NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Quantification and Storage Studies with Total Phenols Content and Percentage Antioxidant Activities of *Piper Guineense* Leaves

[1]Dibie N. Edward and [2]Ukhun E. Mark

[1,2]Department of Chemistry, Food Chemistry Research Unit, University of Benin, Benin City, Nigeria

Correspondence Email: edward.dibie@uniben.edu

GSM : [1]+2347033113395

Article Info

***Keywords*:** *Total phenols, antioxidant activity, spectrophotometer, water activity, Piper guineense*

Received 6 June 2022
Revised 20 June 2022
Accepted 23 June 2022
Available online 2 Sept 2022

https://doi.org/10.37933/nipes/4.3.2022.18

https://nipesjournals.org.ng

Abstract

The concern of this work in part, was to investigate the total phenols levels and antioxidant activity of Piper quineense leaves. Another portion of this study entailed the determinations of the responses of total phenols levels and antioxidant activity of Piper quineense leaves to sun drying, grating and storage using different facilities. Piper quineense leaves samples used in this study were obtained from some markets in Benin City, Edo State. Samples were stored under ambient conditions in the open laboratory and at water activities (a_w) of 0.23, 0.52 and 0.97. Storage duration was two months. All determinations carried out in this study were done in accordance with standard methods. Findings indicated that in raw Piper guineense leaves, total phenols level was 0.69±0.14mg. Total phenols content of sun dried and grated Piper guineense leaves was 0.71±0.22mg/g. With respect to the storage studies, at the end of the storage time, values obtained for total phenols in sun dried, grated stored Piper guineense leaves ranged from 2.87±0.83mg/g to 6.11±0.73mg/g for samples stored in the open laboratory, then between 5.63±1.02mg/g and 10.49±1.15mg/g for samples stored at different a_w. It is further discernible from findings that the percentage antioxidant activity of Piper guineense leaves studied was 86.21±2.13%. Equally deducible from results was that sun drying and grating of Piper guineense leaves, then storage of sun dried and grated Piper guineense leaves led to reduced retention of antioxidant activity of Piper guineense leaves. Storage changes noted for total phenols level and percentage antioxidant were statistically significant ($P<0.05$) with respect to the storage conditions studied. It is important that the factors observed in this study which affects the parameter examined, be taken into consideration in the handling of Piper guineense leaves.

1. Introduction

Foods possess distinct nutritive and pharmacological properties. In particular, [1] remarked that foods contain components that contribute to, and in some cases, uniquely provide for biochemical and physiological functions in the body. Unfortunately, the healthful status of foods presently, are

adversely affected by many current food production and handling methods, a lot of which are unstandardized. It is imperative to mention that the standardization of the yet unstandardized food production, processing and storage methods is one way of ameliorating the problem of foods supplies that are associated with reduced quality to consumers. It should also be noted that clear knowledge of the chemistry involved in the yet unstandardized food production, processing and storage methods, is the panacea to successful standardizations of these food handling operations. The concern of this study therefore, is to determine the responses of selected properties of *Piper quineense* leaves to processing and storage conditions that are yet uninvestigated.

Piper quineense leaves are commonly used in many dietary and pharmaceutical herbal preparations in Nigeria. Like other biological materials it is complex in nature, consisting of wide array of chemicals. There are indications that raw and derived products obtained from *Piper quineense* leaves are sold in many Nigerian open markets. Significantly, the production and storage of the aforementioned derived products obtained from *Piper quineense* leaves are based on utilization of unstandardized methods. There is need to investigate the presently used handling methods with respect to *Piper quineense* leaves and its derived products supplies, to consumers if consistency in their quality and safety is to be guaranteed. It is imperative to mention that the increasing scarcity of healthful foods supplies is an issue of global concern that calls for urgent attention.

In this work, sun drying and grating as processing methods, were used to produce the stored samples of *Piper quineense* leaves investigated. Though sun drying and grating of *Piper quineense* leaves are used by some handlers of *Piper quineense* leaves as processing methods, their applications are yet uninvestigated particularly, with respect to the responses of total phenols and total antioxidant properties of *Piper quineense* leaves. There is need to fill this gap in knowledge. This study focuses on the determinations of total phenols and total antioxidant properties of raw, then processed and stored *Piper quineense* leaves. Literature reports with respect to the concern of this work are scarce if in existence.

