RESEARCH ARTICLE

Total Phenol, Percentage Antioxidant Activities, Selected Vitamins and Mineral Elements Constituents of Raw, Processed and Stored *Aframomum sceptrum* Seeds

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ABSTRACT

Aframomum septrum seeds are used as additive in food and many enthnomedicinal preparations. This study investigated Aframomum sceptrum seeds procured from open markets in Benin City, Edo State, for total phenols, copper [Cu], Cobalt [Co], Zinc [Zn], Fe [Fe]; vitamin C [ascorbic acid] and pantothenic acid. Also, its percentage antioxidant activity was determined. Processing involving sun drying and grinding, then storage in the open laboratory and at water activities [aw] of 0.23, 0.52, and 0.92, studies were also carried out. All storage was done in ambient conditions. Samples were stored for two months. All determinations were made in accordance with standard methods. Findings showed that in raw Aframomum sceptrum seeds total phenols level was 1.26±0.41mg/g; percentage antioxidant activity was 94.28±1.17%; ascorbic acid was 13.941±0.885; pantothenic acid was 1.985±0.113. The range of values obtained for the mineral elements was Cu [4.369±1.287 to 7.667±1.287] mg/kg; Co [0.124±0.008 to 0.362±0.106] mg/kg; Zn [6.533±0.214 to 14.713±1.679] mg/kg; [12.170±0.613 to 19.280±1.083] mg/kg and Na [3.073±0.247 to 5.687±0.709] mg/kg. Processing and storage led to increases in total phenols, but led to reduction in antioxidant activity, ascorbic acid and pantothenic acid. Statistically, the storage changes noted with phenol investigations were at $P<0.05$ found to be significant. Also, at $P<0.05$ the reductions in percentage antioxidant, ascorbic acid and pantothenic acid due processing and storage were statistically significant. These findings would be relevant in policy formulation of standard methods for handling Aframomum sceptrum seeds.

Keywords: Aframomum sceptrum, water activity, Processing, Storage

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INTRODUCTION

Aframomum sceptrum is a terrestrial rhizomal herb and belongs to the family Zingiberaceae [1]. According to [2] the plants are widely found in tropical regions of Asia and Africa. Additionally, [3] reported that in English, *Aframomum sceptrum* is commonly known by the names black amomum, Guinea grains or grains of paradise. In Nigeria, the seeds of *Afromomum sceptrum* are widely used for ethnomedicinal and food additive purposes. For instance, in south-south Nigeria, they are used to prepare soup for newly delivered and lactating mothers, as well as the sick apparently because of their health values. The whole plant is reportedly used for ethno, dietary, medicinal and spiritual effects [3]. Furthermore, [4] also reported that the seeds of *Aframomum sceptrum* are commomly used in herbal medicine. It would appear however, that literature reports on the compositional chemistry of *Aframomum sceptrum* seeds are scarce despite their aged long recognized nutritional and therapeutic values. It is necessary that this frontier of knowledge been improved upon.

Domestically, the pods of fresh *Aframomum sceptrum* are crushed to remove the seeds after which the whole seeds are ground and subsequently used for various preparations. It is reported by [3] that locally, *Aframomum sceptrum* seeds are extracted from pods, fermented, dried and ground, then used as spices for food preparation. Additionally, [4] reported that locally, the pods are crushed to remove the seeds which are then fermented and that the species is conserved *in-situ* in the wild and on farmers' fields. What is obvious here is that postharvest handling methods for *Aframomum sceptrum* seeds are unstandardized. With respect to food safety and quality, the adverse consequences of using unstandardized methods to handle foods postharvest cannot be over emphasized. Findings from this study would, even if partly, be relevant in the development of standard methods for postharvest handling of *Aframomum sceptrum* seeds.

This work in part examined *Aframomum sceptrum* seeds for total phenols, selected mineral elements viz: copper [Cu], Cobalt [Co], Zinc [Zn], Fe [Fe]; selected vitamins viz: vitamin C [ascorbic acid] and pantothenic acid contents, as well as determine its percentage antioxidant activities. Another portion of the study was concerned with processing and storage studies with *Aframomum sceptrum* seeds. With respect to the studied material, these are less investigated areas of research. Processing and storage could affect the compositional chemistry of food materials. Hence it is considered imperative to study the responses of the total phenols, ascorbic acid and pantothenic acids constituents of *Aframomum sceptrum* seeeds to processing and storage. The responses of total antioxidant activities of *Aframomum sceptrum* seeds to the studied processing and storage conditions were also investigated. Sun drying and grinding were the processing techniques used. Storage conditions were both the open laboratory in which samples were stored in uncovered and covered containers and at a_w of 0.23, 0.52 0.97. Information on the responses of total phenols, ascorbic acid and pantothenic acid constituents of *Aframomum sceptrum* seeds, as well as that of their antioxidant activities to water activity are scarce if in existence.

