# Comparative study of the effect of *Piper nigrum* (white and black) and *Piper guineense* on lipids quality of groundnuts pudding


## ABSTRACT

This study investigates the effect of white and black *Piper nigrum* and *Piper guineense* on lipids quality of oil extracted from groundnuts pudding. This work was carried in the Research Unit of Biochemistry, Medicinal plants, Food Sciences, and Nutrition, Department of Biochemistry, Faculty of Science, University of Dschang, Cameroon, between January 2018 and December 2019. The antioxidant activity of these spices was determined. Cooking by steaming of groundnuts (*Arachis hypogaea*) pudding was carried out using groundnuts paste with 0 g, 0.5 g, 1 g, 2 g, and 4 g of spices and 30 ml of warm water respectively. Oils were extracted from the prepared groundnuts pudding using a mixture of chloroform and methanol. The lipid quality of oil samples was studied by the determination of the peroxide, P-anisidine, total oxidation, thiobarbituric and iodine values. Results revealed that these spices possess non negligible antioxidant properties. Black *Piper nigrum* (BPN) presented the highest total phenolic (TPC: 85.00 mg GAE/g) and flavonoids (FC: 271.94 mg CE/g) contents. The lowest TPC and FC was observed with the aqueous extract white *Piper nigrum* (WPN: 52.38 mg GAE/g and 113.32 mg CE/g respectively). The use of these spices in groundnuts pudding preparation contributed to limit the formation of primary and secondary oxidation products of groundnuts pudding oil. It was also observed that white *Piper nigrum* (WPN) better preserve lipids quality of oils at all concentrations because oil extracted from pudding cooked with 0.5 g, 1 g, 2 g, and 4 g presented peroxide values lower than 10 meq$O_2$/kg (2.81 meq$O_2$/kg, 2.99 meq$O_2$/kg, 3.28 meq$O_2$/kg and 5.46 meq$O_2$/kg respectively). In summary these spices especially white *Piper nigrum* can be used to preserve lipids oxidation during cooking by steaming of groundnuts pudding.



*Keywords: black Piper nigrum, cooking by steaming, groundnuts pudding, Piper guineense white Piper nigrum.*


## INTRODUCTION

Lipids are one of the essential macronutrients of our diet. Whether been called oils or fats, they are consumed in their natural form or are present in animal or vegetable food sources [1]. The main oilseeds include peanut (groundnuts) sunflower, soybeans, pumpkin, and palm nuts. Among all these, groundnuts are the main oil seed been used by Cameroonian households [2]. It can be used to produce snack products, to prepare soup, to make groundnuts pudding or cake. In meals, lipids help to increase the flavour, the taste and also contribute to the proper functioning of the body and its development [3].

Studies have shown that groundnuts are complete source of nutrient for some individuals [4]. It contains 48.2% of lipids, 36% proteins [5] and significant amounts of vitamin E, folic acid and minerals such as magnesium, copper, phosphorus, potassium and zinc [6]. Its lipids are rich in linoleic and α-linolenic acids which are known to be essential fatty acids [7]. Groundnuts seeds contain approximately 63.17% of unsaturated fatty acids [7]. Groundnut proteins content is 36% and is composed of essential amino acids, such as

lysine, threonine, valine, isoleucine, leucine and tyrosine, which play an important role in the metabolism [5].

Most of the time, in households before being consumed, groundnut seeds undergo several culinary methods such as boiling, grilling and steaming. However, the colour, flavour, and texture of the seeds are affected by temperature and cooking time during the various culinary processes [8]. During processing, fatty acids can undergo degradation reactions, the main degradation reaction being the oxidation reactions with decomposition of oxidation products. These reactions lead to the reduction of nutritional value and organoleptic properties of end products that have mutagenic, carcinogenic and cytotoxic properties and considered to be risky for human health [9].

In addition, it should be noted that the co-oxidation reactions initiated by the oxidation products of fatty acids can lead to the loss of essential amino acids and a decrease in protein digestibility [10].

During culinary processing, certain spices are added in varyied quantities to improve the taste or flavour of the food [11]. These spices in addition to their organoleptic properties can provide functional properties and pharmacological virtues to the food [12, 13, 14]. It was outlined that *Piper nigrum* (white and black) and *Piper guineense* possess phytochemical and antioxidant properties [15, 16, 17, 18]. Indeed, these *Piper* varieties phytochemical and antioxidant properties have not been outlined during groundnut pudding culinary processing. Thus our study aimed to evaluate the phytochemical and antioxidant activities of white and back *Piper (Piper nigrum*) and *Piper guineense* on the lipids quality of groundnut pudding during cooking by steaming.

## MATERIAL AND METHODS

### Collection of groundnuts and spices

Dry groundnut seeds (*Arachis hypogaea*), and three spices namely white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* seeds were bought in Dschang market, West Region of Cameroon. These spices were later ground and sieved with a 10 mm mail in other to obtain powders.

#### Aqueous extracts preparation for Phytochemical analysis

25 g of powder of each of these three spices was soaked in 500 ml of water for 48 h in other to facilitate the extraction of bioactive compounds. These extracts were regularly submitted to shaking at room temperature. The solutions were then filtered using Wattman N$^{o}$1 filter paper. The filtrate was placed in an oven at 45°C in order to remove water. The extracts obtained were stored at 4°C for further analysis.

### Total Phenolics (TPC) and Flavonoids (FC) Content of spices extract

The Total phenolics content (TPC) was determined using the Folin Ciocalteu colorimetric method as described by Dohou [19]; while the total flavonoids (TFC) content was determined using the methods described by Bahorun [20].

### Evaluation of the Antioxidant Activity by the DPPH Free Radical Scavenging Assay

The radical scavenging ability of the spices extracts was determined according to the method described by Mensor [21].

## Evaluation of Ferric Reducing Antioxidant Power and hydroxyl radical inhibition power

The antioxidant power of the extracts was also determined by their ability to reduce Iron (III) to Iron (II) as described by Oyaizu [22]. The capacity of the extract to inhibit the hydroxyl radical production was done by the methods described by Nagulendran [23].

