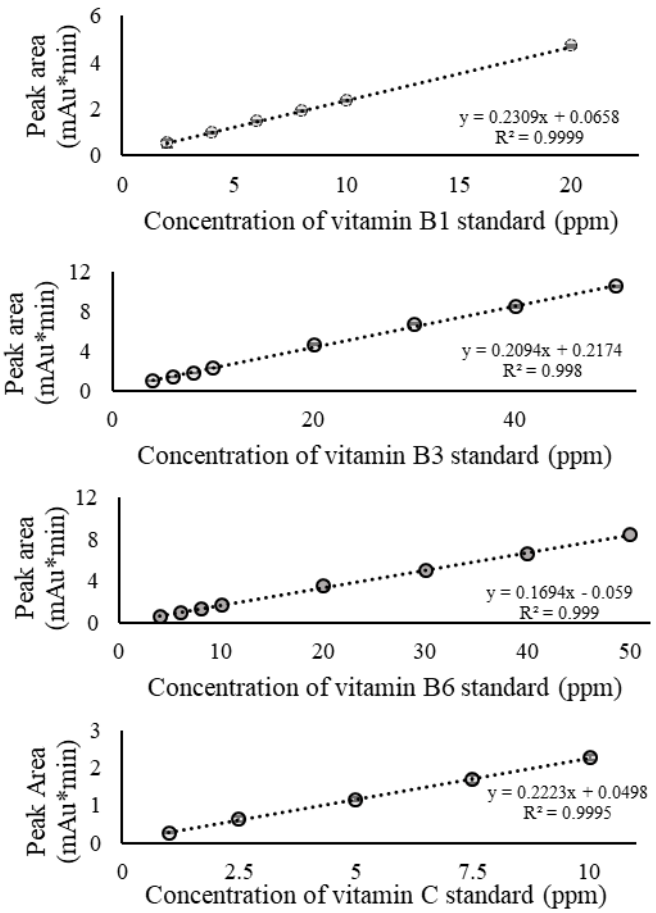

Fig 2. calibration curve for each vitamin standard

Water-soluble vitamin identification, thiamine (B1), niacin (B3), pyridoxine (B6), and ascorbic acid were identified using the retention times of their corresponding standards. Table 2 shows the quantitative values of water-soluble vitamins in different samples of Palmyrah sap and its derived products. For Thiamine, the highest concentration was found in the treacle sample (35.16 ± 6.72) mg/100g followed by crude sugar (27.68 ± 5.54) mg/100g and jaggery (23.31 ± 3.51) mg/100g. The lowest concentration was found in the Palmyrah sap sample (2.81 ± 0.83). For Niacin, the highest concentration was found in the treacle (45.03 ± 3.23) mg/100g and crude sugar (43.52 ± 2.78) mg/100g samples, while the lowest concentration was found in the Palmyrah sap sample, which is less than the LOD. Therefore, it can be stated that the concentration of niacin was not detected by the developed HPLC method. For Pyridoxine, the highest concentration was found in the treacle (87.16 ± 3.94) mg/100g, followed by jaggery (57.78 ± 1.13) mg/100g and crude sugar (55.13 ± 1.97) mg/100g. The lowest concentration was found in the Palmyrah sap sample (4.44 ± 0.17) mg/100g. For Ascorbic acid, the highest concentration was found in the treacle (52.52 ± 3.83) mg/100g followed by crude sugar (50.31 ± 3.18) mg/100g and jaggery (49.84 ± 2.81) mg/100g. The lowest concentration was found in the Palmyrah sap sample (5.42 ± 0.39) mg/100g. The concentration of thiamine increases as the sap is concentrated to form treacle, resulting in higher thiamine levels in treacle compared to sweet sap. It is important to note that the processing temperature should not rise beyond 107 °C, as high temperatures can cause degradation or loss of vitamins. Similarly, the concentrations of other vitamins such as Niacin, Pyridoxine, and Ascorbic Acid also show an increase in their levels as the sap is concentrated to form other products like jaggery and crude sugar. The amount of vitamins in the sap-based products can be used as a reference to understand the impact of temperature on their retention during processing. It may be due to the matrix of sap. Generally, the matrix of food can either protect or degrade the vitamins in it. The matrix effect of food refers to the interactions between the various components of the food, such as vitamins, minerals, and antioxidants, and how these interactions can affect the stability of the vitamins in the food [31]. In the case of sap-based products, the high concentration of antioxidants [32] in the sap can help to protect the vitamins from degradation during higher temperature processing. The antioxidants act as scavengers for reactive oxygen species, which are formed as a result of heat-induced oxidation [33]. This helps to reduce oxidative stress, which can cause the vitamins to break down and lose their activity. Additionally, the presence of antioxidants can also reduce the formation of harmful by-products during high-temperature processing. The matrix effect of sap-based products can play a key role in maintaining the stability and activity of vitamins during processing at higher temperatures.