Phenols possess antioxidant properties hence could be important in the management of oxidative stress in the human body. As noted by [2] phenolic compounds belong to a class of antioxidants that act as free radicals terminators. This is of great interest when viewed from the remark of [3] that the reactive free radicals species foster lipoprotein oxidation, arteriosclerosis, premature cellular aging, and even carcinogenic mutations. Phenols give protection against cardiovascular disease [4]. The imperative of investigating total phenols levels in the samples studied especially as natural sources of phenols could be viewed from this perspective.

Tremendous scientific works on antioxidants are ongoing worldwide. The reasons are obvious. Significantly, these authors [5,6,7,8,9,10,11] posited that when there is failure in the balance between Reactive Oxygen Species (ROS) production and antioxidant defenses, ‘oxidative stress’ occurs, which through a series of events deregulates the cellular functions, and gives rise to various pathological conditions. Notable among which are arthritis, aging, asthma, autoimmune diseases, AIDS, arteriosclerosis, broncho-pulmonary, dysplasia, cataract, carcinogenesis, cardiovascular dysfunction, diabetes, genetic disorders, gastroduodenal pathogenesis, inflammatory diseases, ischemia reperfusion injury, liver disorders, muscular dystrophy, neurodegenerative diseases, Parkinsons dementia, Alzheimer’s disease, pulmonary fibrosis, radiation damage, retinopathy, amyotropic lateral sclerosis, reheumatism stroke, skin disease, porphyria and snile dementia. Major concern with respect to current antioxidant research works focus on the search for natural antioxidants especially plants with potential antioxidant properties. It is hoped that findings from this work will add to the existing list of relevant natural sources of antioxidants.

Some aspects of the concern of this study is to use the open laboratory and water activities (a_w) of 0.23, 0.52 and 0.97 as storage conditions of study. All storages will be at ambient conditions. There is a particular dearth of information on the effect of a_W on the compositional chemistry of *Piper quineense* leaves. According to [12] there is increasing acknowledgement of the central role of water activity in food systems. Furthermore, [13] posited that the concept of water activity is nowadays universally adopted by food scientists and technologists to quantify availability. Also, [14] noted that the storage quality of food does not depend on the water content, but on water activity (a_w). Some other researchers [15,16,17,18,19,20,21,22,23,] have equally shown that food stability, safety and other properties can better be predicted from a_w than from water content. The relevance of water activity in food stability studies cannot be over emphasized.
Spectrophotometric methods would be used in the determinations of total phenols and total antioxidant properties of the *Piper quineense* leaves samples studied. Additionally, data that will be generated in this study will be statistically analyzed. Significantly, aspects of descriptive statistical evaluation of data and statistical evaluation of the relation between variables (ANOVA) will be carried out. International Business Machine (IBM), Statistical Package for Social Sciences (SPSS) will be used in statistical evaluation of data.

2.0.Materials and Methods

2.1 Sample Collection

Piper quineense leaves samples used in this work were obtained from some open markets in Benin City, Edo State.

2.2 Samples Inspection and Cleaning

The *Piper quineense* leaves samples were subjected to pretreatment so as to free them from different forms of contaminants. Significantly, they were not diseased (i.e. they were not affected by viral, bacteria or fungal infection). Furthermore, contaminating plants and/or plants parts were identified and removed. In particular, in this work only healthy *Piper quineense* leaves samples were used.

2.3 Samples Preparation

The samples preparation stage in this work entailed initial sun drying of fresh *Piper quineense* leaves to constant weight. Thereafter, the different dried samples obtained were recorded as the sun dried samples. the sun dried samples were grated using Black and Decker 650W, BX550 blender. Subsequently, the grated samples were sieved, with the aid of 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 [24] were initially prepared. Subsequently, three hundred grams of sun dried and grated *Piper quineense* leaves were weighed in triplicates into different 500ml glass beakers (Pyrex glass), and placed in the separate air tight desiccators. Samples were stored for 2 months. On monthly basis, samples were investigated for the examined parameters. All the storage desiccators were kept on laboratory bench at ambient conditions.

2.5 Measurement

2.5.1. Determination of Total Phenols Content

Total phenol was determined spectrophotometrically by the Folin – Ciocalteau method as described by [25].