## Evaluation of the Oxygen radical absorbance capacity (ORAC)

The peroxyl radical ($ROO^{\bullet}$) scavenging activity of extracts was determined according to the methods described by Ratnasari [24].

## Preparation of groundnuts pudding and oil extraction

Dried groundnuts (*Arachis hypogaea*) were roasted for about 10 to 15 minutes and after air-cooling, they were ground in other to obtain a paste. 100 g of paste was mixed with 0.5 g; 1 g; 2 g; and 4 g of various powders of white *Piper nigrum*, black *Piper nigrum*, *Piper guineense* and 30 ml of warm water respectively. These quantities were selected due to a survey carried out in some cities of Cameroon on groundnut pudding sellers. The paste obtained were tied in banana leaves and boiled separately for about 90 minutes in a pot placed on an electric heater. The bottom of the pot was covered with plantain leaves in other to prevent water from entering the pudding and burning of pudding during cooking. After cooking the groundnut pudding was removed and allowed to cool at room temperature for 30 minutes. Lipids were then extracted using Bligh and Dyer [25] method. The obtained oil samples were put in dark bottles and stored at -20°C for lipids quality assessment.

## Lipids quality assessment of oils extracted

The lipids quality of oils extracted from groundnuts pudding was done by the determination of the peroxide value (PV) using the IDF 74A:1991 standard spectrophotometer method [26], the p-anisidine value (p-AV) using the official AOCS Cd 18-90 « *p*-anisidine value » [27]; the Total oxidation value (TOTOX) as described by Shahidi and Wanasundara [28]; the TOTOX (calculated using the equation TOTOX = 2PV + p-AV), the thiobarbituric acid (TBARS) using the method described by Draper and Hadley [29] and the iodine value (IV) using the AOCS Cd 1-25 official method [27].

## Statistical analysis

Results expressed as mean ± standard deviations were subjected to analysis of variance (ANOVA) using the Student Newman-Keuls Multiple Comparison Test, to determine the statistical significance at 0.05% probability level. In addition, Pearson's correlation between the total phenolic and flavonoid contents and antioxidant properties was calculated using SPSS version 20.

## RESULTS AND DISCUSSION

### Total phenolic (TPC) and flavonoid (FC) contents of spices

Table 1 presents the TPC and FC of white *Piper nigrum* (WPN*),* black *Piper nigrum* (BPN) and *Piper guineense* (PG) aqueous extracts. It appears that, the TPC and FC differ significantly ($p<0.05$) in all extracts with values ranging from 52.38 to 85.00 mg GAE/g and from 113.32 to 271.94 mg CE/g for white *Piper nigrum* and black *Piper nigrum* respectively. Fajobi [18] with methanolic extract of *Piper guineense* obtained a TPC of 141.50 mg GAE/g. This result is significantly higher ($p<0.05$) than that obtained in this study. However, the TPC of white *Piper nigrum* was significantly higher ($p<0.05$) than that obtained by Asha [15] (5.04 mg GAE/g) with methanolic extract. Goswami [17] obtained a TPC of black *Piper nigrum* aqueous extract of 598.00 mg GAE/g. This TPC is significantly higher ($p<0.05$) than that obtained in this study. The FC obtained in this study with the aqueous extract of *Piper guineense,* black *Piper nigrum* and white *Piper nigrum* are significantly higher compared to that of Fajobi [18] (86.09 mg CE/g), Asha [15] (9.16 mg CE/g) and Farzana [30], (13.64 mg CE/g). The variation in TPC and FC registered in this work might be due to genotypic and environmental differences such as climate, location, temperature, fertility, diseases, pest exposure within species, parts of plant tested, time of taking samples, determination methods and the type of solvent used for extractions [31, 32].

**Table 1:** Total phenolic (TPC) and flavonoids (FC) content of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

*Values with different superscripts in the same column differ significantly at $p<0.05$*

### Antioxidant activity of extracts

The free radical scavenging activity of extract is showed in figure 1. It appears that, all extracts presented at all concentrations low antioxidant activity against the DPPH free radical. The highest activity was recorded with black *Piper nigrum* with values ranging from 32.63% to 86.02% at 25 µg/ml and 200µg/ml respectively compared to that of white *Piper nigrum* and *Piper guineense*. However, no significant different was noted at 200 µg/ml with the activity of black *Piper nigrum* and *Piper guineense* compared to that of Butylhydroxytoluene (BHT). The lowest activity was observed with the aqueous extract of white *Piper nigrum* at 25 µg/ml (20.80%) and at 200 µg/ml (34.27%). The highest free radical scavenging activity obtained with black *Piper nigrum* and *Piper guineense* extract might be attributed to their high TPC (85.00 mg GAE/g and 65.71 mg GAE/g) [33]. This result is in line with that of other authors [34, 35, 36] who showed that there exist significance correlation between the total phenolic content and the antioxidant activity of extracts. Results obtained with white *Piper nigrum* extract at all concentrations are lower than those of Gayatri and Sahu [37], which was 28% ; 31.00% ; 34.30% and 36.61% at 25 ; 50 ; 100 and 200 µg/ml respectively.

**Figure 1**: DPPH Free Radical Scavenging Assay of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

### Ferric Reducing Antioxidant Power (FRAP) and inhibition of hydroxyl radical (ROH) production

The FRAP and hydroxyl radical activities of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense* extracts at different concentrations compared to that of BHT is presented in figure

2 and 3 respectively. It appears that white *Piper nigrum,* black *Piper nigrum* and *Piper guineense* extracts have presented FRAP and inhibition of hydroxyl radical activities significantly lower ($p<0.05$) compared to that of BHT at all concentrations. However, black *Piper nigrum* at 100 µg/ml showed a FRAP activity significantly higher than that of white *Piper nigrum,* and *Piper guineense* while at 200 µg/ml *Piper guineense* presented a FRAP activity higher than that of white *Piper nigrum* and black *Piper nigrum*. The aqueous extract of white *Piper nigrum* showed the lowest FRAP activity and inhibition of hydroxyl radical at all concentrations. This result is in line with that of Asha [15] who showed that white *Piper nigrum* has a poor FRAP.