TABLE II. QUANTITATIVE VALUES OF WATER-SOLUBLE VITAMINS IN SAMPLES

Vitamins	Palmyrah sap and its derived products (mg/100g)			
	Sweet sap	Treacle	Crude sugar	Jaggery
Thiamine	2.81±0.83^{b}	35.16±6.72^{a}	27.68±5.54^{a}	23.31±3.51^{a}
Niacin	ND	45.03±3.23^{a}	43.52±2.78^{a}	40.30±3.77^{a}
Pyridoxine	4.44±0.17^{c}	87.16±3.94^{a}	55.13±1.97^{b}	57.78±1.13^{b}
Ascorbic acid	5.42±0.39^{b}	52.52±3.83^{a}	50.31±3.18^{a}	49.84±2.81^{a}

Mean±SD (n=3); Tukey comparison of mean values indicates the means within a row that contains different letters are significantly different (p <0.05). ND-Not Detected

Hence, by controlling the processing temperature and conditions, it is possible to retain vitamins in sap-based products. However, the exact extent of vitamin retention will depend on the specific vitamins and processing conditions used.

IV. CONCLUSION

This study demonstrates the high nutritional value of Palmyrah sap as a natural health drink in Sri Lanka. The results of the analysis showed significant differences in the content of water-soluble vitamins among palmyrah sap, sugar, treacle, and jaggery, with treacle having the highest concentration of vitamins B1, B3, B6, and C. The accuracy and sensitivity of the high-performance liquid chromatography

method used in this study confirmed the validity of the results. The results indicate that the matrix effect of sap can play a role in retaining vitamins in sap-based products during processing, which are rich in antioxidants. However, the temperature should be controlled and kept within a safe range as high temperatures can lead to degradation and decreased concentrations of vitamins. The findings highlight the importance of incorporating traditional health drinks such as palmyrah sap into modern diets, especially for those seeking natural sources of essential vitamins and minerals. This study provides valuable information for future research in the field of natural health drinks and the development of nutritional products from traditional sources.

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REFERENCE

[1] Jansz, E. R., Wickremasekara, N. T. and Sumuduni, K. A. V. (2002) 'A review of the chemistry and biochemistry of seed shoot flour and fruit pulp of the palmyrah palm (borassus flabellifer L)', Journal of the National Science Foundation of Sri Lanka, 30(1–2), pp. 61–87. doi: 10.4038/jnsfsr.v30i1-2.2562.

[2] Naguleswaran, S., Vasanthan, T., Hoover, R. and Liu, Q. (2010) 'Structure and physicochemical properties of palmyrah (Borassus flabellifer L.) seed-shoot starch grown in Sri Lanka', Food Chemistry. Elsevier Ltd, 118(3), pp. 634–640. doi: 10.1016/j.foodchem.2009.05.046.