2.5.1.1 Reagents

Folin – Ciocalteau reagent, gallic acid, methanol and sodium carbonate were purchased from Sigma – Aldrich (Steinheim, Germany). Distilled water was obtained using a Mill – Q system (Millipore – Bedford, MA, U.S.A). Except otherwise stated, all chemicals used were analytical grade.

2.5.1.2 Procedure

To 1ml of methanolic extract 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 separate test tubes, were added 5ml each of Folin–Ciocalteau reagent (1:10 dilution with distilled water) and thoroughly mixed. After which 4ml of 1M Na_2CO_3 was added to each of the test tubes and again, thoroughly mixed. Thereafter, the solution was left to stand for 30min in the dark at room temperature. Blank was also prepared. This was followed by absorbance reading at 765nm, 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 were expressed as gallic acid equivalent (mg/g), which is a common reference compound.

2.5.2 Determination of Total Antioxidant Activity

The free radical scavenging activity of the extracts was determined, using the 1, 1-diphenyl–1–picrylhydrazyl (DPPH) assay, in accordance with the method described by [26]. Ascorbic acid and gallic acid were used as reference standards.

2.5.2.1 Reagents

1,1-diphenyl-1-picrylhydrazyl (DPPH), and methanol were obtained from Sigma – Aldrich (Steinheim, Germany). Gallic acid and ascorbic acid were purchased from Merck (Germany). Distilled water was obtained using a Milli – Q system (Millipore – Bedford, MA, U. S. A).Except otherwise stated, all chemicals used were analytical grade.

2.5.2.2 Procedure

The reference standards viz – ascorbic acid and gallic acid were dissolved in methanol. The concentration of test extract and standards used for the determination was 250µg/ml, obtained by serial dilution. To each of separate 2.5ml of methanolic plant extracts and standards in different test tubes were added 1.0ml of freshly prepared DPPH solution (5.9mg/100ml methanol). Subsequently, the various reaction mixtures were incubated in the dark at room temperature for 30mins. Thereafter, the absorbance of the different reaction mixtures was read at 517nm, using Uv/vis spectrophotometer (Jenway spectrophotometer, 6715 Uv/vis). Measurements were carried out in triplicates. A lowered absorbance value (greater discolouration) indicated higher radical scavenging activity. 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 (ascorbic acid and gallic acids). The obtained percent antioxidant activity of the respective test extracts were compared with the positive control.

3.0 **Results and Discussion**

3.1: **Results of qualitative analysis of total phenols**.

The results of qualitative analysis of total phenols constituents of *Piper guineense* leaves samples obtained from some markets in Benin City are presented in Table 1.

Table 1: Qualitative Analysis of Total Phenols in *Piper guineense* Leaves

Phytochemical family	**Aqueous**	**Methanol**	**Ethanol**	**n-Hexane**	**Acetone**	**Ethyl acetate**
*sample 1	+	+	+	+	+	+
*sample 2	+	+	+	+	+	+
*sample 3	+	+	+	+	+	+
*sample 4	+	+	+	+	+	+
*sample 5	+	+	+	+	+	+

+ = Slightly present, ++ = Largely present, - = Absent

It is deducible from findings that in the aqueous, methanol, ethanol, n-hexane, acetone, and ethyl acetate extracts of *Piper guineense*,leaves studied, that phenolics were slightly present. Hence *Piper guineense* leaves could provide natural phenols to consumers of *Piper guineense* leaves. Qualitatively also, results indicated that with respect to *Piper guineense* leaves, solvent types seemed not to present noticeable variations in the amount of phenols extracted. Ethnomedicinal practitioners should find this very relevant. It is further discernible from the results of the qualitative test presented above, that it would appear, *Piper guineense* Leaves are not very rich sources of total phenols. In particular, no largely present (++) indication was obtained for any of the solvents crude extract. However, it is important that *Piper guineense* Leaves contain phenols, as in assessing nutrient in-take, cumulative sources are more relevant than one point source.

