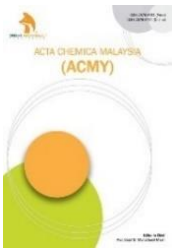
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2576-6724 (Print)
ISSN: 2576-6732 (Online)
CODEN: ACMCCG

Acta Chemica Malaysia (ACMY)

DOI: http://doi.org/10.26480/acmy.02.2025.80.86



RESEARCH ARTICLE

PHYTOCHEMICALS, SELECTED MINERAL ELEMENTS AND VITAMINS CONSTITUENTS OF *AFRAMOMUM SCEPTRUM* SEEDS AND INFLUENCE OF PROCESSING AND STORAGE

Dibie Nduka Edward* and Dibie Chukwudumebi Esther

Department of Chemistry, University of Benin, Benin City.
****Corresponding Author Email: edward.dibie@uniben.edu***

ARTICLE DETAILS

Article History:

Received 7 February 2025
Revised 5 February 2025
Accepted 10 March 2025
Available online 29 April 2025

ABSTRACT

Aframomum sceptrum Seeds used as additives in many foods and enthnomedicinal remedies, are characterized by unstandardized postharvest handling methods. Additionally, their compositional chemistry are not fully investigated. Also, the influence of processing and storage on the compositional chemistry of *Aframomum sceptrum* Seeds are less investigated. This work investigated *Aframomum sceptrum* Seeds obtained from markets in Benin City, Edo State, for tannins, saponins, cyanogenic glycosides, alkaloids, thiamine, riboflavin, Potassium (K), Calcium (Ca), Magnesium (Mg), Chromium (Cr) and Selenium (Se). The influence of sun drying, grinding and storage in the open laboratory and at water activities (a_w) of 0.23, 0.52, and 0.92, on the examined parameters were also determined. All investigations were carried out in accordance with standard methods. In raw *Aframomum sceptrum* Seeds the results were cyanogenic glycosides (0.18±0.04mg/g); saponins (8.174±1.21mg/g); tannins (2.81±0.62mg/g); alkaloids (2.92±0.35mg/g); thiamine (9.980±0.305ppm); riboflavin (0.625±0.031ppm). The range of values obtained for the mineral elements were K (3,850±10.968 - 4,915.783±15.912) mg/kg; Ca (316.677±11.189 - 386.106±5.520) mg/kg; Mg (221.843±5.691 - 253.903±21.004) mg/kg; Cr (1.257±0.045 - 1.457±0.416) mg/kg and Se (0.303±0.017 - 0.421±0.089) mg/kg. Processing and storage positively influenced the values of the phytochemicals, but resulted in decrease in thiamine and riboflavin. Statistically, the reported storage changes in phytochemicals were at $P<0.05$ significant. Additionally, at $P<0.05$ the decreases in thiamine and riboflavin were statistically significant. These findings would be relevant in the formulation of standard methods for the handling of *Aframomum sceptrum* Seeds.

KEYWORDS

Aframomum sceptrum Seeds, water activity, Storage

1. INTRODUCTION

Aframomum sceptrum according to the study is generally found in the tropical regions of Asia and Africa (Adomi et al., 2024). Additionally, the study reported that it is a terrestrial rhizomal herb that belongs to the Zingiberaceae family (Hepper, 1996). In English, the plant is commonly known by the names Guinea grains, grains of paradise or black amomum, but in Nigeria the plant is commonly known as Urioma among the natives (Burkill, 1985). There are wide utilizations of *Afromomum sceptrum* both as food additive and in the preparations of many ethnomedicinal remedies. Remarkably, it posited that the entire plant has been reported to be used for ethno, dietary, medicinal and spiritual purposes (Burkill, 1985). Additionally, it reported that the seeds of the plant are commonly utilized in herbal medicine (Aguda and Gbadamosi, 2019). Furthermore, in south-south Nigeria in particular, the seeds of *Afromomum sceptrum* are incorporated into soup preparation for newly delivered and lactating mothers and the sick, apparently due to their health values. World Health Organization reported that more than 80% of the world's population in poor and underdeveloped countries depends on traditional plant-based medicines for their primary healthcare needs (WHO, 1993). There are indications that a good segment of persons living in Nigeria, rely on plant-based medicines for their medications. *Afromomum sceptrum* in particular, is one of the additives used in various plant-based medicines that have been used to meet the primary healthcare needs of a good segment of Nigerians. However, it would appear that more investigations on the compositional chemistry of *Aframomum sceptrum* seeds are required, as literature reports in this area of knowledge are scarce. There is particular dearth of information on the influence of processing and storage on the compositional chemistry of *Aframomum sceptrum* seeds. It is important that the existing gap in knowledge with respect to *Aframomum sceptrum* seeds be filled. Findings from this work even if partly, would be relevant in filling the identified gap in knowledge.

Unstandardized methods are used to obtain *Aframomum sceptrum* seeds from its pods. Additionally, postharvest handling methods for *Aframomum sceptrum* seeds are unstandardized. This is worrisome. Significantly, from the point of view of food safety and quality, it is imperative that standard methods be formulated for various foods handling, particularly postharvest. Findings from this work would even if partly, provide relevant information for the formulation of standard methods for postharvest handling of *Aframomum sceptrum* seeds.