**Figure 2:** Ferric Reducing Antioxidant Power (FRAP) of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

**Figure 3:** Inhibition of hydroxyl radical of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

## Oxygen radical absorbance capacity (ORAC)

The Oxygen radical absorbance capacity (ORAC) of the aqueous extract of white *Piper nigrum* (WPN)*,* black *Piper nigrum* (BPN) *and Piper guineense* (PG) is illustrated on figure 4. It appears that the extract of black *Piper nigrum* presented an ORAC value (410.72 µMTE/g) significantly higher ($p<0.05$) than that of *Piper guineense* and white *Piper nigrum* (340.33µMTE/g and 193.32 µMTE/g respectively). The ORAC value obtained with PG in this study is higher than that of Ojimelukwe and Ukom [38] (316.74 µMTE/g). Masuda [39] obtained an ORAC value of white *Piper nigrum* of 170316.74 µMTE/g. This value is lower than that recorded in this study.

**Figure 4:** Oxygen radical absorbance capacity of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

*Values with different superscripts differ significantly at $p<0.05$*;
PG = *Piper guineense*; WPN = white *Piper nigrum*; BPN = black *Piper nigrum*

## Pearson's correlation between the total phenolic (TPC), flavonoids contents (FC), Antioxidant activity (DPPH), Ferric Reducing Antioxidant Power (FRAP), inhibition of hydroxyl radical (ROH) and Oxygen radical absorbance capacity ($ROO^o$) of extracts

Table 2 present the Pearson's correlation between the TPC, FC, DPPH, FRAP, ROH and $ROO^o$ of the aqueous extracts of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*. It appears that there is a positive correlation between the TPC and FC ($p<0.01$; $r = 1.000$). FRAP presented a positive and significant correlation with DPPH ($p<0.01$: $r = 0.951$). Also, ROH presented a positive and significance correlation with DPPH and FRAP ($p<0.01$: $r = 0.915$ and $r = 0.995$ respectively). A significant and positive correlation at $p<0.01$ was also observed between $ROO^o$, TPC, FC and DPPH. However, no significant correlation was observed between ROH, TPC and FC. Looking at these observations it is clear that there is a relation between the antioxidant activities and the total phenolic contents of the extracts since extracts that presented a high antiradical activity with regards to DPPH were those that had high phenolic and flavonoid contents. Because of their antiradical activity [40], phenolic compounds might be responsible of the antioxidant activity of the extracts. These results confirm those of many authors [34, 35, 36] who showed that there is a significant correlation between total phenolic content and antioxidant activity.

**Table 2:** Pearson's correlation between the TPC, FC, DPPH, FRAP, ROH and $ROO^{o}$ of extracts

***. Correlation is significant at 0.01 (bilateral)*
***TPC** = Total phenolic contents; **FC** = flavonoids contents; **DPPH** =DPPH free radical scavenging assay; **FRAP** = Ferric Reducing Antioxidant Power; **ROH** = inhibition of hydroxyl radical; **$ROO^{o}$** = Oxygen radical absorbance capacity*

### Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the lipids quality of groundnuts pudding

#### Effect on the peroxide value (PV)

The determination of the peroxide value (ppm) is the appropriate method used to measure primary oxidation compounds such as peroxide. The effect of spices on the peroxide values of oil extracted from groundnut pudding cooked by steaming respectively with 0 g, 0.5 g, 1 g, 2 g and 4 g of white *Piper nigrum* (OGCWPN), black *Piper nigrum* (OGCBPN) and *Piper guineense* (OGCPG) is presented on table 3. It appears that oil extracted from samples enriched with spices powders presented significantly lower ($P < 0.05$) PV compare to that of oil extracted from groundnuts pudding cooked without spices (OGC: 18.39 $meqO_2/kg$). Also, these samples exhibited PV < 10 $meqO_2/kg$ which is the recommended range [41]. The lowest PV was recorded with oil extracted from pudding cooked with 0.5 g of *Piper guineense* (OGCPG: 2.40 $meqO_2/kg$). Looking at the effect of concentration on the lipids quality, it appears clear that oils extracted from samples cooked with 0.5 g and 1 g white *Piper nigrum,* black *Piper nigrum* and *Piper guineense* presented peroxide values significantly lower than that of oils extracted from samples cooked with 2 g and 4 g of these spices. The low increase in the peroxide values observed might be due to the presence of natural antioxidants in spices that may have prevented or limited the formation of hydroperoxides in oils by scavenging free radicals formed in groundnut pudding oil during the preparation [42, 43, 44, 45]. These results are in line with those of Yanjun [46] who showed that turmeric and black piper decrease lipid peroxidation in meat patties during cooking. Ibrahium [47] also showed that *Syzigium aromaticum* essential oil can be used to reduce the peroxide value in cake.

**Table 3:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the peroxide value of lipids extracted from groundnuts pudding

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

#### Effect on the p-anisidine value (p-AV)

The anisidine value is a parameter that is used to measure secondary oxidation products when hydroperoxides are being transformed into aldehydes, ketones and other products at high temperature [48]. The effect of spices on the p-AV of lipids extracted from groundnut pudding cooked by steaming with 0 g, 0.5 g, 1 g, 2 g, and 4 g is shown in table 4. It appears that, oil extracted from groundnut pudding cooked with 0.5 g of *Piper guineense* (OGCPG: 94.45) presented a p-AV significantly higher ($p<0.05$) than that of oil extracted from pudding cooked without spices (OGC: 49.49) while those extracted from pudding cooked with 0.5 g of white *Piper nigrum* (OGCWPN: 12.75) and 0.5 g of black *Piper nigrum* (OGCBPN: 24.01) were significantly lower ($p<0.05$) than that of OGC. Only oils extracted respectively from pudding cooked with 1 g (OGCWPN: 16.71) and 2 g (OGCWPN: 15.82) of white *Piper nigrum* presented p-AV significantly lower ($p<0.05$) than that of OGC. The p-AV of samples

OGCBPN 1 g and 2 g respectively 70.06 and 73.37 and that of OGCPG 53.25 and 56.25 was significantly higher ($p>0.05$) than that of the control. No significant difference ($p>0.05$) was observed between the p-AV of samples OGCWPN, OGCBPN and that of the control. Sample OGCWPN cooked with 4 g of white *Piper nigrum* presented a p-AV significantly higher than that of oil extracted from groundnut pudding cooked with 0.5 g; 1 g and 2 g of white *Piper nigrum*. The high p-AV of oil extracted from OGC may be the consequence of rapid decomposition of hydroperoxides and alkoxyl radicals under the effect of temperature in favour of secondary oxidation products such as carbonyls, 2 alkenals and 2,4-decadienals; it may be also due to the absence of antioxidants in this sample [43, 49]. The low increase in p-AV observed with 0.5 g, 1 g, and 2 g of white *Piper nigrum* might be due to the variable mechanisms of action of phenolic compounds and the antioxidant properties of these spices in preventing lipid peroxidation.

