

Prevention of Oxidation in Palm Oil Using Plants Extract

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Abstract

Palm oil is one of the major edible oils widely consumed in Nigeria. Palm oil sold in the markets has been known to be high in free fatty acid which indicates oxidation and many synthetic chemical used to combat oxidation; though effective have been considered not safe. The effect of extracts from ginger rhizome and basil leaves on the lipid oxidation was studied. Ethanolic extract (70%) of ginger and aqueous extract of basil leave were added to fresh palm oil at concentration 100ppm, 200ppm and 300ppm and were stored under room temperature for 60 days. The antioxidant activities of the extract, the effect of the extracts on the peroxide value, free fatty acid value and the antioxidant effectiveness of the extracts were evaluated at interval 14 days using standard methods. The DPPH radical scavenging activity, ferric ion reducing power, and total phenolic content of the ginger and basil extracts were 76% and 58.4%; 13.3 and 86 in 100mg/g; 133 and 48 in 100mg/g respectively. The sample treated with 300ppm of ginger extract was the lowest in peroxide values of 14.5meq/kg compared with the untreated sample with 25meq/kg. It also showed the highest antioxidant effectiveness at the 60th day of storage. Sample treated with 200ppm of basil extract shows the least of free fatty acid value of 8.58% at the end of 60th day as compared to the untreated sample with 11.47%. There are significant differences ($p < 0.05$) in the peroxide value, free fatty values, antioxidant effectiveness of the untreated and treated palm oil. It was concluded that ginger and basil extract reduced lipid oxidation in palm oil.

Keywords: Palm oil, Storage duration, Natural additives, Antioxidant

1. INTRODUCTION

Oil is an important food component of food plant. They are lipophilic products obtained by processing natural materials of most plants (Hui and Sherkat, 2005). Oil crop and their products are the second most valuable commodities in the world trade (Derek *et al.*, 2006). Nutritionally, oil obtained from plant sources provides the calories, vitamins, and essential fatty acid in the human diet. They are in easily digested form and at a relative low cost. Palm oil is derived from one of the tropical trees and this oil has been to be very important in the world's diet. Palm oil is edible plant oil, derived from the mesocarp of the fruit of the oil palm *Elaeis guineensis*. It is one of the most widely consumed cooking oils in Africa and sub-saharan Africa. As the one of the most widely consumed cooking oils in the tropics, it has been known to have problem with oxidation which may be due to heat, moisture, oxygen, sunlight and temperature during processing and storage (FAO, 2015). Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades the quality of oil. Oxidative deterioration of lipids results in rancidity in food stuffs, accompanying off flavours and smells. Such reactions are more pronounced in food product like mayonnaise, margarine and cooking oils. Palm oil is susceptible to quality deterioration, especially under oxidative stress. For this reason, efforts to reduce oxidation have increased. Most often, the best strategy is the addition of antioxidants.

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical (Brewer, 2011). They can be produced from natural sources, for example plants or synthetic chemicals. Many synthetic chemicals such as Butylated Hydroxyl Anisole (BHA) and Butylated Hydroxyl Toluene (BHT), though very effective as antioxidants, have been known to have toxic and carcinogenic effects on humans (Fayisoro, 2006). Numerous studies have shown the antioxidant potentials of aromatic spices and medicinal plants. The use of these plant materials as natural antioxidants for food, cosmetics and other applications become necessary because of food safety issues (Fayisoro, 2006). Natural antioxidants are being used as food additives for inactivation of free radicals because of their scavenging properties and are readily acceptable by consumers (Miliauskas, 2004). Different degrees of antioxidant activities have been reported from extracts of spices and herbs, among them are ginger rhizomes and basil leaves.

Ginger (*Zingiber officinale*) is a flowering plant in the family Zingiberaceae while basil *Ocimum basilicum* is a leafy, fragrant annual with of the family Lamiaceae (USDA, 2008). These plants are widely used as spice, flavourings and have been reported to exhibit antioxidant activities (Brewer, 2011). Ginger is the rhizome of the plant *Zingiber officinale*, consumed as a delicacy, medicine, or spice. It lends its name to its genus and family (*Zingiberaceae*). Basil (*Ocimum basilicum* Lamiaceae), due to its versatile composition of nutrients and bioactive compounds, has been used extensively for many years in the flavouring of confectionary and baked goods, condiments (ketchups, tomato pastes, chilli sauces, pickles, and vinegars), non- alcoholic beverages, sausages and meats and in salad dressings (Zahid, 2011). Scientific studies have established that compounds in basil oil have potent antioxidant activity, it works as a good anti-aging, it is proved that it also has as anti-cancer, anti-viral, and antimicrobial properties (Bozin, *et al.*, 2006). This study was aimed at reducing the

susceptibility of palm oil using basil and ginger extract.