The interest of this work was to in part examine *Aframomum sceptrum* seeds for their cyanogenic glycosides, saponins, tannins and alkaloids constituents; the focus of another portion of this studied entailed

Quick Response Code	Access this article online	
	Website: www.actachemicamalaysia.com	DOI: 10.26480/acmy.02.2025.80.86

Cite the Article: Dibie Nduka Edward and Dibie Chukwudumebi Esther (2025). Phytochemicals, Selected Mineral Elements and Vitamins Constituents of *Aframomum Sceptrum* Seeds and Influence of Processing and Storage. *Acta Chemica Malaysia, 9(2): 80-86.*

investigation of the studied plant part for its thiamine (B1), riboflavin (B2), potassium (K), calcium (Ca), magnesium (Mg), chromium (Cr) and Selenium (Se) constituents. Furthermore, a third portion of the work was concerned with investigations of the responses of the studied phytochemical and vitamins constituents of *Aframomum sceptrum* seeds to processing and storage. The examined parameters exhibit bio-activities and could be contributing to the nutritional and therapeutic relevance of *Aframomum sceptrum* seeds. Hence their retention postharvest and in storage is important. It is imperative to mention that with respect to *Aframomum sceptrum* seeds, the research interests of this study are less investigated. The processing techniques used consist of sun drying and grinding. Samples storage entailed storing them separately in opened and closed containers that were kept in the open laboratory. Additionally, samples were stored at water activity (a_w) of 0.23, 0.52 and 0.97. It would appear that literature reports on the influence of water activity on the investigated phytochemicals constituents of *Aframomum sceptrum* seeds, as well as their thiamine and riboflavin constituents are scarce if available. There is need to expand this frontier of knowledge, as there is dearth of information on the influence of a_w on the compositional chemistry of *Aframomum sceptrum* seeds. The findings from this study will fill the identified gap in knowledge even if partly.

2. Materials and Methods

2.1 Sample Collection

Fresh *Aframomum sceptrum* pods whose seeds were used in this work were purchased from the following markets located in Benin City, Edo State: Santana, New Benin, Uselu, Ikpoba hill (Orogbeni) and Ogida markets. The fresh *Aframomum sceptrum* pods were identified by Prof. Akinnibosun Henry Adewale a taxonomist in the Department of Plant Biology and Biotechnology (PBB) of University of Benin, Benin City; voucher number UBH – A620 was assigned to the *Aframomum sceptrum* pods.

2.2 Samples Inspection and Cleaning

The fresh *Aframomum sceptrum* pods were checked to be sure that they were healthy. Subsequently, they were cut longitudinally into two parts. Thereafter, the seeds and the protective fibrous materials covering them within the pods were mechanically removed and allowed to ferment for five days during which they were detached from the fibrous materials. After the fermentation period, the seeds were cleaned up using distilled water. Consecutively, eight separate washings were done. After each washing the washed seeds were checked for presence of contaminants (mainly the fibrous materials). After the fifth washing, the presence of contaminants was no longer noticed, though the washing was carried out for three additional times as a means of ensuring that the seeds were free of contaminants.

2.3 Samples Preparation

The cleaned *Aframomum sceptrum* seeds were sun dried to constant weight. Subsequently, the seeds were ground with the aid of Black and Decker 650W, BX550 blender. Thereafter, the ground material was sieved using a 16 – mesh standard sieve (Pascall Eng. Co. Ltd. Sussex, England).

2.4 Samples Storage

Air tight desiccators wherein a_w of 0.23, 0.52 and 0.97 were established in accordance with the method prescribed by Rockland (1960) were initially prepared. Thereafter, 300g of sun dried and ground and samples were weighed in triplicates into separate 500ml glass beakers (Pyrex glass) after which they were stored in the separate air tight desiccators. Storage period was 2 months. On monthly intervals, samples were investigated for the examined parameters.

2.5 Measurement

2.5.1 Quantification of Examined Phytochemicals

2.5.1.1 Extraction of phytochemicals

The investigated phytochemicals were extracted using methanol with the aid of soxhlet extractor. The methanol used was product of Sigma –Aldrich (Steinheim, Germany). The extraction process entailed the followings: One hundred grams of ground sample was weighed, subsequently and uniformly, was distributed in a thimble. Thereafter, 400ml of methanol (material: solvent ratio is 1:4) was measured and introduced into the extraction flask after which the extraction was carried out for 20h (noticeably, the solvent in the siphon tube of the extractor became colourless after 18h of extraction, though the extraction was allowed to proceed 2h more to ensure that exhaustive extraction was carried out). After the extraction, the methanol was removed using rotary evaporator (RE52-3 SEARCH TECH INSTRUMENTS).

2.5.1.2 Determination of Total Tannin Content

The determination of total tannin content was colorimetrically carried out in accordance with the method described by Siddhuraju and Manian (2007).

2.5.1.2.1 Reagents

Polyvinyl polypyrrolidone used was product of Sigma – Aldrich (Steinheim, Germany). Every other chemical used except otherwise stated, was analytical grade.

2.5.1.2.2 Procedure

To 0.5ml of the methanol extracts of samples in different test tubes was added 100mg of polyvinyl polypyrrolidone, followed by the addition of 0.5ml of distilled water. Subsequently, the test–tubes were shaken properly, after which the solutions in the different test – tubes were incubated at 4 ^{0}C for 4h. Thereafter, they were centrifuged at 5,000rpm for 5min and left to settle. Subsequently, 0.2ml of the supernatant was measured out, the supernatant has only simple phenolics free of tannins, the tannins would have been precipitated along with the polyvinyl Polypyrrolidone (Senguttuvan et al., 2014). Blank was equally prepared. The phenolic content of the supernatant was determined in accordance with Folin – Ciocalteau spectrophotometric method for total phenols determination as described by Kujala et al. (2000).

From the results obtained, the tannins content of the respective extracts was calculated thus:-

Tannins (mg GAE/g extract) = Total phenols (mgGAE/g extracts) - Free phenols (mgGAE/g extract)

Where: GAE = Gallic acid equivalent.