**Table 4:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the anisidine values of lipids extracted from groundnuts pudding

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Effect on the Total oxidation value (TOTOX)**

The Totox value is used to measure both hydroperoxides and their breakdown products. It provides a better estimation on the evolution of the oxidative deterioration state of fats and oils. Table 5 shows the effect of spices on the TOTOX value of oils extracted from groundnut pudding. The highest TOTOX value was observed with sample OGCPG (99.25) cooked with 0.5 g compared to that of OGC. However, oils extracted from other samples except that of sample OGCBPN cooked with 2 g of black *Piper nigrum* presented TOTOX value that was significantly lower than that of sample OGC. It also appears that oils extracted from samples OGCWPN cooked with 4 g of white *Piper nigrum*, OGCBPN cooked with 2 g of black *Piper nigrum* and that of OGCPG cooked with 0.5 g of *Piper guineense* presented TOTOX values significantly higher than that of other samples cooked with these spices respectively. The low increase in TOTOX value observed with these samples compared to the control might be due to the antioxidant activity and the phytochemical properties of spices added during the preparation of groundnuts pudding. These spices are rich in phenolic compounds that have the ability to limit or prevent lipid oxidation during cooking [15, 16, 43].These results also confirm the good correlation that exists between the antioxidant activity and the antioxidant properties of the spices. These results are in line with those of Yanjun [46] who showed that turmeric and black pepper decrease lipid peroxidation in meat patties during cooking.

**Table 5:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the TOTOX values of lipids extracted from groundnuts pudding

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Effect on the thiobarbituric acid value (TBA value)**

The TBA value is an oxidative parameter that measures the secondary oxidation products, specifically malondiahydehyde [50]. This compound is produced during degradation of polyunsaturated fatty acids during lipid oxidation and leads to the formation of rancid odours [51, 52]. The effect of spices on the TBA values of oils extracted from groundnut pudding cooked by steaming is illustrated on table 6. It appears that the TBA values of oils extracted from groundnuts pudding cooked with 0.5 g, 1 g, 2 g and 4 g of *Piper guineense*; 1 g and 4 g of black *Piper nigrum* and 1 g and 2 g of white *Piper nigrum* was significantly lower ($p<0.05$) than that of oil extracted from pudding cooked without spices that serves as control (OGC: 8.56 meqMDA/kg) and that of other samples cooked with the same spices. The low increase observed may be due to the effect of antioxidant compounds present in the spices which would have limited the transformation of the hydroperoxides into secondary oxidation products such as malondialdehydes and preserve the quality of lipids [18, 53]. These results are in line with those of Goswami [17] who showed that spices can be used to reduce the formation of malondialdehyde during boiling of fish. They results are also in accordance with those of Ibrahium [47] who showed that *Syzigium aromaticum* essential oil can be used to reduce the thiobarbituric acid values in cake.

**Table 6:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the thiobarbituric acid values of lipids extracted from groundnuts pudding

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Effect on the Iodine value (IV)**

The iodine value (IV) is a parameter that gives the deterioration level of fatty acids double bonds of oils. A decrease in the IV value is generally correlated with the destruction of the double bonds of its fatty acids by oxidation, scission or polymerization [54].The effect of spices on the IV values of oils extracted from groundnut pudding cooked with 0 g, 0.5 g, 1 g, 2 g and 4 g of spices is shown on table 7. It appears that all oils extracted from groundnut pudding cooked with spices except that of samples OGBPN (48.53 $gI_2$/100g), OGCPG (49.37 $gI_2$/100g) cooked with 0.5 g and that of OGCBPN (53.06 gI2/100g) presented IV values significantly higher ($p<0.05$) than that of the control (OGC: 49.00 $gI_2$/100g). The low deterioration of the IV values observed in oils extracted from groundnut pudding cooked with spices compared to the control might be the consequence of the protective action of the antioxidants present in these spices on the unsaturated fatty acids of oils [15, 16, 18].The significant decrease observed with the IV of OGC might be due to the absence of antioxidant in this sample. Dhartiben [43] stated that spices and herbs are source of natural antioxidants for foods and this later can limit or prevent lipids oxidation.

**Table 7:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the Iodine values of lipids extracted from groundnuts pudding

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

## CONCLUSION

From this study it can be concluded that white *Piper nigrum,* black *Piper nigrum* and *Piper guineense* possesses non negligible phytochemical and antioxidant properties. However during cooking by steaming of groundnut pudding these spices can be used to limit the primary and secondary oxidation of groundnuts pudding oil. Good results were obtained with white *Piper nigrum* at all concentrations.

## REFERENCES


[1] Gunstone, F., (2004). The Chemistry of Oils and Fats: Sources, Composition, Properties and Uses. Ed Blackwell Publishing, Oxford (UK): Wiley-Blackwell p. 304.

[2] Iyéri-Manjeck, O. and Seignobos, C., (2000). Production arachidière. *In*: Atlas de la Province de l'Extrême Nord Cameroun. Planche 16. IRD (Edit). Pp. 1-16.

[3] Camas, N., C. Cirak and E. Esendal, (2007). Seed yield, oil content and fatty acids composition of safflower *Carthamus tintorius* grown in Northern Turkey condition. *Journal of the Faculty of Agriculture*. 22: 98-104.

