



Comparative study of anti-rancidity using peroxide value and *in-vitro* antioxidant activities of ethanol extract of *Musa Sapientum* and *Musa Paradisiaca* peels treated with palm oil

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Abstract

Objective: Antioxidant and estimation of Peroxide Value (POV) to ascertain anti-rancidity potential of extract of *Musa sapientum* and *Musa paradisiaca* peels was studied.

Methods: The antioxidant properties of *Musa sapientum* and *Musa paradisiaca* peels extract were analysed using standard methods.

Results: The results revealed that *Musa sapientum* peel extracts treated with palm oil contained 15.34±0.012 mgGAE/g of phenol while *Musa paradisiaca* peels extract treated with palm oil contained 10.033±0.043 mgGAE/g of phenol. The peel extracts of *Musa sapientum* treated with oil palm contained 18.5±0.004 mg RTE/g showed a significant increase ($p > 0.05$) in flavonoid content compare to that of *Musa paradisiaca* peels with 4.32±0.004 mg RTE/g. *Musa paradisiaca* peels extract added to palm oil shows significant increase ($p > 0.05$) in tannin content in the palm oil. The carotene content in the palm oil was reduced by *Musa sapientum* and *Musa paradisiaca* peels extract as extracts recorded 122.2±0.02 mg/100 g and 112.3±0.011 mg/100 g respectively compared to 234.0±0.005 mg/100 g recorded in the palm oil. The vitamin E content in the oil palm was also reduced by the two peel extracts.

Conclusions: The two peels extract demonstrated high level of anti-rancidity properties since the extracts were able to reduce the level of peroxide content measured in the palm oil.

Keywords: Anti-oxidation, Anti-rancidity, *Musa sapientum*, *Musa paradisiaca*, ethanol extract, peels extract.

1. Introduction

Since the onset of human existence, plants have been dependable sources of sustenance and medications to human beings (Udofia *et al.*, 2023) [32]. The plant parts and herbs from the olden time have been major sources of treatment for different health abnormalities in man and other living organisms. However, due to modernization and the inability of native medical practitioners to give clean native treatment, there was a change in preference for medical treatment sources (Maqsood *et al.*, 2010) [24].

The use of medicinal plants to manage various ailments is gaining wide acceptance in both developed and developing countries. This is as a result of the abundance and ease of availability of these natural products. Several studies have shown that plants provide a good source of remedy against several diseases and ailment ((Jayaprakasha, *et al.*, 2001; Mokbel *et al.*, 2005) [20, 25]. Antioxidants are substance that can prevent or slow the oxidative damage process toward our body. Free radicals are unstable molecules or highly reactive chemical that often contains oxygen and is produced during oxidation. Free radicals are highly reactive and harmful substances that can damage the tissues of organs and cause various diseases (Mokbel *et al.*, 2005) [25]. Natural antioxidants are compound from plant or animals' sources that retard oxidative rancidity of oil, fats and fat soluble components, thus protecting them while delaying the development of unpleasant flavors and odors resulting from oxidation process. Antioxidants are present naturally in most raw food sources (Zengin *et al.*, 2011) [36].

Processing of the fruit can remove or trigger the degradation of this antioxidant. The defensive effect of natural antioxidant in fruit and vegetable are related to its major group, which are vitamin, phenolic and carotenoid. Antioxidants are abundant in fruits and vegetables, as well as in nuts, grains, and some meats, and fish. Combination of vitamin C with other antioxidants, including vitamin E, β -carotene, and selenium, provides a synergistic antihypertensive effect. Experimental evidences prove that antioxidants can protect human body from free radicals and reactive oxygen species (ROS) effects (Halliwell *et al.*, 1981) [18]. In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells (Baskar *et al.*, 2011) [7]. Substantial evidence has accumulated and indicated key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health (Halliwell *et al.*, 1981) [18]. Normally, antioxidant system occurring in human body can scavenge these radicals, which would keep the balance between oxidation and anti-oxidation. The increment of intake of exogenous antioxidants would ameliorate the damage caused by oxidative stress through inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents (Sahreen *et al.*, 2010) [28]. The antioxidant contents of medicinal plants may contribute to the protection they offer from disease. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin, 2012) [17]. Besides, antioxidants also protect cells from the damage that may cause by free radicals; antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Mahmood *et al.*, 2012). Vegetable is fresh edible portion of a plant consumed in either raw or cooked form. Ekpo *et al.*, (2012) [12] reported that food value of fresh vegetables varies from species to species and also from age to age owing to the large amount of mineral nutrients in each species. The peels of a variety of fruits and vegetables have gained attention as a natural source of antioxidants and anti-rancidity content which are rich in compounds with free radical scavenging activity.

