COMPARATIVE ANTIOXIDANT STUDY OF RIPE AND UNRIPE PLANTAIN AND THE QUALITATIVE ASSESSMENT OF SOME FOOD OIL EXTRACTS

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Abstract
The reaction of oil with oxygen can cause structural degradation with the development of rancid flavours that reduces the organoleptic characteristics and formation of oxidation products with consequent reduction in shelf life, loss of taste, flavour, food quality and detrimental effect on human health. Therefore, the overall quality of oil in foods contributes ultimately to the nutritional quality of that food. Thus, this study was at evaluating the quality of some food oils. The various oils were extracted locally and the quality was evaluated based on the peroxide values, iodine values, saponification number, free fatty acids and cholesterol values. Ripped and unripe plantain was screened for their Dinitrophenylpincryl hydrazine (DPPH) and Nitric oxide (NO) radical scavenging activity and quantitative alkaloid, flavonoids and tannin content. The result showed that out of all the food oil, coconut oil outflanked the others in terms quality as reflected in all the reference chemical parameters. The ripped plantain showed a better antioxidant activity than the unripe. Therefore, the result of this study affirms the quality and oxidative stability of coconut oil and that it could have a longer shelf life. The antioxidant activity of the ripped plantain maybe associated with its higher tannin and flavonoids content.

Keywords: Food oils, Oxidation, Antioxidant, Oil quality, Plantain

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1. INTRODUCTION

Edible oils are important constituents of diet and also serve as energy for various metabolic processes in the body. Furthermore, they are present in essential fatty acids and also function as carriers of fat-soluble vitamins (Zahir et al., 2017). Lipids and triacylglycerol occur naturally in fats and oils, and are chemically composed of saturated, unsaturated fatty acids and glycerides (Zahir et al., 2017). Lipids are important in foods because of their contribution to satiety, palatability and nutrition. Hence, reasonable quality of lipids is essential in food consumption (Givens and Gibbs, 2006). Oxidation, hydrolysis and thermal alteration of oil cause deleterious reactions that result in the rancidity of oil which has been implicated in the polymerization and homolytic β-scission of hydro-peroxides (Frankel, 1998; Otemuyiwa and Adewusi, 2013). Oxidation of lipids especially unsaturated fatty acids result in the formation of conjugates and polymers. In addition, reaction with oxygen can cause structural degradation with the development of rancid flavours that reduces the organoleptic characteristics and formation of oxidation products consequently, reduction in shelf life, loss of taste, flavour, food quality and detrimental effect on human health (Bhattacharya2008; Dobarganes et al., 2000). Therefore, the overall quality of oil in food is essential in the nutritional quality of that food. Oil quality can be evaluated through the measurement of chemical parameters such as peroxide value, iodine value, saponification number, free fatty acids and cholesterol value.
Free radicals are unstable compounds having an unpaired electron in their atomic electron shell. They are usually short-lived species and highly reactive against important biological macromolecules such as the DNA, proteins and membrane lipids (Xia et al., 2003). Free radicals react aggressively with biological molecules, trapping electrons away from them and consequently rendering them unstable to

start a chain reaction. This has been implicated in the pathogenesis of several metabolic disorders such as cancer, cardiovascular disease, neurodegenerative diseases, Alzheimer disease, Parkinson disease and diabetes (Astley, 2003; Jothy et al,200; Abalaka et al., 2011; Gramza et al., 2005)**.** When free radical activity increases beyond the antioxidant threshold of a biological system, oxidative stress ensue (Zima et al., 2001**).** Often time antioxidant based drug formulations are used in order to avoid oxidative stress related ailments (Ishtiaqet al., 2001). Fruits and vegetables are rich sources of natural antioxidant compounds that have been linked with their health promoting effects (Patel et al., 2011). Important compounds include alkaloids, flavonoids, tannins, steroids and phenolics. High antioxidant activity as well as biological effects has been associated with flavonoids and alkaloids that are usually found in medicinal plants (Mbaebie et al., 2012)