[4] Reese, G., and Lehrer S. B. (1999). Food allergens in M. Frieri, & B. Kettelhut (Eds.), Food hypersensitivity and adverse reactions: A Practical Guide for Diagnosis and Management: 69-97. New York: Marcel Dekker, Inc.

[5] Jambunathan, R., Raju, S., Barde, S., (1993). Analysis of Groundnut Content by Nuclear Magnetic Resonance Spectrometry. *Journal of Science and Food Agriculture.* 36:162-166.

[6] Woodroof, J., (1983). Peanuts Production, Processing, Products. 3th ed. Avi Publishing Company Inc. Westport, Connecticut, USA.

[7] Anyasor, G., Ogunwenmo, K., Oyelana, O., Ajayi, D., Dangana, J., (2009). Chemical Analyses of Groundnut (*Arachishypogaea*) Oil. *Pakistan Journal of Nutrition,* 8 (3): 269-272.

[8] Lee, C. M. and Resurreccion, A. V. A. (2006). Predicting sensory attribute intensities and consumer acceptance of stored roasted peanuts using instrumental measurements. *Journal of Food Quality, 29*(4), 319-338

[9] Julia, K., C. Caroline, B. Corinne, A.G. Marizel, B. Ana-Paulina, R. Michel, d.S.S.D. María, S. Robert, N.S. Anne and G. Françoise. (2015). Antiatherogenic and antitumoral properties of Opuntia cladodes: inhibition of low density lipoprotein oxidation by vascular cells, and protection against the cytotoxicity of lipid oxidation product 4-hydroxynonenal in a colorectal cancer cellular model. *Journal of Physiology Biochemistry*. 71(3):557-587.

[10] Cuvelier, M. E. and Maillard, M. N., (2012). Stabilité des huiles alimentaires au cours de leur stockage. OCL; 19(2): 125-132.

[11] Shahidi, F., (2015). Handbook of Antioxidants for Food Preservation. Woodhead Publishing Series in *Food Science, Technology and Nutrition*, UK. pp: 251-285

[12] Veeriah, S., Kautenburger, T., Habermann, N., Sauer, J., Dietrich, H., (2006). Apple fl avonoids inhibit growth of HT29 human colon cancer cells and modulate expression of genes involved in the biotransformation of xenobiotics. Molecular Carcinogen 45:164–174.

[13] Baidez, A.G., Gomez, P., Del Rio, J.A., Ortuno, A., (2007). Dysfunctionality of the xylem in *Olea europaea* L. plants associated with the infection process by *Verticillium dahliae* Kleb. Role of phenolic compounds in plant defense mechanism. *Journal of Agriculture and Food Chemistry,* 55:3373–3377.

[14] Han, X. Z., Shen, T., Lou, H.X., (2007). Dietary polyphenols and their biological signifiance. *International Journal of Molecular Science,* 8:950–988.

[15] Asha Devi S., Umasankar E. M., Babu S. (2012) A comparative study of anti-oxidant properties in common Indian Spices. *International research Journal of Pharmacy*.

[16] Bin, S., Yizhong, Z., Cai, M., Sun, and Harold, C., (2005). Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. *Journal of Agricultural and Foods Chemistry*. 53, 7749−7759

[17] Goswami, P., Mandal, P., Jha, P., Misra, T., and Barat, S., (2013). Antioxidant Activities of Different Spices on the Lipid Oxidation of Cooked and Uncooked Fillet of Two Fish Species Belonging to the Genus *Puntius. Journal of Agricultural Sciences and Technology*, Vol. 15: 737-746.

[18] Fajobi, O., Adeniyi, Fasakin, O., Wilson, Oyedapo, O., and Oluboade. (2017). Phytochemicals, antioxidant potentials and 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Piper guineense* (Schumach & Thonn) seed. *African Journal of Plant Science.* Vol. 11(4), pp. 99-104.

[19] Dohou, N., Yamni, K., Tahrouch, S., Idrissi-Hassani, L., Badoc, A., Gmira, N., (2003). Screening phytochimique d'une endémique ibéro-marocaine, Thymelaealythroides. Bulletin de la société pharmacologique de Bordeaux, 142: 61-78.

[20] Bahorun, T., Gressier, B., Trotin, F., Brunete, C., Dine, T., Vasseur, J., Gazin, J., Boizot, N., Charpentier, J., (2006). Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre foustier. *Le cahier des Techniques de l'Inra*, Pp 79-82.

[21] Mensor, L., Menezez , F., Leitao, G., Reis, A., Dos Santos, T., Coube, C., Leitao, S., (2001). Screning of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 15: 127-130.

[22] Oyaizu, M., (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction. *Japanese Journal of Nutrition,*44: 307–315

[23] Nagulendran, K., Velavan, S., Mahesh, R., Haseena, B., (2007). *In Vitro* Antioxidant Activity and Total Polyphenolic Content of *Cyperus rotundus* Rhizomes. *Journal of Chemistry*, 4(3): 440-449.

[24] Ratnasari, N., Walters, M., and Tsopmo, A. (2017). Antioxidant and lipoxygenase activities of polyphenol extracts from oat brans treated with polysaccharide degrading enzymes. *Heliyon*, *3*(7).

[25] Bligh, E.and Dyer, W., (1959). A rapid method for total lipid extraction and purification. *Canada Journal Biochemistry and Physiology*, 37: 11-917.

[26] International Dairy Federation Standards, (1991). International Dairy Federation, IDF-Square Vergote 41, Brussels, Belgium, sec, 74A.

[27] AOCS Official Methods and Recommended Practices of the American Oil Chemists' Society. (2003). edited by Firestone D, American Oil Chemists' Society, 5th Ed. AOCS Press, Champaign, Illinois, USA.

[28] Shahidi, F. and Wanasundara, U. N., (2008). Methods for measuring oxidative stability in edible oils. In Akoh, C.C. and Min D.B. (Eds). Food Lipids: Chemistry, Nutrition and Biotechnology, p. 387-388. New York: CRC Press

[29] Drapper, H.and Hadley, M., (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymololy*, 186: 421-431.