2. MATERIALS AND METHODS

2.1 Materials

Freshly prepared palm oil was procured from a traditional palm oil mill in Ilorra, Oyo state. Ginger rhizome and basil leaves were purchased from Bodija market, Ibadan, Oyo state.

2.2 Extraction of plant extracts

2.2.1 Extraction of Ginger rhizomes

Fresh ginger rhizome was thoroughly washed with distilled water and drained. It was crushed using high power blender and weighed. The ginger pulp was soaked in solution containing 70% ethanol with 30% distilled water and was constantly shaken every three hour for 48hours. The mass was filter using muslin cloth and Whatzman filter paper size 1. The filtrate was concentrated using rotatry evaporator at 45-50°C (Abdelmegid, 2014).

2.2.2 Extraction of Basil leaves

The basil leaves were extracted according to the method described by Zahid *et al.*, (2011). Fresh basil leaves were thoroughly washed with distilled water and drained. After that, it was blended using high power blender and was later soaked in distilled water for 24hours. It was filter using a sterile muslin cloth and Whatzman paper size 1. The extract was concentrated using rotatry evaporator at 45-50°C

2.3 Sample treatment

The oil samples were treated with the concentrated extract of ginger and basil at various concentration, 100ppm, 200ppm and 300ppm. Treated palm oil together with the control (untreated) was stored in a transparent container under room temperature ($29 \pm 2^\circ\text{C}$) for 60 days. Chemical analysis, such as DPPH test, total phenolic test and FRAP assay were conducted on the plant extracts while peroxide value, free fatty acid, oxidative stability and antioxidant effectiveness of stored palm oil were determined at interval of 14 days.

2.4 Determination of Free Radical Scavenging Ability

The free radical scavenging ability of the ginger rhizome and basil leaves extracts against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated using the method described by Ursini *et al.*, (1994). An aliquot (0.2ml) of 0.5g of the samples was homogenized in 20 ml methanol. The mixture was mixed with 7.6ml of 0.4mM methanolic solution containing 1,1-DPPH radical, the mixture was left in the dark for 30min before measuring the absorbance at 516nm. The percentage of scavenging effect was determined by comparing the absorbance solution containing the test sample to that of blank sample as follows equation.

$$\% \text{DPPH Scavenging activity} = \frac{(A_0 - A_1)}{A_0} \times 100\% \dots \dots \text{Equation 3.1 Type equation here.}$$

Where, A_0 measurement of the blank, A_1 measurement of the sample.

2.5 Total Phenolic Content

The concentration of phenolic in the sample powder was determined using spectrophotometric method (Singleton *et al.*, 1999). The reaction of the mixture was prepared by mixing 1ml of ethanolic solution of extract, 2.5ml of 10% folin ciocalteu's reagent dissolved in water and 2ml of 7.5% NaHCO_3 was added, blank. The samples were thereafter left for 30min. The blank solution was prepared. The absorbance was determined using spectrophotometer at 610nm, the sample were prepared in triplicate for each analysis. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed, based on the measured absorbance, the concentration of phenolic was read (mg/g) from the calibration line; then the content of phenolic in extract was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

2.6 Determination of Ferric Reducing Antioxidant Potential (FRAP)

The reducing property of the extracts were determined by assessing the ability of the extracts to reduce FeCl_3 solution as described by Palido *et al.*, (2000). One millilitre of the extracts solution (0.5g of the sample homogenized in 20ml ethanol) were mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5ml of 1% (w/v) potassium ferrocyanide. The mixture was incubated at 50°C for 20min, thereafter 2.5ml (10% w/v) trichloroacetic acid was added and subsequently centrifuged at 650rpm for 10 minutes. Two and half millilitres of the supernatant was mixed with equal volume of water and 0.5ml of ferric Chloride. The absorbance were measured at 700nm using UV-Visible spectrophotometer. Higher absorbance indicates a higher reducing power.

