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Proximate, Antioxidant, Vitamins, and Total Phenolic Composition of *Piper guineense* Seeds As Affected by Soaking and Air-Resting

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ABSTRACT: This study investigated the effects of soaking and air-resting on the proximate, antioxidant vitamin and total phenol content, and antioxidant activities of *Piper guineense* seeds. The seed extracts were prepared by soaking the seeds in water for four hours, air-resting for 30, 60, 90, and 120 minutes, and re-soaking for another four hours before being milled and extracted. Proximate composition showed that protein ranged from 4.29-7.30%, moisture content (21.90-62.35%), fat (6.93-8.05%), ash (1.75-7.61%), crude fiber (13.77-38.34%), and carbohydrate (3.39-6.83%). Vitamin A, vitamin C, and vitamin E results ranged from 329.05-908.14 IU, 64.92-95.38 mg/100 g, and 3.7-7.5 mg/100 g. The ferric reducing power of the seed extracts ranged from 66.54 mg/100 g-116 mg/100 g, TAC ranged from 2.50-38.08 mg/ml, antioxidant activity with DPPH ranged from 55.76-66.12% at 50 mg/ml, 68.18-80.44% at 75 mg/ml, 74.79-88.43 % at 100 mg/ml. The results showed a loss of carbohydrates, fats, vitamin C, and total phenols. The results for the 30-minute treatment showed minimal nutrient losses and highest antioxidant activities compared to 60, 90, and 120 minutes, respectively. Therefore, the antioxidants of soaked and air-rested *Piper guineense* seed extracts can help reduce free radicals that cause oxidative stress, leading to degenerative diseases.

Keywords: proximate, antioxidant vitamins, total phenols, antioxidant activity, *Piper guineense* seeds, soaking and air-resting

INTRODUCTION

Piper guineense seeds, known as African black pepper, serve as a spice, food preservative, and insecticide. They are also used in herbal medicine to treat bacterial and fungal infections. The seeds have been a traditional condiment in various foods and native remedies for a long time. In Nigeria, they are called 'Uziza' among the Igbo tribe. Initially consumed in the Southern part of Nigeria, *Piper guineense* is now consumed across the entire country and even in neighboring countries (Usenekong *et al*., 2013). Its incorporation into international dishes and high demand by Nigerians abroad have also popularized its use worldwide. *Piper guineense* is rich in fatty acids, protein, and minerals, making it a valuable culinary ingredient. The seeds are particularly high in omega-3 and omega-6 fatty acids, which are essential for heart health, brain function, and overall health (Ifie *et al*., 2014).

Spices derive their desirable flavors from active constituents, with terpenes or terpenoids being the major flavoring agents (Anton *et al*., 2017). They also contain various other plant constituents, such as phenolic compounds, alkaloids, glycosides, anthraquinones, and flavonoids, among others, which contribute to their functional properties beyond flavor enhancement in food and beverages. These active plant constituents offer nutritional, antioxidant, antimicrobial, medicinal, and insect-repellent properties. Nutritionally, most spices and herbs are rich sources of protein, vitamins—especially vitamins A, B, C, and K—and minerals like calcium, phosphorus, sodium, potassium, and iron (Lawrence, 2017). Despite their nutritional value, the contribution of these dietary plants has historically been underestimated, likely due to the relatively small, though increasing, amounts consumed (Carlsen *et al*., 2011).

The antioxidant properties of spices are attributed to polyphenolic compounds such as flavonoids and phenolic acids, which have strong free-radical scavenging abilities. These natural antioxidants often outperform synthetic antioxidants, some of which may be potential carcinogens (Alexander *et al*., 2017). Recently, there has been a growing interest in using spices as a source of phytochemicals to enhance human well-being and combat free radicals (Satish *et al*., 2014; Pandey *et al*., 2017). Previous studies have shown that the antioxidant capacity of spices and herbs can be enhanced through various culinary processing methods. For example, cooking common culinary herbs and spices, such as cinnamon, cloves, fennel, ginger, parsley, rosemary, sage, and thyme, in small amounts (0.2-1g) using techniques like microwaving, simmering, and stewing can increase their

antioxidant capacity by releasing antioxidant compounds due to heat exposure (Ademoyegun *et al*., 2010). Additionally, research on other seeds like *Moringa oleifera* has demonstrated that processing methods such as drying and roasting can significantly impact the levels of essential nutrients and bioactive compounds, highlighting the importance of optimizing processing techniques to preserve their health benefits (Fasuyi, 2006).