[30] Farzana, M., (2016). Quantification of Total Phenol and Flavonoid content, In vitro Investigation of Antioxidant Activity and Cytotoxic Effect of the Seed Extract of *Piper nigrum* Linn (White pepper). Project submitted in the Department of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy. Brack University, Dhaka, Bangladesh.

[31] Kim, I. and Choe, E., (2004). Oxidative stability and antioxidant content changes in roasted and bleached sesame oil during heating. *Food Science and Biotechnology,* 13: 762–767.

[32] Shan, B., Cai, Y. Z., Sun, M., Corke, H., (2005). Antioxidant capacity of 26 spices extracts and characterization of their phenolic constituents. *Journal of Agriculture and Food Chemistry*, 53: 7749-7759.

[33] Hall, C. (1997). Structure-Activities of natural antioxidants. In" Antioxidant Methodology. In vivo and in vitro. Concepts, Ed. by Aruoma, OI and Cuppett, SL, AOAC press: Champaign, IL. 241 pages.
[34] Pietta, P. (1998). Flavonoids as antioxidants. *Journal of Natural Product*, *63*: 1035-1042.
[35] Marfak, A. and Sanchez, M. (2002). Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food science and technology international, 3*: 80.
[36] Katalinic, V., Milos, M., and Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, *94*: 550–557.
[37] Gayatri, N., and Sahu, R., (2011). Phytochemical Evaluation and Antioxidant activity of *Piper cubeba* and *Piper nigrum*. *Journal of Appliied Pharmaceutical Science*. (08); 2011: 153-157
[38] Ojimelukwe, P. C. and Ukom, A. N. (2017). Microbial and Antioxidant Activities of Some Common Spices from Southeast Nigeria. *Journal of Applied Life sciences International*. 13(4): 1-10.
[39] Masuda H., Hironaka, S., Yoko, M., Saori, H., Mami, H., Yushi, H., Makoto, A., and Hitomi, K., (2015). Comparative Study of the Antioxidative Activity of Culinary Herbs and Spices, and Hepatoprotective Effects of Three Selected Lamiaceae Plants on Carbon Tetrachloride-Induced Oxidative Stress in Rats. *Food Science and Technology Research*, 21 (3), 407-418
[40] Kahouli, I. (2010). Effet antioxydant d'extraits de plantes (*laurus nobilis l. rosmarinus officinalis, origanum majorana, oléa europea l.*) dans l'huile de canola chauffée. *Mémoire de Master*, Université Laval Québec, Canada, p. 16-52.
[41] CODEX STAN-211 (Commission du Codex Alimentarius) (2015). Norme pour les graisses animales portant un nom spécifique. Rome, Italie. 7 p.
[42] Choe, E. and Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Revue of Food Science.* Food Safety. v. 8, p. 345-358.
[43] Dhartiben, B., Kapadiya, B., and Kishorkumar, D., (2016). Spices and Herbs as a Source of Natural Antioxidants for Food. *International Journal of Current Microbiology and Applied Sciences.* (5) : pp. 280-288.
[44] Milda, and Ambuscado, (2015). Spices and herbs: Natural sources of antioxidants. *Journal of Functional Foods*. 9 P.
[45] Shermer, W.D., (1990). Effects of oxidation on the quality of ingredients and feed of poultry. 37º Maryland Nutrition Conference. Maryland, United State of America.
[46] Yanjun, Z., Susanne, M., Henning, R., Jianjun, H., Alona, Z., Zhaoping, L., and David He., (2015). Turmeric and black pepper spices decrease lipid peroxidation in meat patties during cooking. *International journal Foods Science and Nutrition*. 66(3): 260–265
[47] Ibrahium, I., Abd El-Ghany, E., Ammar, S., (2013). Effect of Clove Essential Oil as Antioxidant and Antimicrobial Agent on Cake Shelf Life. *World Journal of Dairy and Food Science.* 8: 140-146.
[48] Mc ginely, L. (1991). Analysis and quality control for processing and processed fats. In: Rossell, J. B. and Pritchard, J. L. R., Eds., Analysis of Oilseeds, Fats and Fatty Foods, *Elsevier Applied Science*, New York, United States of America, p. 460–470.
[49] Roman, O. (2012). Mesure et prédiction de la réactivité des lipides au cours du chauffage d'huiles végétales à haute température. Thèse de Doctorat AgroParisTech, Institut des Sciences et Industries du Vivant et de l'Environnement, Paris, Pp 168.
[50] Gray, J., (1978). Measurement of lipid oxidation: A review. *Journal of the American Oil Chemists' Society,* 55: 539-546
[51] Laguerre, M., Lecomte, J., Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Lipid Research*, 46: 244–282
[52] Zhang, Y., Yang, L., Zu, Y., Chen, X., Wang, F., Liu, F. (2010). Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants

[53] Penchalaraju. M., Lavanya, D., and Shwetha M., (2017). Natural antioxidants to reduce the lipid oxidation process of meat and meat products: A review. Internationa Journal of Chemical Studies. 5(5): 1450-1458 during aceelerated storage. *Food Chemistry*, 118 : 656-662.
[54] Tynek, M., Hazuka, Z., Pawlowicz, R., Dudek M., (2001). Changes in the frying medium during deep frying of food rich in proteins and carbohydrates. *Journal of Food Lipids*, 8: 251-261.