2.5.1.3 Determination of Total Saponin Content

Total saponin content of sample extracts were spectrophotometrically determined in accordance with the method described by a researcher (Makkar et al., 2007).

2.5.1.3.1 Reagents

Ethanol and methanol used in this work were products of Sigma – Aldrich (Steinheim, Germany), Vanillin reagent, sulphuric acid and diosgenin were products of Merck (Germany). All chemicals used were analytical grade, except otherwise stated.

2.5.1.3.2 Procedure

Half milliliter of the methanol extract was measured into a test tube. Thereafter, 0.5ml of distilled water was added to the test tube containing the extract. This was followed by the addition of 0.25ml of vanillin reagent (prepared by dissolving 800mg of vanillin in 10ml of 99.5% ethanol), after which 2.5ml of 72% sulphuric acid (v/v) was added. Thereafter, the test tube and its content were shaken thoroughly. Thereafter, test-tube was immersed in a thermostatic water bath maintained at 60 ^{0}C for 10min. Blank was prepared alongside. After the end of 10min of immersion in the water bath, the reaction mixture was cooled in ice cold water bath for 4min. Subsequently, absorbance was read at 544nm using Uv-Vis spectrophotometer (Jenway spectrophotometer, 6715 Uv-Vis) against the prepared blank. The total saponins content was calculated from the standard graph of diosgenin, after which the result was expressed as diosgenin equivalent (mg/g).

2.5.1.4 Determination of Total Alkaloid Content

The method described and used to determine the total alkaloid content in the sample methanol extracts (Shamsa et al., 2008).

2.5.1.4.1 Reagents

Atropine, phosphate buffer (pH 4.7), bromocresol green (BCG), chloroform, hydrochloric acid and sodium hydroxide used in this study were products of Sigma – Aldrich (Steinheim, Germany). All chemicals used were analytical grade.

2.5.1.4.2 Procedure

Standard curve of atropine was prepared and subsequently used to calculate the total alkaloids content of the sample methanol extracts. Stock atropine solution was prepared by dissolving 2mg of atropine in 10ml of distilled water to give 0.2mg/ml. Thereafter, 5ml of 2M HCl was added to 1ml of the methanol extract of the test sample and subsequently shaken thoroughly; after which the test tube was allowed to stand for 1min. Subsequently, the mixture was filtered using a Whatman No. 41 filter paper, and the pH of \the extract neutralized with 0.1M NaOH. Thereafter,

1ml of this solution was measured into a separating funnel after which 5ml of bromocresol green solution was introduced. This was followed by addition of 5ml of phosphate buffer. Mixing of the content of the separating funnel was properly carried out; after which 5ml of chloroform was added and the separating funnel was shaken vigorously. It was subsequently left to stand, which was followed by the collection of the chloroform layer in a 10ml volumetric flask and made up to volume with chloroform. The standard and blank were subjected to the same treatment. Thereafter, absorbance value of the complex in the chloroform extracts was determined with the aid of Uv-Vis spectrophotometer (Jenway spectrophotometer, 6715 Uv-Vis) against a blank, at 470nm. The total alkaloid content was calculated from the standard graph of atropine. Results obtained were thereafter expressed as atropine equivalent (mg/g).

2.5.1.5 Determination of Cyanogenic Glycosides

Cyanogenic glycosides were determined spectrophotometrically in accordance with the alkaline picrate method as described (Onwuka, 2005).

2.5.1.5.1 Reagents

Picrate, sodium carbonate, hydrochloric acid and potassium cyanide used in this study were products of Merck (Germany).

2.5.1.5.2 Procedure

Five grams of ground sample was weighed and put into a conical flask after which 50ml of distilled water was added to the flask. Subsequently, the conical flask was stoppered and left to stand for 16h under room conditions in the open laboratory. This was followed by the filtration of the content of the conical flask. Portion of the filtrate was used for subsequent determination. 1ml of the filtrate was measured and put into a test tube. Thereafter, 4.0ml of alkaline picrate (prepared by dissolving 1g of picrate and 5g of Sodium carbonate in 50ml of distilled water and heated to a temperature of 35^{o}C; after cooling, the solution was transferred to a volumetric flask and made up to 200ml with distilled water) was added. Subsequently, the test tube with its content was shaken thoroughly, after which the tube with its content, were incubated in a thermostatically controlled water bath maintained at 30^{0}C. Thereafter, absorbance reading against a blank was measured at 490nm, with the aid of an ultraviolet-visible spectrophotometer (Jenway Spectrophotometer, 6715Uv-Vis). Using the standard curve obtained with absorbance values of various KCN standard solution extrapolation was made and values obtained were used in calculating the cyanide content of sample.

2.6 Determination of Thiamine and Riboflavin by HPLC

2.6.1 Reagents and Standards.

High – performance liquid chromatography grade methanol, ammonium acetate and hydrochloric acid used were products of Merck, (Darmstadt, Germany). Thiamine hydrochloride, thiamine pyrophosphate, riboflavin and potassium hexacyano ferrate (iii) were products of Sigma – Aldrich (St. Lousi, Mo. U. S. A), anhydrous sodium acetate, sodium hydroxide, absolute ethanol and glacial acetate were products of Panreac (Barcelona, Spain). Except otherwise stated, all reagents were of analytical grade. Deionized distilled water used was obtained with the aid of a Milli – Q system (Millipore, U. S. A). The Clara – diastase (from a lot with a stated activity of 53 µ/mg) was product of Fluka, (Buchs, SwitzerLand). Stock solutions of thiamine in 20ml/100ml ethanol (pH 3.5 – 4.3) and riboflavin in 20 ml/L acetic acid were stored in the dark at 4^{0}C; subsequently, working solutions were prepared daily from the stock solutions by appropriate dilution (Mondragon – Portocarrero et al., 2011).