Musaceae is one of the major fruit crops, cultivated in tropical and subtropical regions of Asia, America, Africa and Australia for food where it's edible fruits. Banana peels and the vegetative parts of the plants are also used in animal feeding (Heuzé *et al.* 2016) [19]. Bananas come in a variety of sizes and colors when ripe, including yellow, purple, and red. Although the wild species have fruits with numerous large, hard seeds, virtually all culinary bananas have seedless fruits (Arias, 2023) [5]. Banana and plantain peels, an underutilized source of phenolic compounds is considered as a good source of antioxidants for foods and functional foods against cancer and heart disease. Banana peel is rich in phytochemical compounds, mainly antioxidants (Singh *et al.*, 1975) [29]. Banana peels are commonly used as home remedy for several skin problems including allergies, bruises and skin irritation (FAO, 2022). Banana and plantain peels (banana and plantain skin) are the outer envelopes (covering) of the fruits. They are the by-product of household consumption and banana processing. Banana and plantain peels are used as food for

animals, an ingredient in cooking, in water purification, for manufacturing of several biochemical products. The removal of the banana and plantain peels generate a significant amount of organic waste (Onwuka *et al.*, 2003; Arias, 2023) [5,27]. Banana peels are sometimes used as feedstock for cattle, goats, monkeys, poultry, rabbits, fish, zebras and several other species, typically on small farms in regions where bananas are grown (Ariani *et al.*, 2018) [4].

It has been reported that banana fruit do help to prevent anaemia, cure the heart burns stress, strokes, ulcers and many other minor illness. The main by-product of the banana processing industry is the peel, which represents approximately 30% of the fruit. This by-product constitutes an environmental problem because it contains large quantities of nitrogen and phosphorus and its high water content makes it susceptible to modification by microorganisms (González-Montelongo *et al.*, 2010) [16]. Palm oil is widely used by food and non-food manufacturers because of its functional benefits, versatility and widespread availability. Palm oil has diverse functional properties. It provides a semi-solid consistency and long-term stability. It also performs well at high cooking temperatures. Its smooth and creamy texture and absence of smell make it a perfect and affordable ingredient in many recipes, supporting flavour and texture. Crude palm oil (CPO, known also as red palm oil, RPO) is known to contain both healthy beneficial compounds, such as triacylglycerol (TAGs), vitamin E, carotenoids, phytosterols, as well as impurities, such as phospholipids, free fatty acids (FFAs) gums and lipid oxidation products. The present study was designed to evaluate the antioxidant activities and anti-rancidity property using peroxide value of palm oil treated with different methanol extract of Musaceae species peel.

2. Materials and Methods

2.1 Sample Collection

Healthy plantain and banana fruits and palm oil were bought from a local market in Akwa-Ibom State, Nigeria.

2.2 Sample Preparation

The peels were removed from the fruits, washed with clean water, sliced into smaller pieces of 2.0 mm thickness using sterile kitchen knife, divided into two portions and sundried for 4 weeks. The portions were later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction in laboratory for analysis.

2.3 Antioxidant Assay

2.3.1. Determination of Total carotenoids

15ml of petroleum ether were pipette into a separating funnel with Teflon stopcock. 15ml of the acetone extract were added and allowed to stand for 15mins. 150ml of distilled water were added by flowing along the walls of the funnel. The mixture was allowed to separate into two phases and the aqueous phase was washed 4 times with 100ml of distilled water to remove residual acetone. The petroleum ether phase was collected in a 25ml volumetric flask by passing the solution sulfate to remove residual water. The separating funnel was then washed with petroleum ether and the washing was collected into the volumetric flask by passing it through the funnel with sodium sulfate. The volumetric flask was then made up to volume with petroleum ether and the total carotenoids content were determined from the molar absorptivity (AOAC, 2005).