[3] Mohd Hassan, R., Bakar, J., Abdul Rahman, R., & Syed Muhamad, S. K. (2019). Flabelliferin removal by sodium salts and sodium hydroxide: Pretreatment in Borassus flabellifer mesocarp. *Malaysian Journal of Fundamental and Applied Sciences*, *15*(2–1), 313–318. https://doi.org/10.11113/mjfas.v15n2-1.1544

[4] Saidi, I. A., Efendi, N., Azara, R. and Hudi, L. (2018) 'Indigenous technology in utilizations and handlings of palmyra palm (Borassus flabellifer L.) sap and its quality from two regions of East Java', IOP Conference Series: Materials Science and Engineering, 420(1). doi: 10.1088/1757-899X/420/1/012067.

[5] Sankaralingam, A., Hemalatha, G. and Mohamed Ali, A. (1999) A TREATISE ON PALMYRAH. Edited by H. Hameed khan. India: All India Coordinated reseach project 88 on Palms, Agricultural College & Reseach Institute.

[6] Hebbar, K., Pandiselvam, R., Manikantan, M., Arivalagan, M., Beegum, S., & Chowdappa, P. (2018) 'Palm Sap—Quality Profiles, Fermentation Chemistry, and Preservation Methods', Sugar Tech, 20(6), 621-634.

[7] Vengaiah, P.C., Murthy, G.N., Sattiraju, M. and Maheswarappa, H.P., (2017) 'Value added food products from palmyra palm (Borassus flabellifer L.)', Journal of Nutrition and Health Science, 4(1), pp.1-3. doi: 10.15744/2393-9060.4.105

[8] Velauthamurty, K., Balaranjan, S., Sashikesh, G. and Lanka, S. (2014) 'A feasibility study for the authentication of Palmyrah Jaggery using NIR spectroscopy', 6(6), pp. 55–60.

[9] Balasuriyan, A., Mahilrajan, S., Wijesinghe, W. A. J. . and Bandara, S. M. I. P. (2016) 'comparative study on quality charactristics of different palm tracle and its antioxidant activity'. Uva Wellassa university of Sri Lanka.

[10] Srikaeo, K., Sangkhiaw, J. and Likittrakulwong, W. (2019) 'Productions and functional properties of palm sugars', Walailak Journal of Science and Technology, 16(11), pp. 897–907. doi: 10.14456/vol17iss2pp.

[11] Harke, S., Pawar, A. and Patil, Y.Y. (2023). 'Quality and Adulteration in Ethnic Spices and Food Ingredients in Local Market', International Journal on Food, Agriculture and Natural Resources, 4(3), pp.21-26. doi: https://doi.org/10.46676/ij-fanres.v4i3.155

[12] McDowell, L. R. (2000) Vitamins in Animal and Human Nutrition. second, Iowa. second. Iowa state University Press. doi: 10.1093/ajcn/74.3.413.

[13] Holden, R. M., Ki, V., Morton, A. R. and Clase, C. (2012) 'Fat-Soluble Vitamins in Advanced CKD/ESKD: A Review', Seminars in Dialysis, 25(3), pp. 334–343. doi: 10.1111/j.1525-139X.2012.01084.x.

[14] Zempleni, J., Rucker, R. B., McCormick, D. B. and Suttie, J. W. (eds) (2007) Handbook of Vitamins. fourth. CRC Press. doi: 10.1080/07315724.1992.10738201.

[15] Bender, D. A. (2003) Nutritional Biochemistry of the Vitamins. second. New York: Cambridge University Press.

[16] Oviedo-Rondon, E., López-Bote, C., Litta, G. and Hernandez, J.M.. (2023), 'Optimum Vitamin Nutrition for More Sustainable Swine Farming'. 5m Books Ltd.

[17] Schonfeldt, H.C. and Hall, N. (2013) 'Fish, chicken, lean meat and eggs can be eaten daily': a food-based dietary guideline for South Africa. South African Journal of clinical nutrition, 26, pp.S66-S76.