2.7 Determination of peroxide value

The method reported by AOAC (2010) was used. 2g each of the oil samples were respectively weighed into

different conical flasks and 15ml of the mixture of $\text{CH}_3\text{COOH} - \text{CHCl}_3$ in the ratio of 3:2 respectively was added to the oil sample. 0.5ml of saturated potassium iodide was added to each conical flask and allowed to stand for 5minutes, thereafter, 0.5ml of starch was added and 15ml of distilled water was added and filtrated with 0.1N $\text{Na}_2\text{S}_2\text{O}_3$ to a colourless end-point. The peroxide value was calculated from the equation
$$\text{Pv} = \frac{1000 (V_2 - V_1) T}{M}$$

Where, M = mass of oil taken (2g), V_2 = volume of 0.1N $\text{Na}_2\text{S}_2\text{O}_3$, V_1 = volume of 0.1N blank and T = normality of $\text{Na}_2\text{S}_2\text{O}_3$ (0.1N).

2.8 Determination of free fatty acid value

25ml of ethanol was added to 1.5g of each oil sample contained in the different conical flasks. The mixture was brought to boil in a water bath then cooled. Two drops of phenolphthalein indicator was added to the solution. 0.1M NaOH was used to titrate the mixture with constant shaking for proper mixing and the FFA was calculated from the expression, $\% \text{ FFA} = v \times 0.0256 \times \frac{100}{W}$

(W is the weight of the sample), where V = sample titre, w = weight of the sample, 1cm³ of 0.1 NaoH contains 0.0256 of palmitic acid. This method was reported by Ekop *et al.*, 2007.

2.9 Determination of Antioxidant effectiveness

The percentage antioxidant effectiveness during storage test period was monitored using the method described by Adegoke and Gopalakrishna, (1998) in "Extraction and identification of antioxidants from the spice *Aframomum danielli*.

$$\text{Antioxidant effectiveness} = \frac{\text{Pv of control} - \text{PV of test sample}}{\text{PV of control}} \times 100\%$$

2.10 Statistical Analysis

The data collected from all the analysis conducted was tested for significant difference using Analysis of Variance (ANOVA) and the significant difference between the means was separated by Duncan Multiple range test using SPSS statistical package at 5% significant level.

3.0 RESULTS AND DISCUSSION

3.1 DPPH Scavenging Activity

The DPPH scavenging activity of the 70% ethanolic extract of ginger rhizomes and the aqueous extract of basil were 75% and 58% respectively as represented (Table 1). Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts was due to these compounds (Abdelmegid, 2014). Also, based on the results obtained, it is possible that several phenolic compounds of different polarities may contribute to the antioxidative properties of ginger rhizome and basil leaves extracts. Part of the antioxidative activity may be due to these components or flavonoids. In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may be present in the plants. This antioxidant activity may be due to the phenolic compounds redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides that might be present in the palm oil.

3.2 Total Phenolic Compound

The level of phenolic compounds in extracts of ginger *Zingiber officinale* and basil *Occisimu gratissimum* were 133 and 48 in 100mg/g are presented (Table 1). Comparing these extracts, it was found that ginger had higher contents of total phenols than basil extract. This high level of total phenolic compounds was reported in ginger rihzomes by different studies (Ghasemzadeh *et al.*, 2010; Maizura *et al.*, 2011). Harish and Shivanandappa (2006) reported opines that non phenolic antioxidants might also contribute to the antioxidant activity of plant extract.

Table 1. DPPH Scavenging Activity, Ferric Reducing Antioxidant Power and Total Phenolic Content of the Ginger and Basil Extract

| Antioxidant activities | Extracts | |
|---|----------|-------|
| | Ginger | Basil |
| DPPH Scavenging Activity (%) | 75 | 58 |
| Ferric Reducing Antioxidant Power (100mg/g) | 13 | 86 |
| Total phenolic content(100mg/g) | 133 | 48 |

3.3 Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing power of extract from ginger rhizome and basil leaves were 13 and 86 in 100mg/g (Table 1).

The reducing power was higher in aqueous extract of basil leaves than 70% ethanolic extract of ginger rhizome. The reducing power of bioactive compounds is associated with antioxidant activity (Siddhuraju et al., 2002). Hence, the determination of the reducing power of phenolic constituents to explain the relationship between their antioxidant effect and their reducing power. As reported by Chen et al., (2008), the reducing power of solvent extract of 18 different species of ginger ranged from 0.34 to 1.6 nm in 100 mg of sample. Antioxidant components and activity are highly dependent on extracting solvent and concentration of solvent (Turkmen et al., 2006), but they also vary within the samples. Many researchers have reported the relationship between phenolic content and antioxidant activity. In some studies, they found a correlation between the phenolic content and antioxidant activity (Velioglu et al., 1998), whereas others found no relationship (Hodzic et al. (2009).