2.6.2 Extraction

The extraction procedure used was a re-modified version of the method described and reported by the researchers (Sanchez – Machado et al., 2004; Mondragon – Pµortocarrero, et al., 2011). The sample was homogenized, after which 10g sample was placed in 250ml amber – coloured flask. Subsequently, 50ml of 0.1M HCl was added, and the flask immersed in a shaking water bath for 30 min at 100^{0}C. Thereafter, the flask with its content was cooled to room temperature; and the resulting solution brought to pH of 4.5 by addition of 2.5M sodium acetate, after which 10mg of Clara – diastase was added, and then shook. Subsequently, the mixture was incubated in an oven for 3h at 50^{0}C, with magnetic stirring. After incubation, the mixture was allowed to cool to room temperature after which the mixture was transferred to a 100ml volumetric flask, and made to mark with the purified water obtained from Milli- Q system. Subsequently, the mixture was filtered with a fiberglass filter (No. 8. Schleicher and Schuell, Germany) to obtain the sample solution.

2.6.3 Sample Clean up for Quantitation of Riboflavin

The sample clean up in readiness for the quantitation of riboflavin using HPLC was carried out by passing portion of the sample solution through a waters 0.2-µm nylon filter.

Further Treatment of Sample Solution for Thiamine Quantitation

To quantify thiamine in the sample solution, its thiamine content was derivatized to its fluorescent derivative (thiochrome). This was carried out in accordance with the method described by Mondragon – Portoccarero *et al*. (2011) as described below:- Five milliliters of the sample solution (filtered extract) was measured into a 10ml volumetric flask, and a subsequent addition of 2.5ml of 0.375M sodium hydroxide containing 1% potassium hexacyanoferrate (iii) made. The mixture was stirred for 5s and left to stand for 60s. Thereafter, 0.25ml of 3.75M HCl was added, and the content of the 10ml volumetric flask mixed thoroughly.

Prior to HPLC determination, the samples were cleaned by loading them onto a waters C_{18} Sep – Pak column, that had been activated by successive passage of 5ml of methanol, followed by 5ml of 0.005M ammonium acetate. This was followed by the addition of 5ml of sample solution. Possible interferences were subsequently removed by passage of 5ml of a 95: 5 (v/v) mixture of 0.005M ammonium acetate (pH 6.0) and methanol. Thereafter, the vitamins were eluted with 5ml of a 60: 40 (v/v) mixture of 0.005M ammonium acetate and methanol, after which the eluate was further passed through a Waters 0.2µm nylon filter prior to HPLC. 5ml aliquots of standard and blank solutions were taken through the entire sample preparation procedure.

2.6.4 Quantification of Thiamine and Riboflavin

Determination of thiamine and riboflavin contents of the prepared sample was carried out using an HP1000 quaternary pump, equipped with an HP1000 fluorescence detector product of Hewlett-Packard (Waldbronn, Germany), and a 20µl Rheodyn injection loop (Rohnert park; CA); all controlled by Hewlett – Packard HPCHEM software. Also fitted was a 250 × 4.6mm (ID) kromasil 100 C_{18} column packed with 5-µm particles (Teknokroma, Barcelona, Spain). The column was thermostatted at 35^{0}C, using a Mod. 8792 column thermostating system product of Spectra-Physics (San Jose, CA); the mobile phase was a 72 : 28 (v/v) mixture of 0.005M ammonium acetate (pH 6.0) and methanol, delivered at a flow rate of 1.2ml/min. The excitation/emission wavelengths are 366/435 for thiochrome and 440/510 for riboflavin; Peaks were identified by comparison of their retention times with those of the standards, and the peak areas were measured using Jasco software. Each sample was prepared and injected in triplicate

2.7 Selected Mineral Elements Content Determinations

The levels of Ca, Mg, Cr, and Se in the sample digests were quantified by atomic absorption spectrophotometer, while the levels of K in the sample digests were quantified by flame photometry.

2.7.1 Sample Digestion

Wet digestion of sample was carried out using concentrated tri-acid (HNO_3-H_2SO_4-$HClO_4$) as digestion mixture; in accordance with the method of Estefan et al. (2013). The tri- acid mixture (HNO-H_2SO_4-$HCLO_4$) used consists of concentrated HNO_3, H_2SO_4 and $HClO_4$ mixed in 10:1:4 ratio (Estefan et al., 2013) and allowed to cool. The digestion process entailed a series of steps, with an initial weighing of 2g of dried and ground sample into a 300ml digestion flask. Subsequently, 10ml conc. H_2SO_4 was added and swirled carefully. Thereafter, the flask was placed on thermostatically controlled Gallenkamp hot plate. The temperature was gradually raised to 145^{0}C at which the heat was maintained for 1 hour. This was followed by the addition of 10ml tri-acid mixtures (HNO_3–H_2SO_4–$HClO_4$) and the temperature setting slowly increased to 240^{0}C. Heating at the latter temperature was continued for a further 1 hour, within which time a clear solution was obtained. The digestion flask and its content were allowed to cool to room temperature. Subsequently, the digest was filtered using Whatman No. 42 filter paper. Thereafter, the filtrate was made up to 100ml in a glass volumetric flask with deionised distilled water. Reagent blank (no plant material) which was subjected to the same sample digestion, filtration and volume make up with deionised distilled treatment was prepared.