The traditional processing of *Piper guineense* seeds involves soaking in water, rinsing, and other methods like drying, roasting, and boiling to reduce bitterness and improve digestibility and taste. However, these methods can diminish the seeds' nutritional and antioxidant properties, reducing their health benefits (Chiawa *et al*., 2022). While some studies have explored the malting of spices, the specific effects of soaking and air-resting on the antioxidant vitamins and antimicrobial properties of *Piper guineense* seeds have not been thoroughly investigated. For instance, while Ademoyegun *et al*. (2010) focused on antioxidant capacity in common culinary herbs, there is a gap in research regarding the impact of soaking and air-resting, specifically on *Piper guineense*. Studies on similar spices, such as *Capsicum annuum* and *Zingiber officinale*, have shown that pre-treatments like soaking and air-drying can alter their phytochemical composition, suggesting potential similar effects on *Piper guineense* (Sarka *et al*., 2021).

This research aims to evaluate how soaking and air-resting impact the antioxidant vitamins, total phenolic content, and antimicrobial effects of *Piper guineense* seeds, providing valuable data on their antioxidant and antimicrobial properties. By filling this gap in the literature, this study seeks to contribute to the understanding of how traditional processing methods affect the health benefits of this important spice, thereby offering insights that could optimize its use in culinary and medicinal applications.

MATERIALS AND METHODS

Material Procurement and Preparation

The *Piper guineense* seeds were obtained from Ogbete Market Enugu State and other laboratory reagents were procured from the Sigma-Aldrich Company (Food Science and Technology Laboratory, Madonna University Nigeria, Akpugo Campus), and were of analytical grade. The seeds were sorted to remove the unwholesome ones, washed in water to remove any adhering dirt, and then dried.

Determination of the time course variation in water absorption capacity of P. guineense seeds.

The maximum water absorption capacity of the seeds was determined using 10 seeds. The seeds were soaked in water and placed on a filter paper to dry off the surface water and the diameters along the short and long axes of the seeds were measured using a vernier caliper. The weight of the seeds was also taken. This was done at zero hours and subsequently at one-hour intervals until a constant weight was obtained when the seeds could no longer absorb water.

Proximate Analysis

The proximate composition of the sample was evaluated using standard methods described by AOAC (2015). The carbohydrate content of the sample is calculated by difference (Onwuka, 2005).

% carbohydrate = 100 – (% Moisture + % Protein + % Fat + % Ash + % Fiber)

Extraction for antioxidant vitamin determinations.

Two grams (2 g) of each blended sample were placed in test tubes, followed by the addition of twenty milliliters (20 ml) of methanol to each tube. The mixtures were vigorously shaken for ten (10) minutes, allowed to stand for 30 minutes, and then centrifuged at 3000 rpm using a Jinotech centrifuge, model 800 D (Beijing, China), for 10 minutes. The resulting supernatant was transferred to another test tube, and the precipitates were washed off. The supernatants were then left to stand for 24 hours before being used for antioxidant analysis.

Antioxidant Vitamins

The antioxidant vitamins (vitamin C and vitamin E) were determined by the standard spectrophotometric method AOAC (2015) while vitamin A was determined using the Nelson (2002) method.

Ascorbic Acid Content

Five grams (5 g) of each sample was placed in an extraction tube, and 100 ml of the extracting solution (containing meta-phosphoric acid and acetic acid in a 2:1 ratio) was added. After thorough shaking for 30 minutes, the mixture was transferred to a centrifuge tube and centrifuged at 3000 rpm for 20 minutes. The resulting sample extract was transferred to a 100 ml volumetric flask and brought up to the mark (100 ml) with the extracting solution. Subsequently, 20 ml of the extract solution was pipetted into a 50 ml conical flask and titrated with a 2,6-dichloro-indophenol dye solution until a faint pink color appeared. The vitamin C content of each

sample was then calculated (in mg/100g) using a specific formula.

$$Asorbic\ acid\ content\ (mg/100\ g) = C \times V \times DF/WT$$

Where

C = mg of ascorbic acid extract used for titration

V = mg of dye used for titration of diluted sample

DF = Dilution factor

WT = weight of the sample

Vitamin E Content

Five grams (5 g) of each sample was weighed into a 50 ml volumetric flask and mixed with 10 ml of absolute ethanol and 20 ml of sulphuric acid. The flask was wrapped with aluminum foil (to protect it from sunlight) and kept for 40 minutes at the end of which the mixture was agitated and transferred to a separating funnel. Fifty milliliters (50 ml) of distilled water was used to ensure that the content of the flask was completely washed out into the separatory funnel. After the separation, the unsaponifiable matter was extracted (from the mixture) with the addition of 20 ml of diethyl ether. The extract was dried in an oven (Gulfex Scientific DHG 9202, England) for 20 minutes at 30 ℃ and then cooled in a desiccator. The dried and cooled extract was re-dissolved in 10 ml ethanol and used for the assay. The standard known vitamin E concentration solution was prepared from the stock and kept separately. Two pairs of test tubes were added the following: 5 ml of the extracted (test) sample was added to each pair of test tubes, and 5 ml of the standard solution was added to the other pair of test tubes. Then, 5 ml of ethanol and 1 ml of concentrated nitric acid was added to each of the four (4) test tubes. The four labeled test tubes were placed in a boiling water bath for 5 minutes. They were rapidly cooled in running water, and the absorbance of each mixture (in the four test tubes) was measured using a UNICAM Ultraviolet spectrophotometer at 470 nm. The vitamin E content of each sample was calculated (in mg/100g) from the formula.