3.4 Effect of treatment on Peroxide Value of Palm Oil

The peroxide values of the samples over the period of 60days are shown in Tables 2 and 3. Initial peroxide value of the oil was 7.5Meq/kg, this is lower than the quality standard recommended by SON/NIS standard for peroxide value of edible vegetable oils (10Meq/kg). Reduction in peroxide value was noticed within the first 30 days in samples treated with 200ppm and 300ppm of ginger extract and in all samples treated with basil extract up to the 15th day and after this increase in their peroxide value was noticed during the storage period. A steady increase was noticed in the untreated sample throughout the storage period, it gradually increases from 7.5Meq/kg to 25Meq/kg at the 60th day of storage. The reduction in peroxide value indicates lag in the rate of formation of peroxides at first during an induction period that varies with time. This indicates that the extracts started to prevent the oil samples from the formation of hydro peroxides in the early stage

Table 2. Effect of Ginger Extract Treatment on the Peroxide Value of palm oil

| Storage Duration | Peroxide value (Meq/kg) | | | |
|------------------|--------------------------|-------------------------|-------------------------|--------------------------|
| Days | Control | 100ppm | 200ppm | 300ppm |
| 1 st | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} |
| 15 th | 10.50±0.00 ^{fg} | 8.00±0.00 ^{de} | 5.00±0.00 ^{ab} | 5.08±1.52 ^{ab} |
| 30 th | 17.50±0.00 ^k | 6.75±1.06 ^c | 5.75±1.06 ^{bc} | 7.50±0.71 ^{de} |
| 45 th | 20.00±0.0 ^l | 7.50±0.71 ^{de} | 13.75±1.06 ⁱ | 10.50±0.71 ^{fg} |
| 60 th | 25.00±0.00 ^m | 16.75±0.61 ^k | 15.50±0.71 ^j | 14.50±0.71 ^{ij} |

Means with different superscripts are significantly different at P < 0.05

Table 3. Effect of Basil Extract Treatment Peroxide Value of Palm Oil

| Storage Duration (Days) | Peroxide value (Meq/kg) | | | |
|-------------------------|-------------------------|-------------------------|---------------------------|-------------------------|
| | Control | 100ppm | 200ppm | 300ppm |
| 1 st | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} | 7.50±0.00 ^{de} |
| 15 th | 10.5±0.00 ^{fg} | 3.95±0.07 ^a | 4.00±0.70 ^a | 5.00±0.00 ^{ab} |
| 30 th | 17.50±0.00 ^k | 8.25±0.75 ^e | 11.75±1.06 ^{gh} | 10.25±0.35 ^f |
| 45 th | 25.00±0.0 ^m | 13.75±1.02 ⁱ | 11.00±0.00 ^{fgh} | 12.00±0.00 ^j |
| 60 th | 20.00±0.00 ^l | 15.15±0.21 ^j | 17.25±0.35 ^k | 20.00±0.00 ^l |

Means with different superscripts are significantly different at P < 0.05

3.5 Effect of Treatment on Free Fatty Acid Value of Palm Oil

The free fatty acid values of the untreated and the treated palm oil samples during sixty (60) days of storage ranges between 8 and 11% are shown in Table 4 and 5. This value is higher than quality characteristics of vegetable oil as recommended by SON/NIS which is 2% for unrefined oil. The high initial free fatty acid value of the oil value is an indication of onset of primary oxidation in oil; this may be due to exposure to moisture, light and heat during the processing. Slight reduction was noticed in the free fatty acid value of samples treated with 100ppm and 200ppm ginger extract and in 200ppm basil extracts at the 15th day of the study, while other samples gradually increased. Increase in free fatty acid was highest in control sample and least in 200ppm and 300ppm of the samples treated with ginger extract, also in samples treated with 200ppm of basil extract. The order of increase was as follows: control > 100ppm of ginger > 200ppm of ginger > 100ppm of basil > 300ppm of ginger > 300ppm of basil > 200ppm of basil.