2.7.2 Measurements

The atomic absorption spectrophotometer (Buck Scientific Model 210 VGP) and flame photometer (Sherwood model 410) used were operated in accordance with the instructions provided for the equipment. Suitable standards of concentration 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20mg/l (ppm) were prepared and run, after which the values obtained were used to construct the respective standard curves. Subsequently, Ca, Mg, Cr, and Se

contents of the respective filtered and diluted digests were analyzed using the atomic absorption spectrophotometer. On the other hand, K levels of the respective filtered and diluted digests were analyzed by flame photometer.

2.8 Statistical Analysis

In this study, all investigations were carried out in triplicates. Calculations were done for the arithmetic means and standard deviations using the data generated from the various investigations. Thereafter, the results were expressed as mean ± SEM. With respect to the raw, sun dried and stored samples, then the mineral elements levels determinations, data generated were analyzed for statistical significance by two way ANOVA with the aid of International Business Machine (IBM) Statistical Package for Social Sciences (SPSS). Differences were considered significance at $P<0.05$.

3. Results and Discussion

Results for quantitative determinations of cyanogenic glycosides, saponins, tannins and alkaloids in raw, sun dried, ground and stored Aframomum sceptrum seeds are presented in Table 1 below. Findings indicated that cyanogenic glycosides, saponins, tannins and alkaloids occurred in varying amounts in raw Aframomum sceptrum seeds. As shown in Table 1, amongst the phytochemicals examined in raw Aframomum sceptrum seeds, saponnins with a value of (8.17±1.21mg/g). (2.92±0.35mg/g) occurred highest. In decreasing order, the rest are alkaloids (2.92±0.35mg/g), tannins (2.81±0.62mg/g), and cyanogenic glycosides (0.18±0.04mg/g). According to the study, the factors which determine the composition of foods of plant origin include genetic constitution, method of propagation, growing conditions, age or maturity at the time of harvest, as well as length and condition of storage before use (Joslyn, 1970; Yamaguchi and Wu, 1975). Additionally, the study reported that studies have revealed that the nature and quantity of the phytochemicals differ according to the season and geographical location (kumar et al., 2017). Presumably therefore, the reported levels of occurrence of the investigated phytochemicals in samples of Aframomum sceptrum seeds examined in this work, can even if partly, be attributed to the above mentioned factors.

Table 1: Results for Quantitative Analysis of Cyanogenic glycosides, Saponins, Tannins and Alkaloids in Raw, Sun-dried and Ground *Aframomum sceptrum* seeds

S/N	Parameter	Raw (fresh) sample	Sun dried and ground sample
1	Cyanogenic glycosides (mg/g)	0.18 ±0.04	0.21±0.06
2	Saponins (mg/g)	8.17 ±1.21	8.25±1.61
3	Tannins (mg/g)	2.81± 0.62	3.08 ±0.19
4	Alkaloids (mg/g)	2.92±0.35	3.14±0.28

It also reported the occurrence of alkaloids, saponins and tannins in samples of Aframomum sceptrum they worked on (Adomi et al., 2024). The occurrence of cyanogenic glycosides in Aframomum sceptrum seeds is worrisome, as according to the study, cyanides occur in many plants mainly in the form of cyanogenetic glycosides (Montgomery, 1969). Cyanide is toxic reported that the lethal dose of HCN for man is in the order 0.5-3.5mg/kg body weight (Wogan, 1976). According to the study tropical ataxic neuropathy and goiter have been associated with cyanide intake (Osuntokun, 1969).

Results for quantitative analysis of cyanogenic glycosides, saponins, tannins and alkaloids in stored Aframomum sceptrum seeds are presented in Table 2. It is discernible from results (Table 2), that storage of sun dried and ground Aframomum sceptrum seeds in the open laboratory under ambient conditions did not inhibit further biosynthesis of the examined phytochemicals in samples kept in both closed and opened storage containers. Also indicated in Table 2 was that at the end of the two months of storage, samples kept in the closed storage containers had higher amounts of the determined phytochemicals. It would appear that the conditions in the closed containers favoured further biosynthesis of the investigated phytochemicals more, compared to the conditions that prevailed over samples kept in the opened containers.

Table 2: Results for Quantitative Analysis of Cyanogenic glycosides, Saponins, Tannins and Alkaloids in Stored *Aframomum sceptrum* Seeds

S/N	Parameter	Sun dried and pre-stored sample	Stored samples			
			Storage conditions/time (months)			
			Open Laboratory			
			Covered container		Opened container	
			2-months	1-month	2-months	1-month
1	Cyanogenic glycosides (mg/g)	0.21± 0.06	0.61 ± 0.05	0.38± 0.16	0.29± 0.07	0.23 ± 0.04
2	Saponins (mg/g)	8.25± 1.61	17.58± 2.29	12.09± 1.44	11.89± 2.05	9.24 ± 1.36
3	Tannins (mg/g)	3.08 ± 0.19	6.86 ± 1.53	4.88 ± 036	5.50 ± 1.13	3.64 ± 0.55
4	Alkaloids (mg/g)	3.14± 0.28	19.02± 2.14	10.74± 1.12	8.75± 1.49	4.12± 1.08

The results for water activity (a_w) studies with cyanogenic glycosides, saponins, tannins and alkaloids constituents of sun- dried, ground and stored *Aframomum sceptrum* seeds are presented in Table 3 below. It is discernible from findings that biosynthesis of the studied phytochemicals continued in all samples irrespective of storage water activity. It was however noted that samples stored at a_w 0.23 recorded the highest increase in value of the examined phytochemicals. It was particularly observed that with increasing storage a_w, the incremental value of the investigated phytochemicals decreased. Remarkably, the observed increases were lowest in the examined phytochemicals constituents of samples stored at the very high a_w of 0.97.