Musa paradisiacae (Plantain) is an important staple food crop in West Africa. Its starchy fruits (ripe or unripe) can be cooked, fried, roasted or processed for consumption (Chinedum, et al., 2011; Shodehinde and Oboh, 2013). Plantain is a good source of dietary fibre, starch, proteins, fat and ash (Shodehinde and Oboh, 2013). It is also a rich source of calcium, vitamins A, B1, B2, B3, B6, C and minerals such as potassium and phosphorus (Osundahunsi, 2009). Because of the low glycemic index of plantain, the unripe powder is commonly consumed by diabetics in Nigeria to reduce after-meal glucose level (Eleazu et al., 2010; Eleazu et al., 2012). Furthermore, plantain is employed in the folklore management of chronic wounds and ulcer. It has also been reported for its antimicrobial, anti-urolithiatic and analgesic properties (Kumar et al., 2012). Shodehinde and Oboh (2013) identified different phenolics such as apigenin, luteolin, myricetin, capsaicin and isorhaemnetin in plantain, some of which have been related with anti-thrombotic, anti-pyretic, anti-inflammatory, hypolipidemic, hypocholesterolemicand analgesic properties (Min-Ji et al., 2012). Hence, this study assessed the 2, 2 dinitrophenylpincryl hydrazine (DPPH) and Nitric oxide (NO) radical scavenging activity of ripe and unripe plantain as well as the quantitative alkaloid, tannin and flavonoids composition.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The seeds of soybean, groundnut, melon, fruit of coconut and plantain were purchased from a Market in Ogbomoso, Oyo State. The samples were oven dried at 40°C and grinded to powder. Groundnut and melon oil was extracted locally by pressing. The coconut oil was extracted in boiling water. Grinded sample of soybean was macerated in n-Hexane for 72hours, filtered through Whatman's No 1 filter paper and the filtrate was concentrated under pressure to obtain the crude oil. The oil samples were kept in an airtight container until use. All chemicals were of analytical grade (Sigma Aldrich) and were used without any further purification.

2.2 Qualitative oil analysis

2.2.1 Peroxide value

Peroxide value was determined according to the method described (AOCS, 2003). Five gram (5g) of the oil sample was weighed into a conical flask, dissolved with 30ml of solvent mixture containing glacial acetic acid and chloroform in the ratio 3:2 respectively. Half (0.5ml) of saturated potassium iodide (KI) was added, mixed and allowed to stand in the dark for 5min. After 5min., 30ml of distilled water and 1ml starch indicator was added. The solution was immediately titrated with 0.01N sodium thiosulfate solution until yellow color was discharged. Blank sample was also titrated following the same procedure with the exclusion of oil. Peroxide value was calculated using the following expression:

$$\text{Peroxide Value (meq. peroxide/Kg)} = \frac{(V1\text{-}V2)\cdot N\cdot 1000}{W}$$

where:

V1 = volume of $Na_2S_2O_3$ consumed for sample;
V2 = volume of $Na_2S_2O_3$ for blank;
W = weight of the sample in g;
N = normality of the sodium thiosulphate.

2.2.2 Iodine value

Ten millilitres (10ml) of chloroform was added to 100mg (0.1g) of sample weighed into an iodine flask using a dispensing device and shaken to dissolve the sample, thereafter 10ml of Wij's solution. Stopper the flask and kept in the dark for 30min. After 30min., 8ml of 10% KI solution and 100ml of distilled water were added. The mixture was titrated immediately with 0.1N $Na_2S_20_3$ until the aqueous layer begins to lighten. This was followed by the addition of 2ml starch solution and continuous titration until the blue colour of the aqueous solution begins to disappear. The iodine flask was securely closed and vigorously shaken to extract any iodine remaining in the chloroform layer. When the end point (colourless) appear to have been reached, the flask was shaken again, followed by the addition of 2 drops of starch solution to verify that no more blue colour is formed in the aqueous layer. The burette reading was recorded. The blank was prepared with only 10ml chloroform (AOAC, 1998).

The Iodine Value (IV) was calculated with the equation:

$$\text{Iodine Value} = \frac{\text{ml } Na_2S_2O_2 \text{ consumed for blank} - \text{ml } Na_2S_2O_2 \text{ consumed for sample} \cdot N \cdot 12.69}{\text{Weight of sample in g}}$$

N = Normality of $Na_2S_20_3$.