2.3.2. Determination of Vitamin E

The presence of vitamin E in the two peels extract were determine by thin layer chromatography were prewashed in chloroform: methanol (1:1). After drying, the plates were activated at 100 °C for 10min. the peels extract and the pure standard dissolved in methanol were subjected to thin layer chromatography using a mixture of cyclohexane; diethyl ether (4:1) as mobile phase. The mobile phase was allowed to run a distance of 100mm in the saturated tank. The developed plate was left to dry at room temperature, then oven dried for 15 min at 100°C. the plate was sprayed with 10% CuSO₄ phosphoric acid followed by charring at 190 °C for 10min. Vitamin E gave a black spot and was identified in the peels extract by comparison of the R_f (0.53) value with that of corresponding pure standard (Gulcin, 2012; AOAC, 2005) [17].

2.3.4. Estimation of total phenolic content

The Total phenolic contents in the two portion of peels extract were determined by the spectrophotometric method (Kim, 2003). 1 ml of sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na₂CO₃ solution was added to the mixture followed by the addition of 13 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing garlic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of garlic acid equivalents (GAE) per g of dried sample (Wolfe *et al.*, 2003) [35].

2.3.5. Estimation of Total Flavonoid Content

Total flavonoid content was determined following a method. In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30% methanol, 0.15 ml of NaNO₂ (0.5 M) and 0.15 ml of AlCl₃.6H₂O (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using Rutin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of Rutin equivalents per g of dried fraction.

2.3.6. Test for Tannins

50 mg of extract was boiled in 20 ml of distilled water and filtered. A few drops of 0.1% FeCl₃ was added in filtrate and observed for colour change; brownish green or a blue-black colouration was taken as evidence for the presence of tannins (Tinko, *et al.*, 2001; Alves, 2001) [31].

2.4. Anti-Rancidity Assay

2.4.1. Peroxide Value Analysis

This was determined through iodometric titration according to standard methods for the oils analysis AOCS (David, 1998; Gichau *et al.*, 2019) [9, 15] and the results were expressed in meq O₂/kg oil. Two ml of oil samples were weighed into 250 ml stoppered conical flask. Thirty milliliter of acetic acid chloroform solvent mixture was added in each and swirled to dissolve. Then 0.5 ml saturated potassium iodide solution was added with a Mohr pipette and left to stand for 1 minute in the dark with occasional shaking then added about 30 ml

of distilled water. This was titrated with 0.01 N sodium thiosulphate solution, with vigorous shaking until yellow color was almost gone. 0.5 ml starch solution was added as an indicator and titration was continued until the blue color disappeared indicating the end point. Calculation of the peroxide value was done as below:

$$PVC = \frac{\text{Titre} \times N \times 100}{w} \quad (1)$$

Where W is the weight of the sample.

Titre = ml of Sodium Thiosulphate used.

N = Normality of sodium thiosulphate solution.

2.5. Statistical Analysis

The tests were done in triplicate for each sample and mean values determined using One Way Analysis of Variance (ANOVA). Differences at $p \leq 0.05$ were considered significant by applying Duncan multiple range post hoc tests as outlined.

3. Results and Discussion

3.1 Results

The results of the antioxidant and anti-rancidity measured by the Peroxide value (POV) of *Musa paradisiaca* and *Musa sapientum* peels on palm oil are presented in Table 1 and 2. The total phenolic content of *Musa sapientum* and *Musa paradisiaca* peels extract on palm oil were determined according to Folin-ciocalteu method described by (Kim, 2003; Wolfe *et al.*, 2003) [35] and expressed in garlic acid equivalent as shown in Table 1. Result showed that the levels of phenolic content in the *Musa sapientum* peel extracts with palm oil (15.34±0.012) was significantly ($p > 0.05$) higher than the phenolic content recorded in peels extract of *Musa paradisiacal* (10.033±0.043) and the control (0.044±0.02) meq O₂/kg.

The result in Table 1 indicated that the flavonoid content in the peel extracts of *Musa sapientum* mixed with oil palm (18.5±0.004) shows a significant increase ($p > 0.05$) compare to that of *Musa paradisiaca* peels which recorded 14.32±0.004 mg Rutin equivalent/g) of flavonoid. The tannin content obtained in Table 1 shows a significant increase ($p > 0.05$) in the tannin content Pof oil palm when the *Musaceae* species peels were added. The tannin content in the *Musa sapientum* peel extracts (1.34±0.023) recorded lower level of tannin while the tannin content in the *Musa paradisiaca* peels extract (3.17±0.011) was higher than that of *Musa sapientum* and the palm oil (0.031±0.011).