Findings indicate that in raw *Piper guineense* leaves, total phenols level was 0.69±0.14mg. It is also discernable from results presented in Table 2 that in the sun dried *Piper guineense* leaves samples studied, total phenols level was 0.71±0.22mg/g. It would appear therefore that sun drying of *Piper guineense* leaves led to increase in its total phenol content. Apparently, the reported increase in total phenol level in *Piper guineense* leaves, following the sun drying of *Piper guineense* leaves, is an indication of continued biosynthesis of this secondary metabolite postharvest in *Piper guineense* leaves. This is worthy of note particularly, in the formulation of standard methods involving the use of *Piper guineense* leaves, in order that consistency in concentrations of total phenols value in the products are maintained. Findings also revealed that the biosynthesis of phenols was not disrupted by sun drying of *Piper guineense* leaves. Handlers and users of *Piper guineense* leaves will find this information relevant.

3.2: Results of Quantitative Determinations of Total Phenols

Results of quantitative analysis of total phenols levels in raw, sun dried, grated and stored *Piper guineense* leaves samples investigated are presented in Table 2.

Table 2: Quantitative Analysis of Total Phenols Levels in Raw, Sun Dried, Grated and Stored *Piper guineense* Leaves samples

S/N	Parameter	Raw (fresh) sample	Sun dried and pre-stored sample	Stored samples									
				Storage conditions/time (months)									
				a_w 0.97		a_w 0.52		a_w 0.23		Open Laboratory			
										Covered container		Opened container	
				2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month
1	Phenols (mg/g)	0.15 $\pm$ 0.06	0.19 $\pm$ 0.40	5.40 $\pm$ 0.91	1.73 $\pm$ 0.38	7.16 $\pm$ 1.05	2.41 $\pm$ 0.17	100.05 $\pm$ 1.12	3.98 $\pm$ 0.41	6.29 $\pm$ 1.05	2.08 $\pm$ 0.31	2.75 $\pm$ 0.40	0.79 $\pm$ 0.18

It is discernible from open laboratory storage studies with sun dried and grated *Piper guineense* leaves, that storage changes occurred in the levels of total phenols at the end of two months storage time. Significantly, it was observed that with respect to sun dried and grated *Piper guineense* leaves samples stored in opened container and kept in open laboratory, the total phenols level was 2.87±0.83mg/g at the end of the storage period. On the other hand, the value of total phenols in samples stored in closed container and kept in the open laboratory was 6.11±0.73mg/g. Obviously these results indicate that storing sun dried and grated *Piper guineense* leaves in either opened or close d containers and kept under open laboratory conditions, favoured continued biosynthesis of phenols. The reported findings that storage of sun dried and grated *Piper guineense* leaves in closed container led to higher total phenols level compared to samples stored in opened container, is suggestive that at the open laboratory conditions, the series of reactions that led to increased total phenols level, were favoured more by the conditions in the closed containers. Significantly, if simultaneous occurrence of total phenols degradation and synthesis reactions took place in samples stored in the open laboratory under ambient conditions, then, storing sun dried and grated *Piper guineense* in closed containers, tended to favour synthesis reaction more than storing in closed containers. It could also mean that in the closed container, less of degradation reactions involving the phenolics took place, compared to the degree of degradation reactions that probably took place in the sun dried, and grated *Piper guineense* leaves, stored in the opened container, when both were kept in the open laboratory under ambient conditions.

Water activity studies with total phenols in sun dried, grated and stored *Piper guineense* leaves indicate that water activity fostered the continued biosynthesis of the phenolics in sun dried, grated and stored *Piper guineense* leaves. The value of total phenols in sun dried, grated and stored *Piper guineense* leaves, at the end of the storage period, was highest in the samples stored at a_w of 0.23. Significantly, at the end of the storage period the value of total phenols in samples stored at a_w 0.23 was 10.49±1.15mg/g. On the other hand, at the highest storage a_w of 0.97 used in this work, at the end of the storage time, total phenols level in samples stored at this a_w was 5.63±1.02mg/g. The corresponding value of the secondary metabolite examined in sun dried and grated *Piper guineense* leaves then stored at a_w 0.52 was lower than value obtained in samples stored at a_w 0.23, but higher than those of samples stored at a_w 0.97. Obviously, assuming both degradation and synthesis reactions simultaneously took place in sun dried, grated and stored *Piper guineense* leave, what it then means is that biosynthesis reactions, catalyzed by synthase enzymes were favoured over degradation reactions in sun dried and grated *Piper guineense* leaves stored at the different a_w investigated in this work. In particular, the fostering of biosynthesis appeared to be more at the low a_w of 0.23, wherein it seemed also, that the greatest reduction in degradation reactions occurred. Statistical analysis revealed that at $P<0.05$, the increases in total phenols constituent of sun dried, grated and stored *Piper guineense* leaves noted in this work, were statistically significant with respect to the storage conditions studied.