2.2.3 Saponification value

Determination of saponification value was carried according to the combined method of (Evans et al., 1974; Rossel,1984; Rosales,1992 and Kanata and Kande,1993). Two grams (2.0 g) of the oil was transferred into a conical flask to which 20ml of the ethanol was added to dissolve the oil and 50ml of ethanolic KOH to the content of the beaker. The sample was heated on a hot plate for exactly 2min. and allowed to cool to room temperature. 2–4 drops of phenolphthalein indicator was added and titrated to pink end point using 0.1N HCl. A blank was also run and the titer values for both sample and blank were recorded.

The saponification value (SV) was calculated with the equation:

$$\text{SV(mg KOH/g)} = \frac{(V - B) \cdot N \cdot 56.1}{W}$$

SV = Saponification Value;
V = Titter value/volume of 0.1N HCl consumed for sample titration;
B = Blank;
S = Titter value/volume of 0.1N HCl consumed for sample titration;
56.1 = Molecular weight of KOH;
W = Weight of sample in g.

2.2.4 Total FFA by titration

One gram (1g) of the test sample was taken into a conical flask with 20ml of the solvent mixture added to the sample. 0.2ml of phenolphthalein indicator solution was added and titrated while shaking with 0.1N KOH till pink colour appears and persists for at least 10sec. A blank titration was also carried out (Mahesar, 2014).

Total FFA was estimated using the equation:

$$\%\text{FFA (as Oleic Acid)} = \frac{(V - B) \cdot N \cdot 28.21}{W}$$

Where:
V = Volume of titrant (KOH) consumed for sample
B = Volume of titrant consumed for blank
N = Normality of titrant (KOH)
W = Weight of sample
28.21 = Value from the molecular weight of Oleic acid (282.1g/mol) after being multiplied by 100 followed by dividing with 1000 (converting weight to mg) in order to convert result to %.

2.2.5 Total Cholesterol

To test for cholesterol, standard cholesterol of the following concentrations 1, 2, 4, 6, 8, 10mg/l were prepared into five volumetric flasks, with the sixth volumetric flask as blank. To each of the flask including the blank flask, 2ml of Liebermann-Burchard reagent was added and diluted to final volume of 10ml with chloroform. The flasks were covered with black carbon paper and kept in the dark for

15min. Absorption was read at 640nm. Same procedure was followed for the oil sample. One (1ml) of oil sample was taken into a flask, 2ml of Liebermann–Burchard reagent and 7ml of chloroform was added to the oil. The graph of absorbance against concentration for the standard was plotted and concentration of cholesterol in mg/1 of the sample was extrapolated (Attarde et al., 2010).

$$\text{Cholesterol (mg/kg)} = \frac{\text{concentration obtained in mg/l volume of sample}}{\text{Sample weight}}$$

2.3 Quantitative phytochemical determination of ripe and unripe plantain

2.3.1 Determination of Tannins

Five gram (5g) of each of the grounded sample was weighed into a conical flask in triplicates and 100ml 2M HCL was added. The content was boiled on a water bath for 30minutes, thereafter cooled and filtered using Whatman No.1 filter paper. The filtrate was taken up twice in 40ml each of diethyl ether. The ether extract was then heated to dryness and weighed. The average of each sample was calculated and their percentage.

2.3.2 Determination of Flavonoids

Five grams (5g) of each sample was extracted with 50ml of 80% aqueous methanol in triplicates repeatedly at room temperature. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was then transferred into a 200ml beaker and evaporated into dryness over a water bath until constant weight was obtained. Total flavonoids were estimated by Chang *et al* (2002) aluminum chloride colorimetric method.

2.3.3 Determination of Alkaloids

Two and half grams (2.5g) of each sample was weighed into a 250ml beaker; 200ml of 20% acetic acid in ethanol was added and allowed to stand for 4 hours, thereafter filtered and the extract was concentrated using a water bath to evaporate about a quarter of the original volume. Concentrated ammonia solution was added drop-wisely to the extract until precipitation was completed. The entire solution was allowed to settle and the precipitate was filtrated and weighed. The average of each sample was calculated and their percentage (Harbone, 1973).