Water activity plays a central role in food systems. Additionally, [5] reported that the storage quality of food does not depend on water content, but on a_w. Literature reports from the works of [6-10] equally indicated that food stability, safety and other properties will be better forecast from a_w than from water content. In fact, it is now generally agreed that a_w is more closely related to the physical, chemical, and biological properties of foods and other natural products, than is total moisture content [11]. Apparently, the need for water activity studies with foods cannot be over emphasized.

Phenols and antioxidants are implicated as relevant agents in the management of oxidative stress in the human body. For instance, [12] reported that phenolic compounds are a group of antioxidants that function as free radicals quenchers. Additionally, [13] reported that phenols give protection against cardiovascular disease. Clearly, the terminating effect of phenols on free radicals is desirable and foods that are sources of phenol could be relevant in the overall human diets. Hence the investigation of the less studied *Aframomum sceptrum* seeds for its total phenols contents, particularly, their responses to processing and storage handlings are important.

The examined mineral elements and vitamins are dietary relevant substances required for many physiological and biochemical processes. For instance, the occurrence of ascorbic acid in the diet enhance the body uptake of non-haem iron, as it reduces Fe^{3+} to the absorbable Fe^{2+} and aids to keep it in the Fe^{2+} state [14]. For many people living in the less wealthy part of the world, dietary sources of the investigated mineral elements and vitamins remain their major sources of supply to the body. The imperative of examining *Aframomum sceptrum* seeds for these dietary relevant factors can be viewed from this perspective, as its consumption could assist in meeting the body requirements for the investigated mineral elements and vitamins.

MATERIALS AND METHODS

Sample Collection

The fresh *Aframomum sceptrum* pods whose seeds were studied were bought from the following open markets located in Benin City, Edo State: Santana, New Benin, Uselu, Ikpoba hill [Orogbeni], Ewosha, Okah, Useh, Egor, Jeromi, Ekae, Agbado and Ogida markets. The fresh *Aframomum sceptrum* pods were identified by Prof. Akinnibosun Henry Adewale a taxonomist in Plant Biology and Biotechnology [PBB] Department of University of Benin, Benin City; voucher number UBH – A620 was assigned to the *Aframomum sceptrum* pods.

Samples Inspection and Cleaning

The fresh *Aframomum sceotrum* pods were inspected to ensure that they were healthy. Thereafter, the pods were gently cut longitudinally into two parts. Subsequently, the seeds along with the fibrous materials that housed them within the pods were mechanically extracted out and left to ferment for five days, in order to free them from the fibrous materials. Following the completion of fermentation duration, the seeds were washed with distilled water. The washes were carried out eight times. After each washing the washed seeds were inspected to check for presence of contaminants [mainly the fibrous materials]. At the completion of fifth time of washing, no trace of contaminants was observed. However, the washing was continued for extra three times to ensure that the seeds were completely free of contaminants.

Samples Preparation

The washed *Aframomum sceptrum* seeds were sun dried to constant weight and ground with the aid of Black and Decker 650W, BX550 blender. Subsequently, the ground samples were sieved, with the aid of a 16 – mesh standard sieve [Pascall Eng. Co. Ltd. Sussex, England].

Samples Storage

Airtight desiccators wherein a_w of 0.23, 0.52 and 0.97 were attained following the method described by [15] were initially prepared. After which 300g of sun dried and ground samples were weighed in triplicates into separate 500ml glass beakers [Pyrex glass] and subsequently stored in the different airtight desiccators. Storage duration was 2 months, and on monthly intervals, they were examined for the studied parameters.

Measurement

Determination of Total Phenols Content

Folin – Ciocalteau spectrophotometric method for total phenol determination as described by [16] was used.

Procedure

Five milliliter of Folin–Ciocalteau reagent [1:10 dilution with distilled water] was added to each of 1ml methanol extracts of sample and standards [gallic acid solutions of concentration: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10mg/l prepared by dissolving gallic acid in a 1:1, v/v mixture of methanol: water] in different test tubes. Subsequently, the

contents of each of the test tubes were thoroughly mixed. Thereafter, 4ml of 1M Na_2CO_3 was added to the various test tubes which were subsequently shaken thoroughly to mix their contents properly; after which the solution was allowed to rest for 30min in the dark at room temperature. Blank was also prepared. Absorbance reading at 765nm of the content of the various reaction test tubes was carried out using Uv-Visible spectrophotometer [Jenway spectrophotometer, 6715 Uv-Vis]. The total phenol content was calculated from the standard graph of gallic acid and the results subsequently expressed as gallic acid equivalent [mg/g].