## Tables

**Table 1:** Total phenolic (TPC) and flavonoids (FC) content of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

| Samples | TPC (mg GAE/g) | FC (mg CE/g) |
|---|---|---|
| White *Piper nigrum* | 52.38 ± 0.11[c] | 113.32 ± 0.06[c] |
| Black *Piper nigrum* | 85.00 ± 0.03[a] | 271.94 ± 0.02[a] |
| *Piper guineense* | 65.71 ± 0.08[b] | 175.71 ± 0.04[b] |

*Values with different superscripts in the same column differ significantly at p<0.05*

**Table 2:** Pearson's correlation between the TPC, FC, DPPH, FRAP, ROH and $ROO^{o}$ of extracts

| Correlations | | | | | | |
|---|---|---|---|---|---|---|
| | TPC | FC | DPPH | FRAP | ROH | $ROO^{o}$ |
| TPC | 1 | 1.000** | 0.769* | 0.534* | 0.447 | 0.954** |
| FC | | 1 | 0.758* | 0.519* | 0.431 | 0.948** |
| DPPH | | | 1 | 0.951** | 0.915** | 0.926** |
| FRAP | | | | 1 | 0.995** | 0.764* |
| ROH | | | | | 1 | 0.695* |
| $ROO^{o}$ | | | | | | 1 |

***. Correlation is significant at 0.01 (bilateral)*

***TPC** = Total phenolic contents; **FC** = flavonoids contents; **DPPH** =DPPH free radical scavenging assay; **FRAP** = Ferric Reducing Antioxidant Power; **ROH** = inhibition of hydroxyl radical; **$ROO^{o}$** = Oxygen radical absorbance capacity*

**Table 3:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the peroxide value of lipids extracted from groundnuts pudding

| Samples | Peroxide value ($meqO_2/kg$) | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| **OGC**WPN | 2.81±0.22$^{bC}$ | 2.99±0.84$^{bC}$ | 3.28±0.00$^{cB}$ | 5.46±0.88$^{bA}$ |
| **OGCBP**N | 2.91±0.32$^{bB}$ | 3.61±0.00$^{bB}$ | 9.31±2.48$^{bA}$ | 6.89±0.36$^{bA}$ |
| **OGCPG** | 2.40±0.19$^{bC}$ | 2.97±0.20$^{bC}$ | 3.94±0.42$^{cB}$ | 4.77±0.03$^{bA}$ |
| **OGC** | 18.39±1.25$^{aA}$ | 18.39±1.25$^{aA}$ | 18.39±1.25$^{aA}$ | 18.39±1.25$^{aA}$ |

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Table 4:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the anisidine values of lipids extracted from groundnuts pudding

| Samples | Anisidine value | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| **OGCWPN** | 12.75±0.00$^{dB}$ | 16.71±2.84$^{dB}$ | 15.82±3.61$^{dB}$ | 52.28±0.00$^{bA}$ |
| **OGCBPN** | 24.01±1.44$^{cD}$ | 70.06±0.00$^{aB}$ | 73.37±0.00$^{aA}$ | 47.70±0.00$^{bC}$ |
| **OGCPG** | 94.45±1.09$^{aA}$ | 53.25±0.00$^{bD}$ | 56.21±0.00$^{bC}$ | 62.91±0.00$^{aB}$ |
| **OGC** | 49.49±2.41$^{bA}$ | 49.49±2.41$^{cA}$ | 49.49±2.41$^{cA}$ | 49.49±2.41$^{bA}$ |

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Table 5:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the TOTOX values of lipids extracted from groundnuts pudding

| Samples | TOTOX value | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| **OGCWPN** | 18.38±0.44$^{dB}$ | 22.71± 4.52$^{cB}$ | 22.39±3.61$^{bB}$ | 63.21±1.77$^{bA}$ |
| **OGCBPN** | 29.84±2.09$^{cD}$ | 77.29±0.00$^{bB}$ | 91.99±4.96$^{aA}$ | 61.49±2.17$^{bC}$ |
| **OGCPG** | 99.25±4.92$^{aA}$ | 59.20±0.05$^{cC}$ | 64.11±0.85$^{bC}$ | 72.46±1.16$^{bB}$ |
| **OGC** | 86.26±4.92$^{bA}$ | 86.26±4.92$^{aA}$ | 86.26±4.92$^{aA}$ | 86.26±4.92$^{aA}$ |

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Table 6:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the thiobarbituric acid values of lipids extracted from groundnuts pudding

| Samples | Thiobarbituric acid value (meqMDA/kg) | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| **OGCWPN** | 7.29±0.55$^{aB}$ | 4.88±0.41$^{bC}$ | 2.13±0.34$^{bD}$ | 9.43±0.39$^{aA}$ |
| **OGCBPN** | 7.35±0.01$^{aA}$ | 6.15±0.51$^{bA}$ | 7.18±2.12$^{aA}$ | 6.73±0.97$^{bA}$ |
| **OGCPG** | 5.77±0.36$^{bA}$ | 3.90±0.62$^{bcA}$ | 5.91±1.32$^{bA}$ | 5.64±0.33$^{bA}$ |
| **OGC** | 8.56±0.16$^{aA}$ | 8.56±0.16$^{aA}$ | 8.56±0.16$^{aA}$ | 8.56±0.16$^{aA}$ |

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

**Table 7:** Effect of white *Piper nigrum*, black *Piper nigrum* and *Piper guineense* on the Iodine values of lipids extracted from groundnuts pudding

| Samples | Iodine value (gI2/100g) | | | |
|---|---|---|---|---|
| | 0.5 g | 1 g | 2 g | 4 g |
| **OGCWPN** | 54.42±1.10$^{aA}$ | 53.93±3.58$^{aA}$ | 61.86±0.44$^{aA}$ | 54.17±1.57$^{aA}$ |
| **OGCBPN** | 48.53±0.69$^{aA}$ | 54.88±1.34$^{aA}$ | 53.06±3.26$^{bA}$ | 52.86±2.15$^{aA}$ |
| **OGCPG** | 49.37±0.17$^{aC}$ | 56.33±0.19$^{aA}$ | 55.85±0.95$^{aB}$ | 57.65±0.00$^{aA}$ |
| **OGC** | 49.00±0.77$^{aA}$ | 49.00±0.77$^{bA}$ | 49.00±0.77$^{bA}$ | 49.00±0.77$^{bA}$ |

*Values with different superscripts in small letters in the same column and that of capital letters in the same line differ significantly at p<0.05;* **OGC =** Oil extracted from groundnuts cake; **OGCWPN** = Oil extracted from groundnuts cake cooked with white *Piper nigrum*; **OGCBPN** = Oil extracted from groundnuts cake cooked with *black Piper nigrum*; **OGCPG** = Oil extracted from groundnuts cake cooked with *Piper guineense*