2.4 Antioxidant determination of ripe and unripe plantain

2.4.1 DPPH radical scavenging activity

The DPPH antioxidant activity was determined spectrophotometrically using reported method of Brand-William et al (1995) with slight modification. 0.1 mM solution of DPPH was prepared in methanol. 150 and 300µg/ml of the sample and L-Ascorbic acid were made in methanol, the initial absorbance of the DPPH in methanol was measured at 517 nm and did not change throughout the period of assay. The sample was added to 3 ml of methanolic DPPH solution and incubated for 30mins. The change in absorbance at 517 nm was measured after incubation. The antioxidant capacity is based on the DPPH free radical scavenging ability as the percentage decrease in absorbance. All measurements were performed in triplicate and the result was an average of three values.

$$\%\ \text{inhibition of DPPH} = \{(\text{AB} - \text{AA})/\text{AB}\} \cdot 100$$

Hence, AB is the absorbance of blank sample and AA is the absorbance of tested extract solution.

2.4.2 Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was assessed using the method described by Bagepalli et al (2010) with slight modification. Firstly, 50µL Sodium nitroprusside (10mM) in phosphate buffer saline was incubated with 150 and 300µg/ml respectively for ripe and unripe plantain for 15min. at room temperature. 125µL of Griess reagent was thereafter added and incubated for 10min. at room temperature and the absorbance was read at 546nm. The experiments were performed in triplicate and quercetin was used as standard.

$$\%\ \text{inhibition} = \{(\text{AB} - \text{AA})/\text{AB}\} \cdot 100$$

3. RESULTS AND DISCUSSION

Lipid oxidation is the major deteriorative process that affects the quality and value of fats and oil, especially in relation to the development of off-flavour as a consequence of auto-oxidation (Xiuzhu et al., 2007). The quality of oil in foods is essential in the overall nutritional quality of that food and can be evaluated by the measurement of chemical parameters like peroxide value, iodine value, saponification value, free fatty acid and cholesterol value.

Peroxide value (PV) is commonly used to access primary oxidation of lipids as the amount of peroxides formed in fats and oils during oxidation (Gulcan and Bedia, 2007). It is a measure of the extent of rancidity reactions that has occurred and can be used as an indication of the quality and stability of fat and oil (Ekwu and Nwagu, 2004). Peroxide value (PV) could increase with the storage time, temperature and contact with air of oil samples (Erun et al., 2017). High peroxide value means that oil can easily go rancid and therefore has short shelf life (Ibeto et al., 2012). The PV of Groundnut oil (2.90 meq.pero./kg), Melon oil (2.40 meq.pero./kg), Coconut oil (0.23meq.pero./kg), and Soybean oil (1.40 meq.pero./kg) are shown in Table 1. Oils having high percentages of peroxide are unstable and go rancid easily (Nziokwu et al., 2007). The result in Table 1 shows the quality and stability of Coconut and Soybean oil and is likely to have longer shelf life than Groundnut and Melon oil.

Iodine value (IV) measures the degree of un-saturation of fat and oil. It could be used to ascertain the stability of oils to oxidation, and permit the determination of the overall un-saturation of the fat qualitatively (AOCS, 1993; Asuquo et al., 2012). Oils with IV above 125 are classified as drying oils while those between110 and 140 are classified as semidrying oils. Oil with IV less than 110 is anon-drying oil. This implies that oils susceptible to drying are also susceptible to rancidity, hence cannot be preserved for a long period of time (Ibeto et al., 2012). From Table 1, it can be observed that groundnut oil had IV of 93.36g/100g, Melon oil (123.27g/100g), Coconut oil (23.37g/100g) and Soybean oil (191.62g/100g). The high IV of Melon and Soybean oil indicates high degree of un-saturation, which may imply high degree of susceptibility to oxidation. The result affirms the quality and oxidative stability of Coconut oil. Therefore, the low iodine values may contribute to its oxidative storage stability.

Saponification value is an index of average molecular mass of fatty acid in oil sample and can be used to check adulteration. It indicates the presence of high percentage of fatty acids in the oil (Omolara and Dosunmu, 2009) and the tendency to soap formation (Ibeto et al., 2012). High saponification value is an indication of low molecular weight of the triglyceride. The lower the saponification number, the higher the molecular weight (Omari et al., 2015). It also means that, the shorter the saponification value the higher the molecular weight and the longer the fatty acid chain length. The result in Table 1 shows that Groundnut, Melon, Coconut and Soybean oil had a saponification value of 142.40(mgKOH/g), 145.48(mgKOH/g), 150.30(mgKOH/g) and 135.52(mgKOH/g) respectively.