$$\boldsymbol{Vitamin\ E\ (mg/100g) = \frac{Au}{As} \times C \times F}$$

$$\boldsymbol{F = \frac{100 \times Vf \times DF}{W \times Vs}}$$

Where

F = Experimental factor

Df = Dilution factor

Au = Absorbance of a test sample

As = Absorbance of standard solution

Vf = Total volume of extract

Va = Volume of extract analyzed

W =Weight of the sample

Vitamin A Content

The Vitamin A content of the piper seed extract was determined using the Nielson (2002) method. The sample was weighed out, homogenized, and saponified with ethanolic potassium hydroxide (KOH), (an acid oxidant) for 30 minutes. The saponification mixture was transferred to a separatory funnel, and water was added. Using 1-1.5 volume of hexane, extraction was done and repeated, and the sample extract was later combined. The sample extract was filtered [through a paper containing 5 g (anhydrous) Na_2SO_4] into a volumetric flask, and hexane was used to rinse the filter paper and make the filtrate up to volume. A standard curve was prepared. Vitamin A content in the sample was determined at Absorbance 620 (A620). The hexane in the sample extract and standard solutions were evaporated. Calculations:

$$Vitamin\ A\ (\mu l/g) = C \times \left(\frac{DV}{WT}\right)$$

Where

C = Vitamin A concentration resulting from the sample and standard peak heights or area of determination,

DV = Final dilution volume of sample,

WT = Sample weight (g)

Antioxidant Activity by DPPH Assays and FRAP Assays

Antioxidant activities (AOA) were performed using DPPH (2,2-diphenyl-1-picrylhydrazyl; Sigma Aldrich, MO, USA) according to the methods reported by Orsavová *et al*. (2019). Regarding the DPPH assay, the absorbance was measured at 515 nm using Lambda 25 (PerkinElmer, Waltham, MA, USA). Trolox (Sigma Aldrich, MO, USA) was applied as a standard, and the results were expressed as grams of Trolox equivalent kg-1 (g Trolox kg-1 dw).

Ferric Reducing Antioxidant Power

The Ferric reducing antioxidant power of *Piper guineense* seed extract was evaluated according to the Quechers method as developed by Obied *et al*. (2017). The FRAP

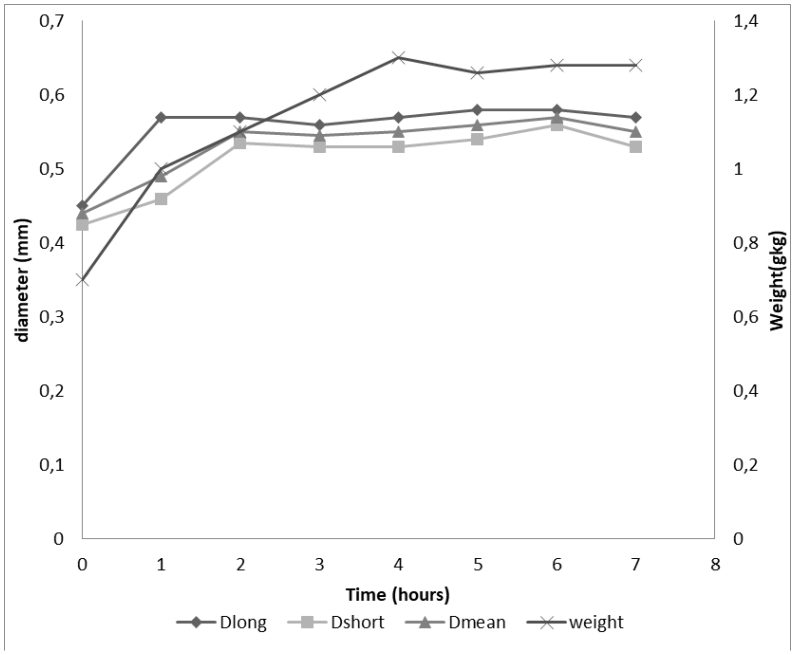

Figure 1: The time course variation in water absorption capacity of *P. guineense* seeds