From Table 1, the carotene content in the control (palm oil) shows a significant increase ($p > 0.05$) which differs from the result observed when the two *Musaceae* species peel extracts were added. The palm oil recorded 234.0±0.005 level of carotene while *Musa sapientum* and *Musa paradisiaca* recorded 122.2±0.021 and 112.3±0.011 respectively. It was observed that *Musa sapientum* peels demonstrated higher antioxidant property compare to *Musa paradisiaca* peels.

The vitamin E content in the two Musacae species peels as shown in Table 1 were significantly decrease ($p < 0.05$) compare to the vitamin E content of the two Musa species. The level of vitamin E in *Musa sapientum* and *Musa paradisiaca* peels extract were 23.54±0.003 and 21.42±0.001 respectively as compared to Vitamin E levels in the palm oil (35.23±0.003). It was observed that the Vitamin E content in the palm oil was reduced when the two peels

extract were added. The anti-rancidity properties of the two *Musaceae* species peels on oil palm were determined by measuring the peroxide values of the samples. From Table 2,

the peroxide value of the Palm oil was 18.31 ± 2.00 while the peroxide value of the *Musa sapientum* and *Musa paradisiaca* recorded 10.21 ± 0.003 and 14.43 ± 0.021 respectively.

Table 1: Antioxidants properties of *Musa paradisiaca* and *Musa sapientum* peels on palm oil

Samples	Phenol (mgGAE/g)	Flavonoid (mg RTE/g)	Tannin (mg/100g)	Carotene (mg/100g)	Vitamin E (mg/100g)	Significant
Oil	0.044 ^c ±0.02	0.04 ^d ±0.003	0.031 ^c ±0.011	234.0 ^a ±0.005	35.23 ^a ±0.003	Yes
M.S + OIL	15.34 ^a ±0.012	18.5 ^a ±0.004	1.34 ^b ±0.023	122.2 ^b ±0.021	23.54 ^b ±0.003	Yes
M.P + OIL	10.033 ^b ±0.043	14.32 ^b ±0.004	3.17 ^a ±0.011	112.3 ^c ±0.011	21.42 ^c ±0.001	Yes

*Means with different subscripts along the same row are significantly different (Duncan's test) $p < 0.05$

*M.S-P + Oil – *Musa sapientum* peels extract with Oil palm

*M.P-P + Oil – *Musa paradisiaca* peels with Oil palm

Table 2: Measurement of peroxide value (POV) of *Musa sapientum* and *Musa paradisiaca* peels on palm oil

Samples	Palm Oil (PO)	<i>M. sapientum</i> P+ PO	<i>M. paradisiaca</i> P+ PO	Significant
Peroxide value (meq/kg)	18.31 ^a ±2.00	10.21 ^c ±0.003	14.43 ^b ±0.021	Yes

*Means with different subscripts along the same row are significantly different (Duncan's test) $p < 0.05$

*P+ PO= Peel extract with Palm oil

3.2 Discussion

Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari, 2008) [33]. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage. The results of the study revealed that both *Musa sapientum* and *Musa paradisiaca* peels had effective capacity of scavenging for superoxide radical and correlated with total phenolic and total flavonoid content thus suggesting its antioxidant potential. Strong antioxidant activity of *Musa sapientum* and *Musa paradisiaca* peels statistically similar to total carotene and vitamin E indicates strong antioxidants in the extract.

The result also revealed that *Musa sapientum* peel extract is a good antioxidant compare to *Musa paradisiaca* as supported by the work of Duh and Yen (1997) [10] who reported that the total phenolic compound in plant extract exceeded 14.72GAE/100g give the highest antioxidant activity. The increase in the phenolic content of the *Musa sapientum* peels compare to that of *Musa paradisiaca* may be as a result of the phenolic hydroxyls present in the *Musa sapientum* peel extracts (Gulcin, 2012) [17]. The presence of phenolic compounds (containing phenolic hydroxyls) found in these two extracts could be led to high antiradical properties of these extracts. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability (Gulcin, 2012) [17]. Kähkönen (1999) [21] noted that plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food. Ecocrop (2010) [11] in his work affirmed that banana and plantain peels are rich in phytochemical compounds, mainly antioxidants

Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoidic derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities (Montoro, 2005) [26]. Findings from this study revealed that the two peels extract increase the level of flavonoid content in the palm oil even though *Musa sapientum* peels had a higher value compare to *Musa paradisiaca*. This may be as a result of other bioactive

compounds such as anthocyanins, terpenoids, glycosides present in the peels extract that can increase the level of the flavonoids (Cao *et al.*, 1997) [8]. Susana *et al.*, (2004) observed that antioxidant activity originates from the secondary metabolites contained in the banana peel extract, namely alkaloids, flavonoids, tannins, and saponins.