Determination of Total Antioxidant Activity

The free radical scavenging activity of the extracts was investigated, using the 1, 1-diphenyl–1–picrylhydrazyl [DPPH] assay, in accordance with the method described by [17]. Ascorbic acid and gallic acid were used as reference standards.

Procedure

The reference standards that is, ascorbic acid and gallic acid were dissolved in methanol. The concentration of test extract and standards used for the investigation was 250µg/ml which was obtained by serial dilution. To 2.5ml each of methanol plant extracts and standards in separate test tubes was added 1.0ml of freshly prepared DPPH solution [5.9mg/100ml methanol]. Subsequently, the individual reaction mixtures were incubated in the dark at room temperature for 30mins. Absorbance reading of the respective reaction mixtures was, thereafter, read at 517nm, using UV-Vis spectrophotometer [Jenway spectrophotometer, 6715 Uv-Vis]. In each case, triplicate measurements were carried out. A lowered absorbance value was an indication of greater radical scavenging activity. The value of the percentage antioxidant activity was calculated, using the expression below:-

% Antioxidant Activity = 100 – [[Abs sample/n Abs control] × 100]

Methanol was used as the blank.

Abs control = absorbance of DPPH radical + methanol.

Abs sample = absorbance of DPPH radical + sample extract.

The positive controls were the values obtained with the reference standards. The obtained percent antioxidant activity of the different studied extracts was compared with those of the positive controls.

Vitamins determination

Determination of pantothenic acid using HPLC

Extraction

The extraction of pantothenic acid from the samples was done following an adaption of the procedure described by [18]. Details are given below: -

Two grams of finely ground sample were weighed into a 25ml conical flask. Thereafter, 10ml of 8% [v/v] aqueous solution of trichloroacetic acid was added while using magnetic stirrer, the contents of the 25ml conical flask were mixed for 10 min and subsequently, centrifuged for 5 min at 2000rpm to separate the proteins. The supernatant solution was filtered through Whatman No. 41 filter paper into a 20ml volumetric flask and made up to the 20ml mark with type 1 ultrapure water, to obtain the sample solution.

Standard preparation

Preparation of the stock standard solution was done in accordance with the method described by [18] as described below:-

Ten milligrams of D-Pantothenic acid hemicalcium salt was weighed and placed in a 100ml volumetric flask. Subsequently, 4ml of 0.002M NaOH was added, and the volumetric flask was shaken until the D- Pantothenic acid dissolved completely. After this, the solution was made up to 100ml with 0.001M sodium dihydrogen phosphate solution [pH = 5.5]. The standard solution was prepared daily and kept in the dark at 4^0C.

Sample cleanup for Quantitation of Pantothenic Acid

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

Quantification of Pantothenic Acid

The HPLC equipment used for the measurement of pantothenic acid consisted of a series HP1000 quaternary pump, equipped with an HP 500 uv-vis absorption detector product of Hewlett – Packard [Waldbronn, Germany], and a 20µl Rheodyn injection loop [Rohnert Park, CA]. All controlled by Hewlett – Packard HPCHEM software. A 250 × 4.00mm [ID] Kromasil $100C_{18}$ column packed with 5-µm particles [Teknokroma, Barcelona, Spain] was used. The column was thermostatted at 20^0C, using a Mod. 8792 column thermostatting system, product of Spectra – Physics [San Jose, CA]: the mobile phase was 0.025% trifluoroacetic acid [v/v in water], delivered at a flow rate of 1.0ml/min. The pantothenic acid concentration measurement was based on ultraviolet light absorption at 210nm. Injection volume was 40µl. Peaks identification were done by comparison of their retention times with those of the standards, and the peak areas were measured using Jasco software. Triplicate determinations were carried out for each sample.

Determination of Ascorbic Acid with HPLC.

Extraction

Extraction solvent used was 6% meta phosphoric acid containing 1×10^{-6}M EDTA and 1×10^{-7}M diethylthiocarbamate in accordance with the method of [19] as prescribed by [20]. Extraction process used is described below:-

Two grams of ground sample were transferred into a 250ml conical flask protected from light. Thereafter, 10ml of the extracting solvent mixture: 6% meta phosphoric acid, 1×10^{-6}M EDTA and diethylthiocarbamate [18:1:1] was added, with constant but slow bubbling of nitrogen gas into the flask, which was stirred gently with the aid of magnetic stirrer for 30mins. Thereafter, the flask was left to stand for 1min, after which the sample – HPO_3, EDTA and diethylthiocarbamate macerated mixture was filtered through Whatman No 1 filter paper into a 100ml volumetric flask, protected from light. Nitrogen gas bubbled into the volumetric flask before it was stopped. The extraction and filtration processes were repeated three times; and each time, 10ml of the extracting solvent mixture was used. The mixture in the volumetric flask was made up to the 100ml mark with purified water obtained from Milli-Q system, to obtain the sample solution.