Findings from storage studies in this work, have in particular, revealed the relevance of standardizing storage conditions for sun dried, grated and stored *Piper guineense* leaves especially with respect to its total phenols content, if the beneficial effects of such practices are to maximized. Significantly, while storage of sun dried and grated *Piper guineense* leaves particularly at low a_w will enhance the availability of total phenols, there is need to strike a balance between the desired consequences of enhanced availability of total phenols on one hand, and the relevance of toxicity on the other hand. This in particular, should be considered in the utilization of stored sun dried and grated *Piper guineense* in dietary and pharmaceutical preparations especially, with respect to the long term effects of consumption of stored sun dried and grated *Piper guineense* leaves products.

The total phenols content of *Piper guineense* leaves as well as the noted storage increase in sun dried grated and stored *Piper guineense* leaves could be therapeutically relevant. Significantly, phenols are antioxidants. According to [2] phenolic compounds are a class of antioxidants, which act as free radicals, terminators. Worthy of note is the remark of [3] that the reactive free radicals

species foster lipoprotein oxidation, arteriosclerosis, premature cellular aging, and even carcinogenic mutations. It would appear therefore, that the consumption of *Piper guineense* leaves, especially in their sun dried, grated and stored form, can help furnish the body with defense agents against deleterious oxidation reactions.

Plant phenols can protect against lipoprotein oxidation [27]. It is also imperative to mention that phenols retard oxidative degradation of lipids, hence improve the quality and nutritional value of foods, especially when in storage. It would appear therefore, that *Piper guineense* leaves especially the sun dried, grated and stored form could be used as ingredients in certain food processing, where amongst other function they can supply natural antioxidants. It is imperative to mention that [28] noted that several studies have demonstrated the antimicrobial activity of phenols and /or phenolic extracts. Therefore, the suggested relevance of the use of *Piper guineense* leaves as food additives can also be viewed from this perspective.

3.3: Results of Percentage Antioxidant Activities Determinations

Results of determinations of percentage antioxidant activities of raw, sun dried, grated and stored *Piper guineense* leaves extracts are presented in Table 3.

Table 3: Percentage Antioxidant Activity of Raw, Sun Dried, Grated and stored *Piper guineense* Leaves Extracts.

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)	85.16± 1.41	78.35± 0.91	72.08± 1.20
2	a_w 0.52 , stored sample extract (0.25mg/ml)	85.16± 1.41	75.79± 1.15	66.70± 0.74
3	a_w 0.97 , stored sample extract (0.25mg/ml)	85.16± 1.41	72.39± 1.23	60.08± 0.25
4	Open Laboratory (covered container) , stored sample extract (0.25mg/ml)	85.16± 1.41	74.09± 0.61	64.46± 1.81
5	Open laboratory (uncovered container) , stored sample extract (0.25mg/ml)	85.16± 1.41	68.98± 0.21	55.18± 0.35
6	Sun dried pre-stored sample extract (0.25mg/ml)	85.16± 1.41	ND	ND
7	Raw sample extract (0.25mg/ml)	86.21± 2.13	ND	ND
8	Ascorbic acid (0.25mg/ml)	93.61± 4.15	93.41 ± 2.95	92.17 ± 1.55
9	Gallic Acid (0.25mg/ml)	91.74± 1.88	91.68 ± 0.74	90.25± 1.02