Table 1. Proximate composition of soaked and air-rested *Piper*

Sample	Protein	Ash	Fats	Moisture	Fiber	Carbohydrate
SA_{30}	5.99±0.01^{c}	1.99±0.01^{c}	7.56±0.00^{a}	62.35±0.07^{a}	18.74±0.00^{b}	3.39±0.00^{e}
SA_{60}	4.29±0.00^{e}	1.81±0.00^{d}	7.45±0.01^{a}	60.90±0.00^{b}	13.77±0.00^{e}	11.75±0.00^{d}
SA_{90}	6.68±0.01^{b}	3.48±0.00^{b}	6.93±0.00^{a}	53.04±0.00^{d}	17.23±0.00^{c}	12.35±0.00^{b}
SA_{120}	5.50±0.00^{d}	1.75±0.00^{e}	7.08±0.00^{a}	57.89±0.00^{c}	15.58±0.00^{d}	12.2±0.00^{c}
R_{pg}	7.30±0.00^{a}	7.61±0.00^{a}	8.05±0.07^{a}	21.90±0.00^{e}	38.34±0.00^{a}	16.83±0.00^{a}

Values are mean ± standard deviation (SD) of triplicate determinations. **KEY:** SA_{30}= soaked and air-rested *Piper guineense* seeds for 30 min, SA_{60} = soaked and air-rested *Piper guineense* seeds for 60 min, SA_{90} = soaked and air-rested *Piper guineense* seeds for 90 min., SA_{120} = soaked and air-rested Piper guineense seeds for 120 min R_{pg} = Raw *Piper guineense* seeds.

assay was prepared by mixing 0.3 M acetate buffer, 10 mM TPTZ solution with 40 mM HCl, and 20 mM ferric chloride solution. Ten microliter (10 µL) of *Piper guineense* extract is mixed with 200 µL of the prepared FRAP solution, and the absorbance is measured at 693 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) after 30 minutes of incubation. The FRAP value is calculated using a standard curve, and the results were expressed as ferrous sulfate equivalent per gram dry weight (Fe (II)/g dw)

Total Phenolic Content Assay

Total phenolic content was determined using the Folin-Ciocalteu method using $NaNO_2$, $AlCl_3 \cdot 6H_2O$, and NaOH. The method was conducted following the protocol described by Orsavová *et al*. (2019) using a UV/VIS spectrophotometer Lambda 25 (PerkinElmer, Waltham, MA, USA). The results were expressed as μg of gallic acid equivalents per 1 mg of extract using the following equation obtained from a standard gallic acid graph (R2 = 0.9878).

Statistical analysis

Statistical analysis, including Analysis of Variance (ANOVA), was conducted to compare different sources of variance within a data set and to determine significant differences between groups. Statistical Package for Social Sciences (SPSS) Version 17.0 for Windows, SPSS Inc. Illinois, USA was used for the analysis. Mean separation was performed using the Least Significant difference (LSD) test, with statistical significance set at a 0.05 level of probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

Moisture content and thickness of the seeds increased with longer air-rest periods, suggesting that the seeds absorbed more water, leading to greater thickness and diameter. This occurs as the seeds' interior layers continue to absorb water during air-resting, causing internal swelling while surface moisture diminishes (Gutierrez *et al*., 2020). These findings are consistent with studies on other seeds and grains. Adetola *et al.* (2024) reported similar moisture absorption and expansion in cowpeas during air-resting, while Ogundele *et al*. (2019) observed increased thickness and mass in maize kernels after soaking and air-resting. These results reinforce the role of air-resting in enhancing seed hydration and morphology.

However, the rate of moisture absorption and seed expansion varies by seed type, soaking conditions, and air-rest duration. For instance, Gutierrez *et al*. (2020) observed a steady increase in moisture content in soaked and air-rested barley seeds, but the increase in thickness was more gradual compared to *Piper guineense*. This variation may be due to differences in seed structure and composition, which affect how water is absorbed and distributed within the seed.

Proximate composition of Piper guineense seeds

Table 1 presents the proximate composition of *Piper guineense* seeds affected by soaking and air-resting at different durations. The analysis reveals significant protein, ash, moisture, and crude fiber content changes due to these processing methods. The protein content of *Piper guineense* seeds ranged from 4.29% to 7.30%. Raw seeds (Rpg) had the highest protein content (7.30%), while seeds soaked and air-rested for 60 minutes (SA60) exhibited the lowest (4.29%). Seeds soaked and air-rested for 30 to 120 minutes exhibited decreased protein content, with reductions ranging from 8.4% (SA90) to 41% (SA60). The SA90 sample retained more protein than others, but all values were significantly different (P<0.05). Compared to commonly consumed spices, such as pepper (2-9%), ginger (5-7%), turmeric (4-5%), and cinnamon (4-6%) (Ifie *et al*., 2019), the protein content in *Piper guineense* seeds is relatively high. However, the soaking and air-resting treatment had a significant negative impact on protein content, suggesting that these methods are not recommended for enhancing protein levels in *Piper guineense* seeds.