The sample clean up in readiness for the quantitation of ascorbic acid using HPLC was carried out by passing portion of the sample solution through a Waters 0.2-µm nylon filter. 5ml aliquots of standard and blank solutions were taken through the entire sample preparation steps.

Quantification of Ascorbic Acid

Determination of ascorbic acid content of the prepared sample was carried out using an HP1000quaternary pump, equipped with an HP500 uv-vis absorption detector product of Hewlett-Packard [Waldbronn, Germany], and a 20µl Rheodyn injection loop [Rohnert Park,

CA]; all controlled by Hewlett – Packard HPCHEM software. A 250 × 4.00mm [ID] Kromasil 100C_{18} column parked with 5-µm particles [Teknokroma, Barcelona, Spain] was used. The column was thermostatted at 25^{0}C, using a Mod. 8792 column thermostatting system, product of Spectra – Physics [San Jose, CA]; the mobile phase was 1.5% NH_4 H_2PO_4, pH3, delivered at a flow rate of 4ml/min. The ascorbic acid concentration was based on ultraviolet light absorption at 254nm. Injection volume was 20µl. Peaks were identified by comparing their retention times with those of the standards; and the peaks areas were measured using Jasco software. Each sample was prepared and injected in triplicate.

Selected Mineral Elements Content Determinations

The levels of Cu, Co, Zn, and Fe in the various sample's digests were quantified by atomic absorption spectrophotometer, while the levels of Na in the various sample's digests were quantified by flame photometry.

Sample Digestion

Wet digestion using concentrated tri-acid [HNO_3-H_2SO_4-$HClO_4$] as digestion mixture, in accordance with the method described by [21] was used to release mineral elements from the samples tissues. The tri- acid mixture [HNO-H_2SO_4-$HCLO_4$] used was obtained by mixing concentrated HNO_3, H_2SO_4 and $HClO_4$ in 10:1:4 ratio [21] and subsequently left to cool. The digestion was carried out by weighing 2g of dried and ground sample into a 300ml digestion flask. Which was followed by the addition of 10ml concentrated H_2SO_4 and swirled carefully. The digestion flask was subsequently clamped with a retard stand and then placed on the surface of a thermostatically controlled Gallenkamp hot plate. With a glass funnel placed in the neck of the digestion flask, the temperature slowly increased to 145^{0}C. Heating at the temperature of 145^{0}C was carried out for 1 hour. Thereafter, 10ml tri-acid mixtures [HNO_3–H_2SO_4–$HClO_4$] was added, and the temperature setting gradually increased to 240^{0}C. Heating at 240^{0}C was carried out for another 1 hour. Clear solution was obtained. Subsequently, the digestion flask and its content were left to cool to room temperature, after which the digest was filtered through Whatman No. 42 filter paper. Then the filtrate made up to 100ml in a glass volumetric flask with deionised distilled water. Each batch of digests contained reagent blank [no sample] that was subjected to the same sample digestion, filtration and volume make up with deionised distilled water stages.

Measurements

At the stage of elemental quantifications, 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. Series of suitable standards of concentration 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20mg/l [ppm] were run, and values obtained were used to produce the respective calibration curves. Thereafter, the atomic absorption spectrophotometer was used to analyze for Cu, Co, Zn, and Fe, contents of the various filtered and diluted digests, Na contents of the filtered and diluted digests were analyzed by flame photometer.

Statistical Analysis

In all cases in this work, triplicate measurements were made. The arithmetic means and standard deviations for the data obtained in the various tests were calculated and the results are expressed as the mean ± SEM. With respect to the raw, processed, and stored samples, as well as mineral elements investigations, data generated were analyzed for statistical significance by two-way ANOVA using the International Business Machine [IBM] Statistical Package for Social Sciences [SPSS]. Differences were considered significance at $P<0.05$.

RESULTS

Results for quantitative determination of total phenols in raw, sun dried and ground*Aframomum sceptrum* seeds are presented in Table 1. It is discernible from results that phenols are present in raw and processed *Aframomum sceptrum* seeds and that processing led to increase in total phenols content of *Aframomum sceptrum* seeds. It is also discernible from results [Tables 2 and 3] that storage of sun dried and ground *Aframomum sceptrum* seeds in the open laboratory, as well as at the examined water activities positively influenced its total phenols content. The observed storage increases in total phenols were progressive with time.

Results [Table 4] for determinations of percentage antioxidant activities of raw, sun dried and ground *Aframomum sceptrum* seeds extracts indicated that the studied samples exhibited antioxidant activity. It is however deducible from Table 4, that the processing methods investigated led to reduced antioxidant activity retention in the samples. Storage losses in antioxidant activity [Tables 5 and 6] were equally noted in the samples stored at the storage conditions investigated. Tables 5 and 6 also revealed that the storage losses in antioxidant activity of the examined samples were progressive with time.