ND = Not Determined

Findings indicated that the percentage antioxidant activity of *Piper guineense* leaves studied was 86.21±2.13%. The obtained percentage antioxidant activity of *Piper guineense* leaves studied is ascribed to their compositional chemistry. According to [29] naturally occurring antioxidants

include enzymes viz: Superoxide dismutase, glutathione peroxidase, catalase, e.t.c; which act as antioxidants by transferring reactive oxygen species and reactive nitrogen species into the stable compounds. Furthermore, [30] noted that high molecular weight compounds such as albumin, transferin, such as ceruplasmin that restrict the production of metal catalysed free radicals are included in the class of natural antioxidants. Also, as posited by [31] low molecular weight compounds involving the lipid soluble antioxidants like tocopherol, quinines, bilirubin and some polyphenols, as well as water soluble antixodiants such ascorbic acid, uric and and some polyphenols belong to the class of natural antioxidants. As noted by [32] minerals such as selenium, copper manganese and zinc are also antioxidants. Additionally, [33] and [34] also remarked that vitamins particularly vitamin A, C and E which play a critical role in preventing peroxidation damage in the biological system are among natural antioxidants. Clearly, from the above classification, the antioxidant factors in plants are numerous, and their presence and quantity could be subjected to different conditions. Consequently, the prevailing conditions with respect to whether favourable or not, will to a large extent determine the availability of a particular antioxidant. Therefore, the obtained percentage antioxidant activity of *Piper guineense* leaves studied could have arisen from different contributory factors.

The joint presence of different antioxidants in plants could have synergistic effect in their actions. Viewed from this perspective, it could mean that synergism contributed to the overall percentage antioxidant activity of *Piper guineense* leaves studied in this work. It is important that *Piper guineense* leaves possessed antioxidants activity. The relevance of this is that *Piper guineense* leaves could be source of natural antioxidants to consumers; and consequently, help to maintain the body's antioxidants composition.

In this work, the reported percentage antioxidant activity of *Piper guineense* leaves studied at a concentration of 0.25mg/ml was lower than those of the standards (ascorbic and gallic acids) at the same concentration of 0.25mg/ml, in the DPPH free radical scavenging activity test. However, *Piper guineense* leaves extracts indicated good antioxidant potentials. Significantly, the studied plant in addition to contributing to the supply of antioxidants to consumers could also be exploited as antioxidants source for industrial applications.

It is also discernible from findings, that sun drying, grating and storage of *Piper guineense* leaves, led to reduction in their percentage antioxidant activity values. With respect to sun drying operation, it could mean that some antioxidant species in *Piper guineense* leaves are photo-sensitive, and consequently, they were adversely affected by sun drying. If some degradation reactions aided or even initiated by light, involving antioxidants constituents of *Piper guineense* leaves occurred during the sun drying of the plant, then this could also led to reduction in percentage antioxidant activity. Grating obviously, would create greater surface area for reactions to take place. Therefore, if the overall consequences of these reactions are degradative in nature, then grating of the samples prior to storage should foster loss in some of the grated plant constituents. In particular, the collapse of cell walls following grating, would promote contact of certain exogenous enzymes with some protected substances. Clearly, this could lead to enzymatic transformation of the protected substances into other forms; which subsequently, and probably, led to certain changes in their biological activities. For instance, the contact of ascorbic acid with ascorbic acid oxidase will foster the oxidation of ascorbic acid and its subsequent conversion to substances with little or no reducing properties. Instructively, ascorbic acid is one of the most powerful antioxidant provided by nature.

Also discernible from results was that in sun dried, grated and stored *Piper guineense* leaves, progressive storage losses with time, occurred in their percentage antioxidant activity values. This was noted in all the samples stored at the various storage conditions. In particular, the magnitude of reduced retention of antioxidant activity, varied with storage condition. Notably, with respect to samples stored in the open laboratory under ambient conditions, those stored in opened containers suffered greater loss in antioxidant activity, compared to samples stored in closed containers. What is anticipated here is that continuous contact of the stored samples with atmospheric factors including oxygen and moisture, fostered greater loss in antioxidant activities of the sun dried, grated

and stored samples. This could mean that the loss in antioxidant activities of stored samples proceeded via some oxidative and hydrolytic degradation processes.