Ash contents varied from 1.75% to 7.61%, with raw seeds (Rpg) having the highest ash content (7.61%) and seeds soaked and air-rested for 120 minutes (SA120) showing the lowest (1.75%). The ash content of raw seeds was significantly (P < 0.05) higher than processed samples. Similar to protein, the SA90 sample showed a lower ash loss (54.3%) compared to SA30, SA60, and SA120, which recorded losses of 73%, 76%, and 77%, respectively. This indicates that soaking and air-resting negatively affected ash values, likely due to soluble ash leaching into the soak water during the 8-hour soaking period and the high moisture content. The high ash content in raw seeds suggests that *Piper guineense* is a rich source of minerals. The ash content range of 1.75% to 7.61% aligns with the FAO (2013) normal range for spices like ground black pepper (4-8%), ground turmeric (4-8%), ground cinnamon (2-6%), and ground ginger (2-6%). These observations suggest that soaking and air-resting may not be ideal for processing *Piper guineense*, as they have an inverse relationship with nutritional enhancement.

Moisture content rose significantly from 21.90% in raw seeds to 62.35% in seeds that were air-rested. The trend indicated that moisture increased with longer air-rest periods, attributed to prolonged soaking and extensive water absorption by the seeds. This is consistent with general principles of seed processing, where water absorption is crucial for further processing steps like germination or fermentation.

However, the trend reversed with further air-resting, leading to decreased moisture due to excessive water loss during the extended resting period. Air-resting, a physiological process, involves exposing soaked grains to atmospheric oxygen for a set period, during which aerobic respiration occurs (Gutierrez *et al*., 2020). The low moisture content of raw *Piper guineense* seeds suggests that the spice can be stored for an extended period without quality deterioration. The high moisture content of the treated *Piper guineense* seeds suggests that they may be more prone to quality deterioration during storage. Increased moisture levels can create a favorable environment for microbial growth, including molds and bacteria, which can lead to spoilage and a reduction in shelf life. Additionally, high moisture content can accelerate chemical reactions, such as lipid oxidation, which can further degrade the quality of the spice. Therefore, treated seeds with elevated moisture levels would require more stringent storage conditions, such as refrigeration or drying, to maintain their quality over time (Ojinaka *et al.*, 2016).

The crude fiber content of *Piper guineense* seeds ranged from 13.77% to 38.34%, indicating significant ($P < 0.05$) variation among the samples. Crude fiber in *Piper guineense*, like other plant materials, primarily consists of indigestible components such as cellulose, hemicellulose, and lignin. These components form part of the plant cell walls and provide structural integrity to the seeds. There was a notable reduction in fiber content from 38.34% in raw seeds to 13.77% in seeds soaked and air-rested for 60 minutes (SA60). The significant reduction in fiber content from 38.34% in raw seeds to 13.77% in the sample soaked and air-rested for 60 minutes (SA60) can be attributed to the effects of the soaking and air-resting processes. Soaking involves immersing the seeds in water, which can cause the cell walls to soften and swell. During this process, some soluble fibers, particularly hemicellulose, may dissolve in the water and leach out of the seeds. Additionally, prolonged soaking can break down the structural integrity of the cell walls, leading to further loss of fibrous material. Air-resting, especially after soaking, can exacerbate this reduction in fiber content. Exposure to air and oxygen can initiate enzymatic and oxidative processes that degrade fiber components.

The crude fiber content of 38.34% in raw seeds is higher than the FAO/WHO (2013) recommended dietary intake (38 g for men and 25 g for women) and values reported by Uzoekwe & Ezenwajiugo (2023) for *Piper guineense* leaves (13.0%) and *Zanthoxyllus zanthozylloides* (*Zanthoxyllus piperitum*) (1.4%). Dietary fiber intake is known to reduce the risk of stroke (Fang *et al*., 2016), hypertension (Liu *et al*., 2019), diabetes (Pan *et al*., 2013), and obesity (Brown *et al*., 2011), and potentially improve the immune system (Zhang *et al*., 2019).

The fat content of *Piper guineense* seeds showed a slight decrease, with crude fat content ranging from 8.05% in raw seeds (Rpg) to 6.93% in seeds soaked and air-rested for various durations. Notably, seeds air-rested for 30 minutes (SA30) and 60 minutes (SA60) exhibited better fat retention, with fat contents of 7.56% and 7.45%, respectively. These values are lower than the 13.34% reported for *Myristica fragrans* but significantly higher than the 3.48% for *Rosmarinus officinalis*, commonly used spices in South Eastern Nigeria (Okonkwo & Ogu, 2014). The minimal reduction in fat content can be attributed to high moisture absorption during the soaking and air-resting processes, which have a limited impact on fat content, possibly due to the hydrophobic nature of fats.