Table 7 indicated that *Aframomum sceptrum* seeds contain ascorbic and pantothenic acids and that sun drying led to reduction in the ascorbic and pantothenic acids levels in the processed samples. It is further deducible from findings [Tables 8 and 9] on storage studies that there were storage losses in ascorbic and pantothenic acids constituents of the samples. The storage losses of these vitamins were noted to be progressive with time. The variation in storage losses in samples stored at different water activity was noted to be statistically significant at $P<0.05$.

Results [Table 10] for the minerals examined indicated variations in their levels of occurrence and that iron occurred most. Statistical analysis using Two-way ANOVA with multiple comparison indicated that at $P<0.05$, there was statistical difference between the values obtained for the individual mineral elements determined in *Aframomum sceptrum* seeds that were obtained from different locations.

DISCUSSION

The reported occurrence of phenols in *Aframomum sceptrum* seeds is consistent with the report of [2]. The diet remains a very significant source of valuable substances to the body. It is therefore desirable that phenols occur in *Aframomum sceptrum* seeds, especially as plant phenols have been recognized for their therapeutic and nutritional relevance. According to [22] plant phenols can protect against lipoprotein oxidation. Additionally, [23] reported that plant phenols are group of antioxidants that inhibit various stages of cancer process. Also, [24] reported that several studies have demonstrated the antimicrobial activity of phenols and /or phenolic extracts. These are instructive enough to consider phenols in foods, as relevant in the maintenance of the quality and nutritional values of foods, especially in storage. Therefore, *Aframomum sceptrum* seeds having been revealed to contain phenols could serve as natural source of phenols to foods when used as additive in their preparations, thereby enhancing stability. Remarkably, the work of [10] revealed that the addition of ground *Aframomum sceptrum* seeds to stored *Elaesis guineense* oil improved the storage stability of the oil. This suggests the need to explore *Aframomum sceptrum* seeds for wider use as ingredients in certain food processing, especially those foods in which their antioxidant and antimicrobial properties can be used to advantage.

It is imperative to say that with respect to the reported value for phenols in *Aframomum*

sceptrum seeds, several factors influence the levels of phytochemicals present in foods of plant origin. Notably, [25]; [26] remarked that the factors which influence the composition of foods of plant origin include genetic constitution, growing conditions, method of propagation, age or maturity at the time of harvest, as well as length and condition of storage before use. Furthermore, according to [27] studies have shown that the nature and quantity of phytochemicals differ according to the season and geographical location. The level obtained for total phenols in the *Aframomum sceptrum* seeds studied could have been influenced by these factors.

The observed storage increases in the level of total phenols in the stored samples is an indication that the investigated storage conditions foster biosynthesis and less possible degradation of total phenols in the stored samples. The higher total phenols value recorded in samples stored in closed containers compared to the values obtained for samples stored in opened containers is an indication that the conditions within the closed containers promoted faster biosynthesis and less of possible degradation of total phenols, than the conditions which prevailed over the samples that were kept in opened container and stored in the open laboratory. The higher increase in total phenols in samples stored at lower a_w compared to those of samples stored at higher a_w, clearly showed that the conditions which prevailed over the samples stored at lower a_w favoured more of the series of reactions that resulted in increases in the total phenols contents of the stored samples, vis-à-vis the degradation reactions; far and above the conditions at the higher a_w.

In milled cowpea flour, [28] posited that physical attributes such as large surface area, high degree of porosity, enzyme decompartimentalization following milling and the milling operation, which is a form of stress, promoted chemical responses. Viewed from this perspective, it could mean that the mechanical disintegration of the cell walls of the examined sun dried samples following the grinding operation, promoted some chemical responses. Clearly, the grinding of *Aframomum sceptrum* seeds before storage would have promoted chemical reactions due to histological disintegration, and enhanced enzyme decompartmentalization which usually accompany grinding operations and the chemical reactions positively influenced the production and accumulation of phenols in the ground and stored samples.

Percentage antioxidant of 55.43±9.41was reported by [4] for *Aframomum septrum* seeds. The difference between the values for percent antioxidant activities reported by the different authors can in part be ascribed to the earlier mentioned factors which affect the chemical composition of .biological materials and also, to possible different postharvest changes that could have occurred in the different *Aframomum sceptrum* seeds examined by the different authors. Significantly, in this work fresh *Aframomum sceptrum* seeds were extracted from the pods, on the other hand, [4] worked on already extracted and open market sold *Aframomum sceptrum* seeds. It is imperative to say that in Nigerian open markets, commodities are exposed to varying weather conditions. Additionally, the length of time the *Aframomum sceptrum* seeds stayed with the sellers before sold could be relevant, sespecially, for antioxidant species that are sensitive to storage conditions. It is imperative to say that postharvest handling methods for *Aframomum sceptrum* seeds are unstandardized. This study has revealed that a negative relationship exists between duration of storage and percentage antioxidant activities of the stored samples. The conditions of storage were also noted to influence the magnitude of loss of antioxidant activities in the samples examined.