[18] Chand, T. and Savitri, B. (2016) 'Vitamin B3, niacin. Industrial Biotechnology of Vitamins', Biopigments, and Antioxidants, pp.41-65. doi: 10.1002/9783527681754.ch3

[19] Kohlmeier, M. (2015) 'Water-Soluble Vitamins and Nonnutrients', in NutrientMetabolism. 2nd edn. Elsevier Ltd, pp. 567–671. doi: 10.1016/b978-0-12-387784-0.00010-9.

[20] Herrmann, W. and Obeid, R. (2016) 'Functions and Deficiencies of B-Vitamins (and Their Prevention)', in International Encyclopedia of Public Health. second. Elsevier Inc., pp. 199–203. doi: 10.1016/B978-0-12-803678-5.00166-1.

[21] Bender, D. (2012) 'Vitamin B6: Physiology', Encyclopedia of Human Nutrition. Elsevier Inc. doi: 10.1016/B978-0-12-375083-9.00275-0.

[22] Kroner, Z. (2011), Vitamins and minerals. Bloomsbury Publishing USA.

[23] Yin, X., Chen, K., Cheng, H., Chen, X., Feng, S., Song, Y. and Liang, L. (2022), 'Chemical stability of ascorbic acid integrated into commercial products: A review on bioactivity and delivery technology', Antioxidants, 11(1), p.153. doi: 10.3390/antiox11010153

[24] Raposo, F. and Ibelli-Bianco, C. (2020) 'Performance parameters for analytical method validation: Controversies and discrepancies among numerous guidelines', Trends in Analytical Chemistry. Elsevier Ltd, 129. doi: 10.1016/j.trac.2020.115913.

[25] Romero-Gonza´lez, R. and Garrido Frenich, A. (eds) (2017) Applications in High Resolution Mass Spectrometry. ELSEVIER.

[26] Sami, R., Li, Y., Qi, B., Wang, S., Zhang, Q., Han, F., Ma, Y., Jing, J. and Jiang, L. (2014) 'HPLC Analysis of Water-Soluble Vitamins (B2, B3, B6, B12, and C) and Fat-Soluble Vitamins (E, K, D, A, and beta-Carotene) of Okra, Journal of chemistry, 2014. Doi: 10.1155/2014/831357.

[27] Nollet, L. M. . and Lambropoulou, D. A. (eds) (2016) Chromatographic Analysis of the Environment Mass Spectrometry Based Approaches. fourth. CRC Press.

[28] Gross, J. (2004) Mass spectrometry, switzerland. Springer International Publishing. doi: 10.1007/978-3-319-54398-7.

[29] Vashist, S. K. and Luong, J. H. T. (2018) 'Bioanalytical requirements and regulatory guidelines for immunoassays', in Handbook of Immunoassay Technologies. Ireland: Elsevier Inc., pp. 81–95. doi: 10.1016/B978-0-12-811762-0.00004-9.

[30] Chan, C. chow, Lee, Y. ., Lam, H. and Zhang, X. (eds) (2004) ANALYTICAL METHOD VALIDATION AND INSTRUMENT PERFORMANCE VERIFICATION, A JOHN WILEY & SONS, INC. New jersey. doi: 10.1385/1-59259-670-3:387.

[31] Aguilera, J.M. (2019) 'The food matrix: implications in processing', nutrition and health. Critical reviews in food science and nutrition, 59(22), pp.3612-3629. doi: 10.1080/10408398.2018.1502743.

[32] Upadhyaya, A. and Sonawane, S.K. (2023) 'Palmyrah palm and its products (Neera, Jaggery and Candy) - A Review on chemistry and technology', Applied Food Research, 3(1), p.100256. doi: 10.1016/j.afres.2022.100256

[33] Toydemir, G., Subasi, B.G., Hall, R.D., Beekwilder, J., Boyacioglu, D. and Capanoglu, E. (2022) ‘Effect of food processing on antioxidants, their bioavailability and potential relevance to human health’, Food Chemistry: X, 14, p.100334. doi: 10.1016/j.fochx.2022.100334