Table 4. Effect of Ginger Extract Treatment on Free Fatty Acid Value of Palm Oil

| Storage Duration (Days) | Free fatty acid value (%) | | | |
|-------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | Control | 100ppm | 200ppm | 300ppm |
| 1 st | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} |
| 15 th | 8.58±0.00 ^{cde} | 7.94±0.35 ^{ab} | 7.86±0.87 ^a | 8.17±0.28 ^{abc} |
| 30 th | 9.78±0.78 ^{jk} | 8.77±0.77 ^{def} | 7.19±0.41 ^a | 9.77±0.61 ^{jk} |
| 45 th | 10.69±0.41 ^k | 8.99±0.11 ^{efgh} | 9.30±0.71 ^{hij} | 8.86±0.31 ^{defg} |
| 60 th | 11.47±0.42 ^l | 10.15±0.71 ^{jk} | 9.34±0.00 ^{ghij} | 8.97±0.25 ^{efgh} |

Means with different superscripts are significantly different at P < 0.05

Table 5. Effect of Basil Extracts Treatment on Free Fatty Acid Value of Palm Oil.

| Storage Duration (Days) | Free fatty acid value (%) | | | |
|-------------------------|----------------------------|---------------------------|--------------------------|---------------------------|
| | Control | 100ppm | 200ppm | 300ppm |
| 1 st | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} | 8.00±0.00 ^{ab} |
| 15 th | 8.58±0.00 ^{ab} | 8.00±0.01 ^{ab} | 7.74±0.18 ^a | 8.02±0.04 ^{ab} |
| 30 th | 9.48±0.78 ^{ij} | 8.43±0.75 ^{bcd} | 8.84±0.67 ^{def} | 8.05±0.21 ^{ab} |
| 45 th | 10.69±0.00 ^{fghi} | 9.15±0.00 ^{fghi} | 8.77±0.00 ^{def} | 9.15±0.00 ^{ghij} |
| 60 th | 11.47±0.42 ^l | 9.12±0.31 ^k | 8.58±0.00 ^{cde} | 8.93±0.03 ^{efgh} |

Means not followed by the same superscripts are different at P < 0.05

3.6 Antioxidant Effectiveness of Extracts on Palm Oil

Tables 6 and 7 show the results of antioxidant effectiveness of extracts on palm oil samples during the 60 days of storage. The antioxidant effectiveness of ginger extract increased averagely from 23% to 60% up to the 30th day and decreases to 40% at the 60th day of the storage. The antioxidant effectiveness of basil extract was evaluated to be 61.9% on the 15th day, declines to 36% was noticed at the 60th day of the storage. The fluctuating antioxidant effectiveness can be as a result of fluctuating peroxide value of the treated palm oil samples. Fluctuation in peroxide value of palm oil was also noticed by Ghazalia *et al.*, (2006). The 200ppm of ginger extract and 200ppm of basil extract have the highest peak antioxidant efficiency (62.85%) and (61.9%) respectively as shown in Tables 6 and 7.

Table 6. Antioxidant Effectiveness of Ginger Extracts on Palm Oil

| Storage Duration (Days) | Antioxidant Effectiveness (%) | | | |
|-------------------------|-------------------------------|--------|--------|--------|
| | control | 100ppm | 200ppm | 300ppm |
| 1 st | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 th | 0.00 | 23.8 | 52.38 | 50.00 |
| 30 th | 0.00 | 57.14 | 62.85 | 60.00 |
| 45 th | 0.00 | 55.50 | 19.40 | 44.40 |
| 60 th | 0.00 | 30.00 | 36.00 | 40.00 |

Table 7. Antioxidant Effectiveness of Basil Extracts on Palm Oil

| Storage duration (Days) | Antioxidant Effectiveness (%) | | | |
|-------------------------|-------------------------------|--------|--------|--------|
| | control | 100ppm | 200ppm | 300ppm |
| 1 st | 0.00 | 0.00 | 0.00 | 0.00 |
| 15 th | 0.00 | 61.90 | 61.90 | 52.38 |
| 30 th | 0.00 | 51.40 | 28.57 | 40.00 |
| 45 th | 0.00 | 19.40 | 38.80 | 33.30 |
| 60 th | 0.00 | 25.00 | 36.00 | 20.00 |

4. CONCLUSIONS

Extract from ginger rhizome and basil leave exhibited good radical scavenging ability and ferric ion reducing potential. The ginger extract and basil extract exhibited an antioxidant effectiveness of 62.85% and 61.9% respectively. The extract from basil leaves and ginger rhizome delayed the onset of primary oxidation in palm oil

by reducing the peroxide value and the free fatty acid value of the treated palm oil.

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