The reported antioxidant activity of *Aframomu sceptrum* seeds extracts is considered high,

especially as the value obtained for percentage antioxidant activity of *Aframomum sceptrum* seeds extracts at the concentration of 0.25mg/ml, is higher than that of the standards [ascorbic and gallic acids] at the same concentration of 0.25mg/ml, in the DPPH free radical scavenging activity test. It is interesting that *Aframomum sceptrum* seeds possessed antioxidants activities, as it is an indication that *Aframomum sceptrum* seeds could be source of natural antioxidants to humans, thereby contribute to the maintenance of the body's antioxidants composition.

The lowered antioxidant activity of *Aframomum sceptrum* seeds following sun drying and grinding is ascribed in part to the instability of some of the antioxidant species in the samples to light. Additionally, the occurrence of photo-aided antioxidant degradation reactions following exposure to sunlight could also have resulted in the reduction of antioxidant retention in the sun-dried samples. Furthermore, grinding increases surface area which promotes reactions; if the overall effects of these reactions are inimical to antioxidant activity retention, then grinding of the sun-dried samples prior to storage should enhance losses of antioxidant species. The collapse of cell wall which accompanies grinding promotes contact between certain exogenous enzymes and some protected substance. The effect of which would be enzymatic transformation of the protected substances into other forms which may not have similar biological activities to the protected material. For instance, the contact of ascorbic acid with ascorbic acid oxidase will promote oxidation of ascorbic acid into derived products that indicate little or no reducing properties.

Ascorbic acid is a reducing agent and in fact one of the most powerful antioxidants provided by nature.

The higher loss in antioxidant activity in samples stored in opened containers compared to the ones stored in closed containers is ascribed to the contact which the samples in the opened containers had with atmospheric factors including moisture and oxygen, especially, if the loss in antioxidant activities of the stored samples proceeded via some oxidative and hydrolytic degradation processes. With respect to water activity studies, the greater loss in antioxidant activity in samples stored at higher a_w is attributed to the greater available water at higher water activity. Significantly, at the higher a_w the increment in the available water would lead to greater solubilisation of the water-soluble antioxidant entities and their subsequent involvement in reactions which resulted in their destruction. It is imperative to say that the breakdown of crystalline regions of the examined materials due to enhanced amount of available water should foster oxygen diffusion into them and would as a result lead to higher antioxidant species degradation via oxidative reactions.

It is nutritionally desirable that ascorbic and pantothenic acids occur in fresh and processed *Aframomum sceptim* seeds, as their consumptions could assist in meeting the body's requirements for these vitamins. *Aframomum sceptrum* seeds may not be among the conventionally recognized sources of ascorbic acid and pantothenic acid in human diets, however, it is imperative to say that in assessing nutrient sources for humans, holistic consideration of different sources could provide better information than information centered on one point source.

The reduction in ascorbic and pantothenic acids following sun drying of the samples is ascribed to adverse consequences of environmental factors that prevailed over them during sun drying. It is, however, desirable, that ascorbic and pantothenic acids though in reduced amount occurred in the processed and stored *Aframomum sceptrum* seeds. Significantly, in computing and compounding food intakes

aimed at meeting recommended daily allowances for various nutrients, [8] reported that 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.

The reported losses of ascorbic and pantothenic acids in sun dried, ground and stored *Aframomum sceptrum* seeds particularly at the elevated $a_{w\ are}$ ascribed to the increased available water especially at the higher storage water activities. Remarkably, the water-soluble nature of these vitamins could foster their involvement in water aided chemical, and biochemical reactions, as well as microbial degradation processes. Clearly, these should lead to their losses. In stored cassava and garri, [8] remarked that increased levels of available water led to increasing solubilization of the water- soluble ascorbic acid, which then led to higher rates of destruction. Additionally, in stored cassava and garri, increased levels of available water could have promoted increased oxygen dissolution in food materials, leading to increased oxidative loss of ascorbic acid [8]. Furthermore, [29] reported that elevated a_w may act to lower the activation energy for ascorbic acid destruction. These could even if partly, explain why there were reductions in the examined vitamins retention in the stored samples, especially those stored at the higher water activities.