Table 3: Results for Water Activity (a_w) Studies with Cyanogenic glycosides, Saponins, Tannins and Alkaloids Constituents of Sun- dried, Ground and Stored *Aframomum sceptrum* Seeds.

S/N	Parameter	Sun dried and pre-stored sample	Stored samples					
			Storage conditions/time (months)					
			a_w0.97		a_w0.52		a_w0.23	
			2-months	1-month	2-months	1-month	2-months	1-month
1	Cyanogenic glycosides (mg/g)	0.21± 0.06	0.56± 0.15	0.31± 0.02	0.74 ± 0.19	0.43± 0.06	1.04± 0.32	0.66± 0.11

Table 3 (cont): Results for Water Activity (a_w) Studies with Cyanogenic glycosides, Saponins, Tannins and Alkaloids Constituents of Sun- dried, Ground and Stored *Aframomum sceptrum* Seeds.								
2	Saponins (mg/g)	8.25± 1.61	16.01± 2.41	11.93± 1.85	21.23± 3.11	14.06± 2.19	29.81± 2.05	15.84± 1.36
3	Tannins (mg/g)	3.08 ± 0.19	6.42 ± 1.66	4.52 ± 0.31	7.30 ± 0.92	5.12± 1.07	10.25± 1.81	6.47± 1.08
4	Alkaloids (mg/g)	3.14± 0.28	17.15± 2.59	8.29± 1,44	22.74± 1.62	13.68± 1.34	31.93± 3.27	18.96± 2.50

Clearly, the conditions at the low a_w of 0.23, promoted the reactions that resulted in increases in the studied phytochemicals more, vis-à-vis degradation reactions; far and above the conditions that prevailed over the samples stored at the higher a_w of 0.52 and 0.97 respectively. They had reported that the storage quality of food does not depend on the water content, but on water activity (a_w) (Belitz et al., 2009). Findings from this work have revealed the relevance of water activity to storage quality of processed *Aframomum sceptrum* seeds.

The reported increases in the levels of the investigated phytochemicals after sun drying, grinding, then in samples stored at the different storage environment, could also be attributed to the non/or reduced utilisations of the studied phytochemicals in some other biological activities; which could have led to their accumulations. It should be emphasised that the grinding of the samples could have created stress on them, aftermath of which could enhance chemical reactions, particularly as histological breakdown and increased enzyme decompartmentalization usually follow grinding operations. As finds indicated, the possible enhancement of chemical reactions following processing, favoured the generation of the studied phytochemicals more than their breakdown or involvement in some other biological activities. In milled cowpea flour, physical attributes such as large surface area, high degree of porosity, enzyme decompartmentalization following milling and the milling operation which is a form of stress, could have promoted chemical responses (Ukhun, 1984).

Presented in Table 4 are the results for thiamine and riboflavin investigations. The occurrence of thiamine and riboflavin in raw and processed *Aframomum sceptrum* seeds is nutritionally desirable. Clearly, it is an indication that the examined plant part would be an additional source of thiamine and riboflavin to consumers.

Table 4: Thiamine and Riboflavin Contents of Fresh and Sun Dried *Aframomum sceptrum* Seeds			
S/N	Sample Description	Thiamine ppm	Riboflavin ppm
1	Fresh	9.980±0.305	0.625±0.031
2	Sun dried	7.725±0.104	0.187±0.067

The results for thiamine and riboflavin investigations in the processed and stored samples are presented in Tables 5 and 6. It is deducible from findings (Tables 5 and 6) that the investigated storage conditions adversely affected the thiamine and riboflavin constituents of the samples. Evidently, the storage losses of the investigated vitamins were progressive with time. Notwithstanding, the retention of thiamine and riboflavin in the stored samples despite their occurrence in reduced amounts is desirable. Apparently, it would appear that the examined processing techniques and storage conditions would provide relevant information for the formulation of postharvest handling methods for *Aframomum sceptrum* seeds. In computing and compounding food intakes aimed at meeting recommended daily allowances for various nutrients, food composition must be considered as it exists naturally, and as it exists post-processing and during storage under various conditions; if reliable nutritional information is to be obtained (Ukhun and Dibie, 1991). This makes it interesting that the examined vitamins occurred in the processed and stored samples.

The results for water activity studies with thiamine and riboflavin constituents of sun dried, ground and stored *Aframomum sceptrum* seeds are presented in Table 5. Findings indicated that there were general losses of the examined vitamins at the investigated water activities. It is also deducible from results, that samples stored at the higher storage water activity had greater losses of the studied vitamins. Remarkably, samples stored at the highest investigated a_w of 0.97 had the greatest losses. The reported variations in thiamine and riboflavin storage losses in samples stored at the studied water activity were noted to be statistically significant at $P<0.05$.

Table 5: Water Activity Studies with Thiamine and Riboflavin Constituents of Sun Dried, Ground and Stored *Aframomum sceptrum* seeds						
Storage water activities(a_w)	Thiamine ppm			Riboflavin ppm		
	0	1	2	0	1	2
a_w=0.23	7.725 ±1.104	6.395 ±0.797	5.224 ±0.963	0.187 ±0.067	0.137 ±0.041	0.106 ±0.018
a_w= 0.52	7.725 ±1.104	5.921 ±0.684	4.288 ±0.269	0.187 ±0.067	0.095 ±0.030	0.071 ±0.013
a_w= 0.97	7.725 ±1.104	4.958 ±0.339	2.275 ±0.362	0.187 ±0.067	0.066 ±0.017	0.042 ±0.008

The reduced retention of thiamine and riboflavin in the samples, especially at the elevated a_w, is attributed to increased available water that was present at the higher water activities. In particular, greater available water would increase dissolution of the studied vitamins, the tendency of which was that their involvement in hydrolytic chemical and biochemical reactions, as well as in microbial degradation processes could have promoted their losses.