Findings from water activity studies with antioxidant activity of the stored samples also revealed that progressive antioxidant losses occurred with time; and the losses occurred more in the samples stored at the higher a_w. Several factors could have been responsible for this. For instance, at the higher a_w increased amount of available water possibly fostered solubilization of water soluble antioxidant entities with a subsequent increase in their rate of destruction. In stored cassava and garri, [22] posited that increased levels of available water could have promoted increased oxygen dissolution in food materials, leading to increased oxidative loss of ascorbic acid. In accordance with this reasoning, it would appear that increased oxygen dissolution particularly at the elevated a_w, fostered increase in oxidation reactions, which probably led to increased antioxidant species losses; especially in samples stored at the elevated water activity. Significantly, breakdown of crystalline regions of the samples following increased levels of available water, should promote oxygen diffusion into them, and would therefore result in increased antioxidant species degrading reactions. Elevated a_w may act to lower the activation energy for ascorbic acid destruction [35]. Hence perhaps, for the stored samples, at the elevated a_w, lowering of the activation energy for various antioxidant species destructions occurred. The reported storage losses noted for percentage antioxidant activity of sun dried, grated and stored *Piper guineense* leaves, were statistically significant ($P<0.05$) with respect to the storage conditions studied.

4.0. Conclusion

In this work findings have shown that *Piper guineense* leaves contain phenols. Findings further indicated that *Piper guineense* leaves possess antioxidant activity. It was also discernible from results, that sun drying and grating led to increases in total phenols contents of *Piper guineense* leaves. Storage increases in total phenols contents of sun dried and grated *Piper guineense* leaves were also recorded. On the other hand, reduced retention of antioxidant activity was recorded following sun drying and grating of *Piper guineense* leaves. Storage losses of antioxidant activities were equally observed in sun dried, grated and stored *Piper guineense* leaves, at the storage conditions studied. Thus, while the processing and storage conditions investigated in this study positively influenced phenols biosynthesis, they however adversely affected antioxidant activity of *Piper guineense* leaves. It is hoped that findings from this study will be relevant to handlers and users of *Piper guineense* leaves. Additionally, policy makers even if partly, will find the outcome of this study useful in the formulation of standard methods for the processing and storage of *Piper guineense* leaves.

References

[1] Lean, E. J. (2006). Fox and Cameron's Food Science, Nutrition and Health (7th ed.). Edward Arnold Publishers London. 10

[2] Sharafati-Chaleshtori, R., Sharafati-Chaleshtori, F., and Rafieian, M. (2011). Biological Characterization of Iranian Walnut (*Juglans regia*) Leaves. *Turk. J. Bio*.35: 635 – 639.

[3] Pamplona – Roger, D. G. (2005). Enc*yclopedia of Foods and Their Healing Power.* Hang Tai Printing Co. Ltd Vols 1, 2 & 3.

[4] Solihab, M.A., Wan Rosli, W. A., and Nurhanan, A.R. (2012). Phytochemical Screening and Total Phenolic Content of Malaysian *Zea mays*. Hair Extracts. *International Food Research Journal*. 19(4): 1533 – 1538

[5] Yagi, K. (1987). Lipid Peroxide and Human Diseases. *Chem. Phys. Lipids*. 45: 337 – 351..

[6] Maxwell, S. (1997). Antioxidant Therapy: Does it Have a Role in The Treatment of Human Diseases? *Exp.Opin Invest Drugs*. 6: 211 – 236.

[7] Thomas, C. E. and Kalyanaraman, B. (1997). *Oxygen Radicals and the Disease Process*. (Thomas, C. E. and Kalyanaraman, b.ed). Harwood Academic Publishers, The Netherlands.

[8] Scandalios, J. G. (1997). *Oxidative Stress and The Molecular Biology of Antioxidant Defenses*. (Scandalio, J.G. ed.) Cold spring Harbour. New York. 268 – 274.

[9] Jose, N., and Janardhan, K. K. (2000). Antioxidant and Antitumour Activity of *Pleurotus florida*. *Curr. Sci*. 79(7): 941 – 943.

[10] Tiwari, A. K. (2001). In balance in Antioxidant Defence and Human Diseases: Multiple Approach of Natural Antioxidants Therapy. *Curr. Sci.* 81(9): 1179 – 1187.
[11] Guest Editorial. (2002). Antioxidant in Health. *Indian J. Physiol. Pharmocol*. 46(1): 1 – 6.
[12] Dibie E. N. (2019). Selected Nigerian Foods: Characterization, Chemical Toxicology, Storage and Processing Chemistry. *Ph.D Thesis*