Foods rich in tannins are considered to be of low nutritional value because they precipitate proteins, inhibiting digestive enzymes and iron absorption and affect the utilization of vitamins and minerals from meals, according to reports by Tinko *et al.*, (2001) [31]. Different in the values of tannin may be as a result of the difference in the bioactive compounds present in the two *Musaceae* species as *Musa paradisiaca* indicated higher level of tannin. The findings of the study affirmed observations of Mokbel and Hashinaga (2001) who investigated the antioxidant effects of crude extracts from green banana and yellow peel. The findings of the study also affirmed observations of Baskar *et al.*, (2011) [7] who investigated the antioxidant potential of 9 local bananas peel varieties which showed that the banana peel extract showed significant antioxidant activity. Aboul-Enein *et al.*, (2016) [1] also reported that banana peel (*Musa paradisiaca* L.) acetone extract of *Musa sapientum* peel extract is a good antioxidant compare to *Musa paradisiaca* which can useful for treating free radical mediated diseases.

Findings from this study revealed that vitamin E content in the palm oil reduced drastically and significantly when treated or amended with *Musa sapientum* and *Musa paradisiaca* peels extract. *Musa paradisiaca* peels extract reduced the vitamin E content of the palm oil significantly compare to *Musa sapientum*. This may be as a result of the anti-nutrient present in the peels extract that contribute to lowering the Vitamin E content of the palm oil. This result coincides with the result obtained by Awak *et al.*, (2017) [6] who observed that anti-nutrients from plant extract can degrade the level of useful minerals.

According to Kaleem (2015) [22], the degree of oil oxidation is determined by the peroxide value. It is a measure of the lipid matrix's freshness and oxidation, especially while the oil is in its primary stage of storage. In the present study, peroxide value (PV) was used to ascertain the anti-rancidity potential of palm oil quality. This study revealed that the two *Musaceae* peel extracts were able to reduce the level of peroxide content measured in the palm oil. The peroxide value obtained from this study ranges from 18.31 ± 2.00

meq/kg in the palm oil; *Musa sapientum* peels extract was able to reduce the peroxide value (PV) of the palm oil to 10.21 ± 0.003 meq/kg compare to the value reduced by *Musa paradisiaca* peel extract (14.43 ± 0.021 meq/kg) which is far higher than the standard value of 10 meq/kg. Therefore, *Musa sapientum* peels shows good antrancidity property compare to *Musa paradisiaca* peels. According to Negash *et al.* (2019), a high peroxide value indicates a higher degree of unsaturated fatty acid and a higher risk of rancidity. Frankel, (1980) ^[14] also affirmed that acidity and a rancid taste are often noticeable in foods when peroxide values are between 20 to 40 meqO₂/Kg.

Thus, in this study, a low peroxide value indicates a lower percentage of unsaturated fatty acids and a lower risk of rancidity (Vanhanen, 2006) ^[34]. A product with PV below 5 meq O₂/kg is classified at low oxidation state, that between 5 and 10 meq O₂/kg at moderate oxidation and above 10 meq O₂/kg is classified at high oxidation state. The PV limit of refined oil according to East African Standard is 10 meq O₂/kg (EAS 795:2013). Codex gives a general PV limit of 15 meq/kg for virgin oils (Kim, 2003). As such the *Musa* species peels extract can increase the shelf life of palm oil due to their low level of peroxide when added to palm oil.

4. Conclusion

The replacement of synthetic with natural antioxidants (because of implications for human health) may be advantageous. The present study showed that *Musa sapientum* peels had a higher value compare to *Musa paradisiaca* of antioxidant activities and free radical scavenging activity and therefore can be the potent source of natural antioxidants. This study also revealed that the two peel extracts were able to reduce the level of peroxide content measured in the palm oil. The degree of oil oxidation is determined by the peroxide value. The peel extracts from these two varieties *Musaceae* may be useful to combat oxidation and rancidity of palm oil during storage resulting from growth and activities of microorganisms, food enzymes activities and chemical reactions within the food.

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6. Competing Interests

Authors have declared that no competing interests exist.

7. Authors' Contributions

The authors worked together to complete this work. The final manuscript was read and approved by the authors.

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