The results of storage studies with thiamine and riboflavin constituents of sun dried, ground and stored *Aframomum sceptrum* seeds at ambient conditions are presented in Table 6. It is discernible from findings that progressive losses of the examined vitamins occurred with increasing storage time. Additionally, results indicated that samples stored in opened containers had higher losses of thiamine and riboflavin compared to the corresponding results obtained for samples stored in closed containers. Presumably, the different weather conditions that prevailed over the samples stored in the various storage containers influenced the results obtained.

Table 6: Storage Studies with Thiamine and Riboflavin Constituents of Sun Dried, Ground and Stored *Aframomum sceptrum* seeds at ambient Conditions		
Storage conditions (open laboratory)	Thiamine (ppm)	Riboflavin (ppm)

Table 6 (cont): Storage Studies with Thiamine and Riboflavin Constituents of Sun Dried, Ground and Stored *Aframomum sceptrum* seeds at ambient Conditions						
	0	1	2	0	1	2
Open laboratory *Covered container	7.725 ±1.104	5.067 ±0.208	3.619 ±0.518	0.187 ±0.067	0.081 ±0.016	0.055 ±0.012
*Uncovered container	7.725 ±1.104	4.760 ±0.189	2.161 ±0.095	0.187 ±0.067	0.060 ±0.018	0.038 ±0.004

The results for the investigations of K, Ca, Mg, Cr and Se, levels in *Aframomum sceptrum* seeds purchased from markets in Benin City are presented in Table 7. Results indicated that the investigated mineral elements occurred in varying amounts. Notably, the occurrence range for K was (3,850.670±10.968 to 4,915.783±15.912) mg/kg; Ca occurred in the range (316.677±11.189 to 386.104±5.520) mg/kg; Mg occurred in the range (221.843±5.691 to 253.903±21.004) mg/kg; Cr occurred in the range (1.257±0.045 to 1.457±0.410) mg/kg and Se occurred in the range (0.303±0.017 to 0.421±0.089) mg/kg. Thus potassium occurred most compared to other mineral elements determined in the samples. On the other hand, as findings indicated, selenium was the least occurring. It is evident from findings that *Aframomum sceptrum* seeds could be a dietary source for the investigated mineral elements assuming bioavailability.

The reported differences in the level of occurrence of the examined mineral elements in samples of *Aframomum sceptrum* seeds investigated, are attributed to differences in agricultural practices particularly, the type and amount of fertilizer utilization. Furthermore, differences in soil types, time of harvest, environmental conditions, species and postharvest handling methods could also have affected their level of occurrence. Statistical analysis which entailed the use of Two-way ANOVA with multiple comparison, indicated statistical difference at $P<0.05$, between the values obtained for the individual mineral elements in *Aframomum sceptrum* seeds that were studied.

Table 7: Selected Mineral Elements Content (mg/kg Dry wt Basis) of *Aframomum sceptrum* seeds Obtained from Markets in Benin City

S/N	Sample Source	Mineral elements				
		K	Ca	Mg	Cr	Se
1	New Benin Market	4464.753±7.022	384.257±7.662	251.913±3.638	1.457±0.410	0.377±0.053
2	Ogida market	3850.670±10.968	386.104±5.520	227.113±12.956	1.427±0.352	0.325±0.021
3	Oliha market	4449.973±18.230	355.009±16.171	221.843±5.691	1.383±0.029	0.370±0.065
4	Ewosha market	4382.440±12.366	348.067±2.961	253.903±21.004	1.379±0.682	0.421±0.089
5	Okah market	3947.095±2.196	361.092±7.483	246.953±1732	1.403±0.088	0.347±0.045
6	Useh market	4731.637±5.176	348.703±12.750	237.027±6.238	1.318±0029	0.383±0.031
7	Uselu market	4660.833±6.771	372.590±20551	230.028±9.028	1.410±0.096	0.350±0.059
8	Ekae market	4538.737±8.952	316.677±11.189	245.707±4.119	1.257±0.045	0.303±0.017
9	Oluku market	4773.310±11.813	349.917±4.235	233.067±4.124	1.390±0.013	0.370±0.008
10	Jeromi market	4491.470±4.080	362.083±8.551	239.527±15.336	1.421±0.065	0.309±0.025
11	Agbado market	4908.437±7.248	377.187±15.122	231.497±3.732	1.377±0.037	0.310±0.073
12	Egor market	4915.783±15.912	353.740±3.662	236.251±1.733	1.391±0.082	0.311±0.085

4. Conclusion

This study revealed that cyanogenic glycosides, saponins, tannins and alkaloids were present in the examined *Aframomum sceptrum* seeds. Furthermore, the occurrence of thiamine, riboflavin, K, Ca, Mg, Cr and Se in *Aframomum sceptrum* seeds were also indicated. Findings further showed that the levels of the examined phytochemicals in *Aframomum sceptrum* seeds, were positively influenced by sun drying, grinding and the various studied storage conditions. The same however, cannot be said of thiamine and riboflavin as their levels were adversely affected by the investigated processing and storage conditions. Variations in the levels of occurrence of the examined mineral elements were noted in this study. It was particularly noted that water activity conditions influenced highest, the levels of the investigated vitamins and phytochemicals in the stored samples. The central role of water activity in food chemistry is thus emphasized. It is important therefore, that users of sun dried and stored *Aframomum sceptrum* seeds be conscious of the influence of postharvest handling conditions on their compositional chemistry, especially, with respect to the investigated vitamins and phytochemicals in this study.