The carbohydrate content in *Piper guineense* seeds experienced a significant decrease, from 16.83% in raw seeds to 3.39% in seeds air-rested for 30 minutes (SA30). The longer the air-resting time, the smaller the reduction in carbohydrate content, with an average loss of 40.9% observed across all soaked and air-rested samples. The sample air-rested for 30 minutes (SA30) had the lowest carbohydrate content. This substantial reduction in carbohydrates is attributed to the high moisture absorption during soaking, which significantly reduced the total dry matter content of the seeds. This finding aligns with existing literature indicating that starch content diminishes during steeping due to diastatic activity (McMillan *et al*., 2017).

In conclusion, the proximate composition analysis of *Piper guineense* seeds shows that soaking and air-resting negatively affect protein, ash, crude fiber, and carbohydrate content, although moisture content increases initially. These methods are not ideal for preserving the seeds' nutritional value. The slight reduction in fat content suggests that these processing methods do not significantly alter the lipid profile, maintaining the seeds' utility as a dietary fat source compared to other spices. However, the marked decrease in carbohydrate content highlights the potential drawbacks, particularly regarding the loss of starch and other carbohydrates, which could affect the seeds' energy value and functional properties in culinary applications. This loss is consistent with diastatic activity during steeping, where enzymes break down starches into simpler sugars that leach into the soaking water. Further research is needed to explore alternative processing methods that retain or enhance the nutritional properties of *Piper guineense* seeds.

Effect of steeping / air-resting on Total Antioxidant activity (TAC), Ferric Reducing Antioxidant Power (FRAP), and Total Phenolic content of Piper guineense seeds

Total Antioxidant activity (TAC) (as α-tocopherol)

The Total Antioxidant Activity (TAC) of *Piper guineense* seeds, measured as α-tocopherol, ranged from 2.5 to 28.08 mg/100 g. As presented in Table 2, the result revealed a notable decrease in antioxidant capacity with prolonged air-resting, which correlates with the observed reduction in vitamin E content across the various extracts. This decline in TAC suggests that air exposure during resting periods may negatively impact the seeds' ability to

scavenge free radicals, likely due to the degradation of key antioxidant compounds such as vitamin E.

Table 2. Vitamin Composition of Raw and Treated *Piper guineense* Seeds

Samples	Vitamin A (IU/100 g)	Vitamin C (IU/100 g)	Vitamin E (mg/100 g)
SA_{30}	496.36±0.04^{c}	84.31±0.00^{b}	6.88±0.00^{b}
SA_{60}	329.05±0.00^{e}	74.65±0.01^{c}	5.15±0.00^{d}
SA_{90}	436.78±0.02^{d}	67.33±0.02^{d}	4.7±0.00^{e}
SA_{120}	636.81±0.01^{b}	64.92±0.00^{e}	5.87±0.00^{c}
Rpg	908.14±0.02^{a}	95.38±0.02^{a}	7.25±0.02^{a}

Values are mean ± standard deviation (SD) of triplicate determinations. SA_{30} = soaked and air-rested *Piper guineense* seeds for 30 min, SA_{60} = soaked and air-rested *Piper guineense* seeds for 60 min,, SA_{90} = soaked and air-rested *Piper guineense* seeds for 90 min., SA_{120} = soaked and air-rested Piper guineense seeds for 120 min, R_{pg} = Raw *Piper guineense* seeds

Despite the observed reduction in antioxidant capacity, *Piper guineense* seeds remain a valuable source of bioactive components, including polyphenols, flavonoids, saponins, and proanthocyanidins. These compounds are well-known for their antioxidant properties and contribute significantly to the overall health benefits of the seeds. However, when compared to standard ascorbic acid (21 µg), the antioxidant capacity of *Piper guineense* seeds was significantly lower. This comparison highlights the relative limitations of these seeds in providing antioxidant protection, particularly in processed forms where TAC might be compromised.

Previous research on similar spices, such as black pepper (*Piper nigrum*) and cloves (*Syzygium aromaticum*), provides a useful context for understanding these findings. For example, black pepper, which also contains polyphenols and flavonoids, has shown a reduction in antioxidant capacity following processing methods like drying and storage, similar to the effects observed in *Piper guineense* seeds. In a study by Parthasarathy *et al.* (2008), the antioxidant activity of black pepper was found to diminish over time due to the degradation of its active components under oxidative stress. Similarly, cloves, known for their high antioxidant content, exhibit reduced TAC when exposed to air and light, as reported by El-Massry *et al.* (2009). These studies emphasize that, like *Piper guineense*, other spices also experience a loss in antioxidant activity due to environmental factors during processing.

In conclusion, while *Piper guineense* seeds retain significant bioactive compounds contributing to their antioxidant potential, the reduction in TAC observed with prolonged air-resting underscores the importance of optimizing processing conditions to preserve these health-promoting properties. Comparing this with similar spices further confirms that antioxidant degradation is a common challenge, necessitating careful handling and storage to maintain the efficacy of such spices as dietary antioxidants.

Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant activity (TAC)

FRAP measures the antioxidant capacity by assessing the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The FRAP values of *Piper guineense* seed extracts ranged from 66.54 mg/100 g to 116 mg/100 g. The highest value was observed in seeds air-rested for 30 minutes. The increased FRAP values during the initial air-resting period can be attributed to the development of antioxidant enzymes (Superoxide Dismutase, Catalase, and Peroxidase) and the high total phenolic content in the seeds (Orjiakor *et al.*, 2019). These enzymes work synergistically to protect the seed cells from oxidative stress, enhancing the overall antioxidant capacity. The high total phenolic content in the seed extracts further contributes to the increase in FRAP values, as phenolics are known for their strong antioxidant properties. The reducing power of air-rested seeds was higher than that of untreated seeds. This is consistent with studies linking antioxidant activities to phenolic content and other bioactive molecules (Aazza, 2011; Ravi Kiran *et al.*, 2012)

The total phenolic content in *Piper guineense* seeds ranged from 108 mg/100 g to 320 mg/100 g (See Appendix 1). Raw seeds (Rpg) had the highest phenolic content (320 mg/100 g), while seeds soaked and air-rested

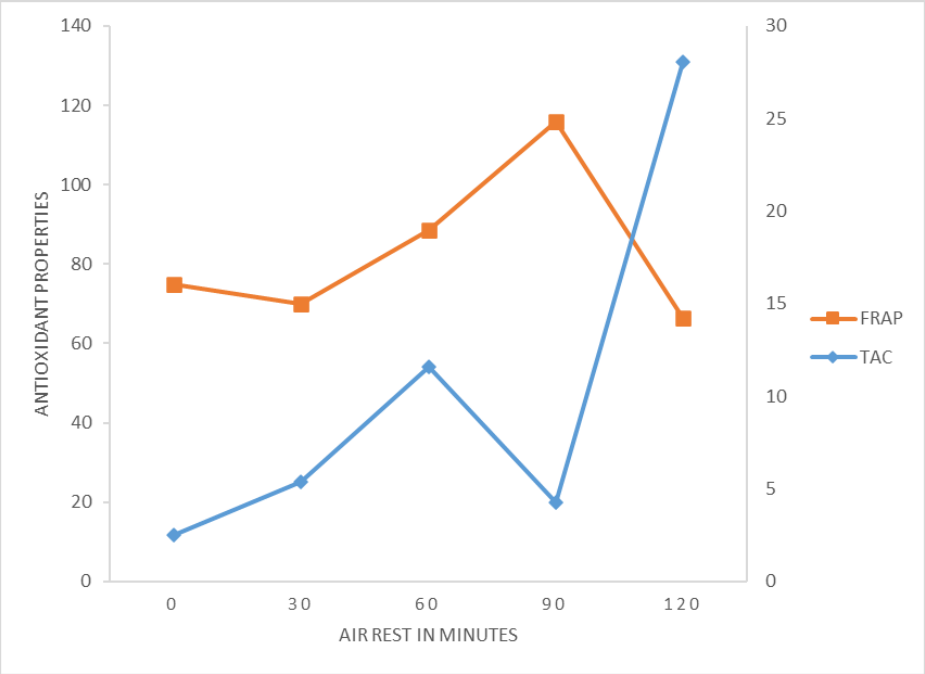

Figure 2. Antioxidant Activities of Raw *Piper guineense*.

for 120 minutes (SA120) had the lowest (108 mg/100 g). Soaking for 8 hours and air-resting for 30 minutes reduced the total phenolic content by 51.83%, with further reductions observed at longer air-resting times. The significant decrease in phenolic content with increased air-resting duration is likely due to the solubility of phenolic compounds in polar solvents, causing them to leach into the soaking water. These findings suggest that prolonged air-resting leads to higher phenol loss, consistent with reports that phenolic compounds' radical scavenging properties are influenced by the presence of hydroxyl groups in polyphenols (Liu *et al*., 2021)

Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant activity (TAC)

FRAP measures the antioxidant capacity by assessing the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The FRAP values of *Piper guineense* seed extracts ranged from 66.54 mg/100 g to 116 mg/100 g. The highest value was observed in seeds air-rested for 30 minutes. The increased FRAP values during the initial air-resting period can be attributed to the development of antioxidant enzymes (Superoxide Dismutase, Catalase, and Peroxidase) and the high total phenolic content in the seeds (Orjiakor *et al*., 2019). These enzymes work synergistically to protect the seed cells from oxidative stress, enhancing the overall antioxidant capacity. The high total phenolic content in the seed extracts further contributes to the increase in FRAP values, as phenolics are known for their strong antioxidant properties. The reducing power of air-rested seeds was higher than that of untreated seeds. This is consistent with studies linking antioxidant activities to phenolic content and other bioactive molecules (Aazza, 2011; Ravi Kiran *et al*., 2012)