In the presence of amino acids, ascorbic acid, dehydroascorbic acid and their degradation products might be changed further by entering Maillard type browning reactions [5]. This could be another factor that contributed to the loss of ascorbic acid in the processed and stored *Aframomum sceptrum* seeds. In storage perhaps, hydrolysis of some of the proteins of the stored samples occurred, with a subsequent production of amino acids, which then reacted with ascorbic acid in Maillard reactions. Clearly, the involvement of ascorbic acid in Maillard reactions will accentuate its reduction in the studied samples.

The occurrence of greater losses of ascorbic and pantothenic acids in samples kept in opened containers and stored in open laboratory, compared to the values obtained for samples kept in closed containers and stored under similar condition, is attributed to the different weather conditions that prevailed over the samples stored in the different storage containers. In particular, the greater amount of oxygen available to the samples in the opened containers should result in greater oxidative losses of their oxidizable constituents.

The examined mineral elements viz: Cu, Co, Zn, Fe and Na, were noted to be present in the samples. Assuming bioavailability, the occurrence of these mineral elements in the samples is an indication that *Aframomum sceptrum* seeds even if partly, could be a dietary source for these mineral elements. The noted variations in the level of occurrence of the determined mineral elements in the samples are ascribed to differences in agricultural practices used to cultivate the parent plants that produced the seeds, as well as differences in soil conditions, time of harvest, environmental factors, plant species and postharvest handling chemistry.

Conclusion

This study investigated *Aframomum sceptrum* seeds procured from open markets in Benin City, Edo State. Findings revealed that the studied material contains phenols, Cu, Co, Zn, Fe, Na, ascorbic acid and pantothenic acid. Additionally, *Aframomum scptrum* seeds exhibited antioxidant activities. Also, findings showed that the processing and storage conditions examined led to progressive increases in total phenols level. On the other hand, the processing and storage conditions studied led to progressive decreases in total antioxidant activities, ascorbic acid and pantothenic acid. It was further observed from

results that the obtained values for the examined mineral elements varied with sample source. Thus, while the processing methods and storage conditions investigated could be used to enhance total phenols contents in *Aframomum sceptrum* seeds the same cannot be implied with respect to antioxidant activities. This is an indication that in addition to phenols, *Aframomum sceptrum* seeds contain some other substances with antioxidant activities, some of which however, were adversely influenced by the processing methods and storage conditions studied in this work.

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Table 1: Total Phenol Contents of Fresh and Sun Dried *Aframomum sceptrum* Seeds

S/N	Sample Description	Phenols [mg/g]
1	Fresh	**1.26±0.41**
2	Sun dried	**1.58±0.26**

Table 2: Open Laboratory Storage Studies with Total Phenol Contents of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds.

S/N	Parameter	Sun dried and pre-stored sample	Samples Storage conditions/time [months]			
			Covered container		Opened container	
			2-months	1-month	2-months	**1-month**
1	Phenols [mg/g]	1.58 ± 0.26	8.05 ± 1.11	4.13 ± 0.37	3.48 ± 0.69	**1.95 ± 0.27**

Table 3: Water Activity [a_w] Studies with Total Phenol Contents of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds Samples

S/N	Parameter	Sun-dried and pre-stored sample	Storage conditions/time [months]					
			a_w 0.97		a_w 0.52		a_w 0.23	
			2-months	1-month	2-months	1-month	2-months	**1-mSonth**
1	Phenols [mg/g]	1.58 ± 0.26	6.83 ± 1.17	3.52 ± 0.22	9.06 ± 0.81	4.39 ± 0.27	12.71 ± 1.63	**6.29 ± 0.97**

Table 4: Percentage Antioxidant Activity of Raw, Sun Dried and Ground *Aframomum sceptrum* Seeds

S/N	Sample Description	Total antioxidant capacity [%]
1	Sun dried pre-stored sample extract [0.25mg/ml]	93.65 ± 2.55
2	Raw sample extract [0.25mg/ml]	94.28 ± 1.17
3	Ascorbic acid [0.25mg/ml]	93.61 ± 4.15
4	Gallic Acid [0.25mg/ml]	91.74 ± 1.88

Table 5: Open Laboratory Storage Studies with Percentage Antioxidant Activity of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds

S/N	Storage Condition / Sample Description	Total antioxidant capacity [%] / Time [months]		
		Pre-storage	1-month	**2-months**
1	Open Laboratory [covered container], stored sample extract [0.25mg/ml]	93.65 ± 2.55	81.48 ± 2.03	**70.89 ± 1.54**
2	Open laboratory [uncovered container], stored sample extract [0.25mg/ml]	93.65 ± 2.55	74.92 ± 1.00	**59.94 ± 2.11**
4	Ascorbic acid [0.25mg/ml]	93.61 ± 4.15	93.41 ± 2.95	**92.17 ± 1.55**
5	Gallic Acid [0.25mg/ml]	91.74 ± 1.88	91.68 ± 0.74	**90.25± 1.02**