This would guarantee concentration uniformity in the various applications of processed and stored *Aframomum sceptrum* seeds.

Acknowledgement

The authors are sincerely grateful to the Department of Chemistry, University of Benin, Benin City for allowing us use some of their facilities.

References

Acker, L.W., 1969. Water Activity and Enzymes Activity. Food Technol. 23(10): Pp. 1257 – 12

Adomi, O. P., Nana, J. M. and Owhe-Ureghe, B. Ubreye., 2024. Phytochemical Properties and Antibacterial Effects of Aframomum sceptrum, Chryosobalanus icaco and Piper guineense Seeds. World J. of Pharm. and Med. Res. 2024, 10(7): Pp. 45-49.

Aguda, O. Y. and Gbadamosi, I. T., 2019. Nutritional and Phytochemical Components of Three Aframomum species. J. of For. Res. and Mgt, 16(1): Pp. 118-128.

Belitz, H. D., Grosch, W., and Schieberte, P., 2009. Food Chemistry (4th Revised and Extended Edition). Springer – Verlag. Pp. 8 – 789.

Burkill, H. M., 1985. The Useful Plant of West Tropical Africa. Families A-D kew Royal Botanic Gardens, Vol 2

Estefan, G., Sommer, R., and Ryan, J., 2013. Methods of Soil, Plant and Water Analyses (3rd Ed.) ICARDA Publ.

Hepper, F. N., 1996. Flora of West Africa. 3rd Edn. Government of Nigeria Publisher.

Joslyn, A.M., 1970. Methods in Food Analysis: Physical, Chemical and Instrumental Methods of Analysis (2nd ed.). Academic Press. New York. Pp. 24 – 402.

Kujala, T. S., Loponen, J. M., Klika, K. D. and Phihlaja, K.., 2000. Phenolics and Betacyanins in Red Beetroot (Beta vulgarias) Root: Distribution and Effect of Cold Storage on the Content of Total Phenolics and Three Individual Compounds. J. Agric. Food Chem. 48: Pp. 5342 – 5388

Kumar, S., Yadav, A., Yadav, M. and Yadav, J. P., 2017. Effect of Climate Change on Phytochemical Diversity, Total Phenolic Content and In Vitro Antioxidant Activity of Aloe vera (L) Burm. F. BMC ResearchNotes, 10(1): Pp. 60

Makkar, H. P. S., Siddhuraju, P., and Becker, K., 2007. Methods in Molecular Biology: 393 Plant Secondary Metabolites. Humana Press Inc., Totowa, New Jersey, Pp. 93 – 100.

Mondragon – Portocarrero, A., Del C., Vazquez – Oderiz, L. and Romero – Rodriguez, Ma. A'., 2011. Development and Validation of an HPLC Method for the Determination of Thiamine and Riboflavin in Green Leafy Vagetables Using Clara – Diastase. Journal of Food Sciences. Institute of Food Technologists Publication. 76: Pp. C639 – C642.

Montgomery, R. D., 1969. Cyanogen. In: Linear IE (ed) Toxic Constituents of Plant Foodstuff. Academic Press, London, Pp. 75.

Onwuka, G. I., 2005. Food Analysis and Instrumentation: Theory and Practice. Naphthali Prints. Pp 137.

Osuntokun, B. O., 1969. Cassava Diet and Chronic Degenerative Neuropathy: An Epidemiological Study. Nig J. Sci. 3: Pp. 3-15

Rockland, L. B., 1960. Saturated Salt Solutions for Static Control of Relative Humidity Between 5 and 40^{0}C. Anal. Chem. 32: Pp. 1375-1376.

Senguttuvan, J., Subramaniam, P., and Krishnamoorthy, K., 2014. Phytochemical Analysis and Evaluation of Leaf and Root Parts of the Medicinal Herb, Hypochaeris radicata L. For In Vitro Antioxidant Activities. Asian Pacific Journal of Tropical Biomedicine, 4: Pp. S359 – S367.

Shamsa, F., Monsef, H., Ghamooshi, R., and Verdian – Rizi, M., 2008. Spectrophotometric Determination of Total Alkaloids in Some Iranian Plants. Thai Journal of Pharmaceutical Sciences, 32: Pp. 17 – 20.

Siddhuraju, P. and Manian, S., 2007. The Antioxidant Activity and Free Radical Scavenging Capacity of Dietary Phenolic Extracts From Horse Grain (Macrotylom uniflorum am.) Seeds. Food Chem, 105(3): Pp. 950 – 958.

Ukhun, M. E., 1984. Fatty Acid Composition and Oxidation of Cowpea (Vigna unguiculata) Flour Lipid. Food Chemistry. 14: Pp. 35

Ukhun, M. E., and Dibie, E. N., 1991. The Ascorbic Acid Contents of Selected Marketed Foods and Influence of Water Activity (a w) During Storage. Food Chemistry. 41: Pp. 277 – 283.

Wogan, G. N., 1976. Undesirable or Potentially Undesirable Constituents of Foods. In: Fennema OR (ed) Principles of Food Science Part 1 Food Chemistry. Marcel Dekker Inc, New York, Pp. 515.

World Health Organization (WHO), International Union for Conservation of Nature (IUCN), and World Wildlife Fund (WWF)., 1993. Guidelines on the conservation of medicinal plants. IUCN Gland. ISBN 2-8317-0136-8.

Yamaguchi, M., and Wu, C.M., 1975. Composition and NutritiveValue of Vegetables for Processing.Commercial Vegetable Processing (Luh and Woodroof Edition). The Avi Publishing Company, Inc; Connectient. Pp. 639 – 693.