[13] Coultate, T. P. (2002). *Food The Chemistry of Its Components* (fourth Edition) Royal Society of Chemistry Publication. 400-4001.
[14] Belitz, H. D., Grosch, W., and Schieberte, P. (2009). *Food Chemistry* (4th Revised and Extended Edition). Springer – Verlag. 8 – 789.
[15] Acker, L.W. (1969). Water Activity and Enzymes Activity. *Food Technol.* 23(10): 1257 – 1270.
[16] Schoebel, T., Tannenbaum, S.R., and Labuza, T.P. (1969). Reaction at Limited Water Concentration .I. Sucrose Hydrolysis.*J. Food Sci.* 34(4): 324 – 329.
[17] Labuza, T.P., Tannenbaum, S.R., and Karel, M. (1970).Water Content and Stability of Low-Moisture and Intermediate – Moisture Foods. *Food Technol.* 24(5): 543-550.
[18] Lajollo, F.S., Tannenbaum, S.R. and Labuza, T.P. (1971).Reaction at Limited Water Concentration. 2. Chlorophyll Degradation. *J. Food Sci.* 36(6): 850 – 853.
[19] Eichner, K., and Karel, M. (1972).The Influence of Water Content and Water Activity on the Sugar – Amino Browning Reaction in Model Systems Under Various Conditions. *J. Agric. Food Chem.* 20(2): 218 – 223.
[20] Ukhun, M. E. (1986). Effect of Storage and Processing on the Nutritive Value of Certain Nigerian Foods. *Experientia* 42: 948 - 950.
[21] Ukhun, M. E., and Uwatse, G. M. (1988). Nutritional Evaluation of Selected Nigerian Rubber Seed Products – a Chemical Approach.*Plant Foods for Human Nutrition*. 38: 309 - 318.
[22] 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: 277 – 283.
[23] Dibie, E. N. and Ukhun, M. E. (2020). Effect of Storage on Selected Physico-Chemical Properties and Fatty Acid Profile of *Aframomum sceptim* Seeds Oil. *Technical Transactions of Materials Science and Technology Society of Nigeria.* 3: 1-10
[24] Rockland, L. B. (1960). Saturated Salt Solutions for Static Control of Relative Humidity Between 5 and 40^0C. *Anal. Chem*. 32: 1375-1376.
[25] 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: 5342 – 5388.
[26] Liyana-Pathiranan, C. M., and Shahidi, F. (2005). Antioxidant Activity of Commercial Soft and Hard Wheat *(Triticum aestivum* L.) as Affected by Gastric pH Conditions. *J. Agric. Food Chem.* 53: 2433 – 2440.
[27] Hollman, P.C.W. (2001). Evidence for Health Benefits of Plant Phenol: Local or Systemic Effect. *Journal of the Science of Food and Agriculture*. 81: 842 – 845.
[28] Cowan, M.M. (1999). Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev.*. 12: 564 – 582.
[29] Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., Lischner, N., Ehlenfeldt, M., Kalt, N., Krewer, G., and Mainland, C. M. (1998). Antioxidant Capacity as Influenced by Total Phenolic and Anthocyanin Content, Maturity and Variety of *Vaccinium species*. J. Agric. Food Chem. 46(7): 2686-2693
[30] Khanam, S., Shivprasad, H. N., and Devi, K. (2004). In vitro Antioxidant Screening Models: A Review. *Indian J. Pharm. Edu.* 38(4): 180-183.
[31] Blois, M. S. (1958). Antioxidant Determination by the use of a Stable Free Radical. *Nature* 181 :1199-1200
[32] Shirwankar, A., Rajendran, K., and Kumar, C. D. (2004). In vitro Antioxidant Studies of *Annona squamosal* linn. Leaves. *Indian J. Exp. Biol.* 42 : 803-807.
[33] Fogliano, V., Verde, V., Randazzo, G., and Ritieni, A. (1999). Method for Measuring Antioxidant Activity and its Application to Monitoring the Antioxidant Capacity of Wines. *J. Agric. Food Chem*. 47 : 1035-1040.
[34] Mantena, S. K., Jagdish, A., Badduri, S. R., Siripurapu, K. B., and Unikrishnan, M. K. (2003). In vitro Evaluation of Antioxidant Properties of *Coco nucifera* Linn. Water. *Nahrung Food*. 2: 126-131
[35] Labuza, T. P. (1972). Processing and Storage Effects on Nutrients in Dehydrated Foods. Crit. Rev. *Food Technical* 3: 217 – 221.