Table 6: Water Activity [a_w] Studies with Percentage Antioxidant Activity of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds

S/N	Storage Condition / Sample Description	Total antioxidant capacity [%] / Time [months]		
		Pre-storage	1-month	**2-months**
1	a_w 0.23, stored sample extract [0.25mg/ml]	93.65 ± 2.55	86.16 ± 1.65	**79.27 ± 0.28**
2	a_w 0.52, stored sample extract [0.25mg/ml]	93.65 ± 2.55	82.41 ± 1.44	**72.52 ± 0.91**
3	a_w 0.97, stored sample extract [0.25mg/ml]	93.65 ± 2.55	77.73 ± 0.80	**64.52 ± 1.12**
5	Ascorbic acid [0.25mg/ml]	93.61 ± 4.15	93.41 ± 2.95	**92.17 ± 1.55**
6	Gallic Acid [0.25mg/ml]	91.74 ± 1.88	91.68 ± 0.74	**90.25± 1.02**

Table 7: Ascorbic Acid and Pantothenic Acid Contents of Fresh and Sun Dried *Aframomum sceptrum* Seeds

S/N	Sample Description	Ascorbic acid [Vitamin C] ppm	Pantothenic acid [B3] ppm
1	Fresh	13.941±0.885	**1.985±0.113**
2	Sun dried	8.136±0.229	**1.392±0.211**

Table 8: Open Laboratory Storage Studies with Ascorbic Acid [vitamin C] and Pantothenic Acid [B3] Constituents of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds

Storage	Ascorbic acid [vitamin C] ppm	Pantothenic acid [B3] ppm

conditions open laboratory/time						
Open laboratory	0-month	1-month	2-months	0-month	1-month	**2-months**
*Covered container	8.136 ±1.042	6.092 ±0.879	5.183 ±0.452	1.392 ±0.178	0.941 ±0.263	**0.758 ±0.121**
*Uncovered container	8.136 ±1.042	5.176 ±0.914	3.946 ±0.313	1.392 ±0.178	0.670 ±0.113	**0.392 ±0.017**

Table 9: Water Activity [a_w] Studies with Ascorbic Acid [vitamin C] and Pantothenic Acid [B3] Constituents of Sun Dried, Ground and Stored *Aframomum sceptrum* Seeds

Storage water activities[a_w]	**Ascorbic acid [vitamin C] ppm**			**Pantothenic acid [B3] ppm**		
	0-month	1-month	2-months	0-month	1-month	**2-months**
a_w= 0.23	8.136 ±1.042	7.641 ±1.186	6.589 ±1.120	1.392 ±0.178	1.178 ±0.429	**1.005 ±0.028**
a_w= 0.52	8.136 ±1.042	6.827 ±1.006	5.725 ±0.981	1.392 ±0.178	1.069 ±0.334	**0.981 ±0.162**
a_w= 0.97	8.136 ±1.042	5.392 ±0.790	4.198 ±0.517	1.392 ±0.178	0.728 ±0.119	**0.446 ±0.091**

Table l0: 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				
		Cu	Co	Zn	Fe	**Na**
1	New Benin Market	4.369±1.287	0.212±0.090	11.993±1.631	15.550±1.389	**3.823±0.852**
2	Ogida market	5.267±1.014	0.124±0.008	6.773±0.154	18.350±1.968	**3.073±0.247**
3	Oliha market	4.760±0.185	0.127±0.001	11.250±1.217	15.253±1.158	**3.553±0.415**
4	Ewosha market	5.547±0.583	0.194±0.019	12.513±0.856	13.457±0.983	**5.687±0.709**
5	Okah market	6.417±1.155	0.305±0.034	10.347±0.505	14.713±1,227	**3.417±0.099**
6	Useh market	6.133±0.928	0.196±0.056	14.320±1.679	12.170±0.613	**4.743±0.977**
7	Uselu market	5.297±0.934	0.362±0.106	10.180±1.364	14.680±1.003	**3.857±0.649**
8	Ekae market	5.820±1,590	0.260±0.085	8.473±0.604	19.280±1.083	**3.437±0.117**
9	Oluku market	5.680±0.212	0.195±0.066	7.863±1.111	16.463±0.716	**3.647±0.351**
10	Jeromi market	5.037±1.098	0.277±0.049	6.533±0.214	18.670±1.210	**3.933±1.166**
11	Agbado market	7.600±0.750	0.313±0.021	9.110±1.813	118.175±0.149	**3.467±0.922**
12	Egor market	7.667±1.287	0.315±0.009	7.483±0.829	1.023±0.990	**4.190±0.784**