The total phenolic content in *Piper guineense* seeds ranged from 108 mg/100 g to 320 mg/100 g (See Appendix 1). Raw seeds (Rpg) had the highest phenolic content (320 mg/100 g), while seeds soaked and air-rested for 120 minutes (SA120) had the lowest (108 mg/100 g). Soaking for 8 hours and air-resting for 30 minutes reduced the total phenolic content by 51.83%, with further reductions observed at longer air-resting times. The significant decrease in phenolic content with increased air-resting duration is likely due to the solubility of phenolic compounds in polar solvents, causing them to leach into the soaking water. These findings suggest that prolonged air-resting leads to higher phenol loss, consistent with reports that phenolic compounds' radical scavenging properties are influenced by the presence of hydroxyl groups in polyphenols (Liu *et al*., 2021)

1, 1 – Diphenyl -2 Picrylhydrazyl (DPPH)

The DPPH assay assesses the ability of *Piper guineense* seed extracts to donate electrons, with inhibitory concentrations measured at 50, 75, and 100 mg/ml, using Vitamin C as a positive control (Rahman *et al*., 2015). The results demonstrated a concentration-dependent increase in antioxidant activity, with higher ethanol extract concentrations showing greater DPPH scavenging

Table 3. Antioxidant properties (DPPH % Inhibition)

Sample Code	Sample conc. 50 mg	Sample conc. 75 mg	Sample conc. 100 mg
SA_{30}	66.12±0.00[b]	80.44±0.00[b]	88.44±0.00[b]
SA_{60}	58.66±0.00[c]	73.07±0.00[c]	81.73±.0.00[c]
SA_{90}	56.83±0.02[e]	71.97±0.00[e]	80.5±0.00[e]
SA_{120}	57.45±0.01[d]	72.34.18±0.00[d]	81.11±0.00[d]
Rpg	55.76±0.00[f]	68.18±0.01[f]	74.79±0.01[f]
Vitamin C	74.15±0.00[a]	85.55±0.00[a]	87.10±0.00[a]

Values are mean ± standard deviation (SD) of triplicate determinations.DPPH = Diphenyl 1,1 - picrylhydrazyl, R_{pg} = Raw *Piper guineense* seeds, SA_{30} = soaked and air-rested *Piper guineense* seeds for 30 min, SA_{60} = soaked and air-rested *Piper guineense* seeds for 60 min, SA_{90} = soaked and air-rested *Piper guineense* seeds for 90 min., SA_{120} = soaked and air-rested *Piper guineense* seeds for 120 min

capacity. At 100 mg/ml, the sample soaked and air-rested for 30 minutes (SA30) exhibited the highest antioxidant activity (88.44 mg/ml), closely followed by the sample soaked and air-rested for 60 minutes (SA60) at 81.73 mg/ml. These values were comparable to that of Vitamin C (87 mg/100 g). The early air-resting periods in SA30 and SA60 likely facilitated the synthesis of components with superior electron-donating abilities, enhancing the antioxidant potential of the extracts. The ethanol extract of *Piper guineense* demonstrated a robust concentration-dependent DPPH scavenging ability, indicative of its hydrogen-donating potential. The highest scavenging effect was observed at 100 mg/ml, while the lowest was at 50 mg/ml, as depicted in Figure 3. This pattern is consistent with findings by Gomathi *et al*. (2016) on alfalfa seeds, which also exhibited increased antioxidant activity with higher extract concentrations. The reducing power of *Piper guineense* is closely linked to its antioxidant activity, as evidenced by its comparable performance to α-tocopherol, a well-known antioxidant (Chang *et al*., 2002). This indicates a strong electron-donating capacity, underscoring the potential of *Piper guineense* as a valuable source of natural antioxidants. In conclusion, the early stages of soaking and air-resting appear critical for optimizing the synthesis of bioactive compounds with strong electron-donating abilities, contributing to the overall antioxidant potential. Nonetheless, extended air-resting periods result in significant losses of total phenolic content and other antioxidants, undermining the nutritional and functional benefits of the seeds. These findings suggest that while *Piper guineense* seeds possess inherent antioxidant properties, alternative processing methods are necessary to better retain these qualities.

CONCLUSION

The study demonstrates that soaking and air-resting negatively impact the nutritional and antioxidant properties of *Piper guineense* seeds, significantly reducing protein, ash, crude fiber, carbohydrate, and antioxidant vitamin content while only slightly affecting fat content. The early stages of processing may optimize the synthesis of bioactive compounds, but extended air-resting results in considerable losses of total phenolic content and other antioxidants. Therefore, alternative processing methods are necessary to retain or enhance the nutritional and functional benefits of *Piper guineense* seeds.

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