Refractive index (RI) reflects the level of un-saturation or conjugation of oil. Higher RI is a reflection of high degree of un-saturation or conjugation and vice versa. In this study, only a slight difference was observed in the RI of the food oil. The Coconut oil showed the least value to corroborate the observation in the iodine value of this same oil,thus Coconut oilhas the least degree of un-saturation of all the oil.

The analytical data of the cholesterol level of each oil are reflected in Table 1.

Table 1: Qualitative chemical parameters of different food oil extracts

Parameters	Groundnut oil	Melon oil	Coconut oil	Soybean oil
Peroxide Value (meq.pero./kg)	2.90±0.001	2.14±0.001	0.23±0.01	1.40±0.02
Iodine Value (g/100g)	93.36±0.23	123.27±0.25	23.37±0.12	191.62±0.28
% Free Fatty Acid (as Oleic)	3.41±0.002	3.66±0.001	0.30±0.01	1.15±0.01
Saponification Value(mgKOH/g)	142.40±0.32	145.86±0.40	150.30±0.32	135.52±0.33
Refractive Index (Brix%)	71.00±0.22	72.40±0.19	65.50±0.35	72.50±0.23
Cholesterol (g/100ml)	0.01±0.00001	0.01±0.01	0.00073±0.00	0.024±0.000

Important functions of cholesterol in the body include providing essential components of membrane, serving as a precursor of bile acids, steroid hormones and vitamin D (Dimberu and Belete, 2011). Also, cholesterol may be associated with increased risk of cardiovascular related disease. LDL (low density lipoproteins) is termed bad cholesterol because it is responsible for allowing fatty plaques to develop in the arteries (Mishra and Manchanda, 2012). Consumption of cholesterol in diet increases the level of LDLs (Dimberu and Belete, 2011). The result of this study showed that cholesterol is present in all the oil samples but in small proportion. Among all the oils, Coconut oil recorded the least cholesterol content therefore, reasserts the quality of this oil.

Oxidative damage occurs when there is an imbalance between the oxidants and antioxidants in a system. Antioxidants functions to protect against free radical induced damage. Table 2 presents the DPPH and Nitric oxide (NO) radical scavenging activity of ripped and unripe plantain. The DPPH is a purple colour free radical that is stable at room temperature. It is a widely used model to study antioxidant activities in a relatively short time. Reduction of DPPH by antioxidants causes discoloration of the radical with a consequence loss of absorbance. The ripe (67%) plantain demonstrated a better DPPH radical scavenging activity than the unripe (45%) at 150 and 300µg/ml. However, ascorbic acid scavenged 87% of the DPPH radical. In addition, the NO radical scavenging activity was observed to be higher for the ripped than the unripe plantain. The reference antioxidant (quercetin) scavenged almost completely the NO radical.

Table 2: DPPH and NO scavenging activity of ripe and unripe plantain

Sample	% DPPH inhibition		Sample	% NO inhibition	
	150(µg/ml)	300(µg/ml)		150(µg/ml)	300(µg/ml)
Unripe	34.10±0.08	45.01±0.20	Unripe	61.88±0.20	68.45±0.11
Ripe	58.89±0.01	67.55±0.01	Ripe	63.90±0.15	79.91±0.20
Ascorbic acid	80.01±0.01	87.96±0.01	Quercetin	85.99±0.23	97.53±0.26

Table 3: Quantitative phytochemical composition of ripe and unripe plantain

Sample	Phytochemicals (%)		
	Tannins	Alkaloids	Flavonoids
Ripe	0.48±0.07	0.01±0.06	10.16±0.12
Unripe	0.44±0.08	0.01±0.06	6.20±0.12

Furthermore, the quantitative assessment of the phytochemical composition revealed the tannin, alkaloid and flavonoids content (Table 3). Ripe plantain contain higher tannin and flavonoids than the unripe, hence, may contribute significantly to the higher antioxidant activity observed for the ripped plantain in this study.

4. CONCLUSION

The outstanding quality of coconut oil as observed in this study reflects it edibility and might be a good alternative for consumption in households. Consequently may reduce the prevalence of oil induced disorders. Also, the antioxidant activity of the ripped plantain can be associated with its flavonoids and tannin content.

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