## Figure captions

**Figure 1**: DPPH Free Radical Scavenging Assay of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

**Figure 2:** Ferric Reducing Antioxidant Power (FRAP) of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

**Figure 3:** Inhibition of hydroxyl radical of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

**Figure 4:** Oxygen radical absorbance capacity of the aqueous extract of white *Piper nigrum,* black *Piper nigrum* and *Piper guineense*

**Figure 1**

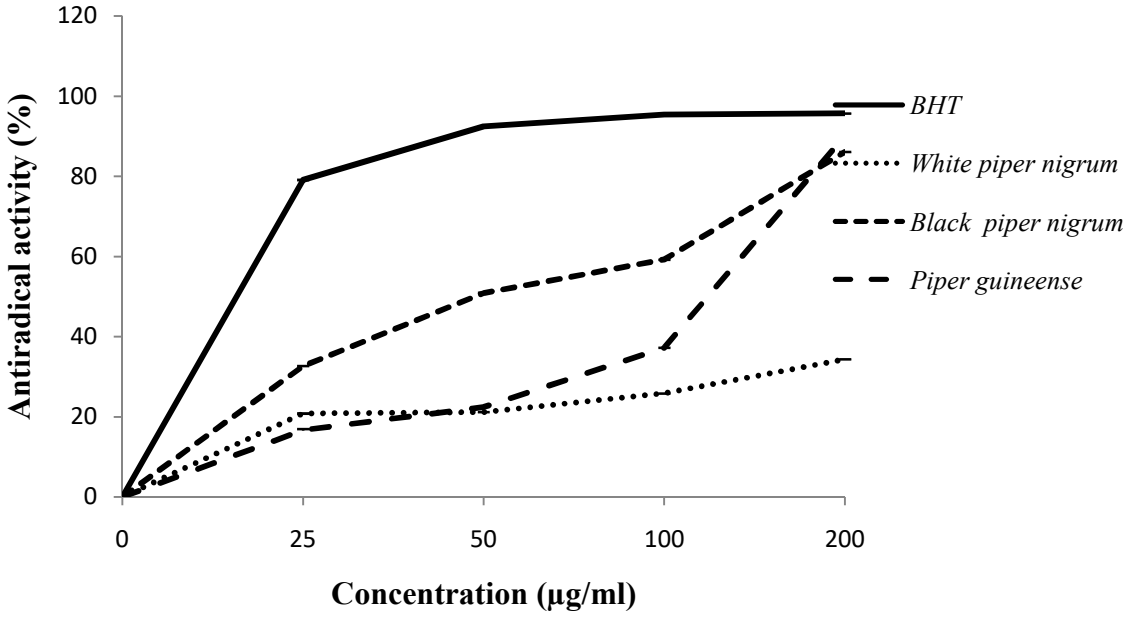

120
100
80
60
40
20
0
Antiradical activity (%)
0
25
50
100
200
Concentration (µg/ml)
BHT
White piper nigrum
Black piper nigrum
Piper guineense

**Figure 2**

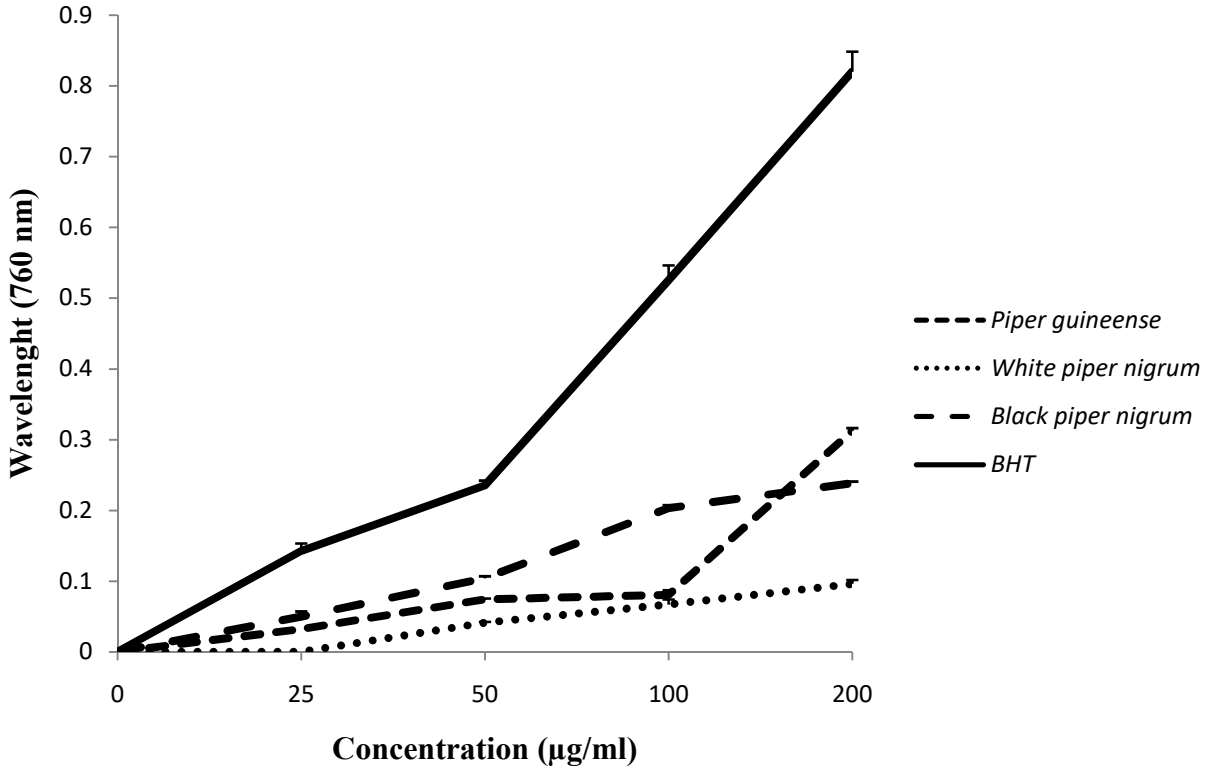

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Wavelenght (760 nm)
0
25
50
100
200
Concentration (µg/ml)
Piper guineense
White piper nigrum
Black piper nigrum
BHT

**Figure 3**

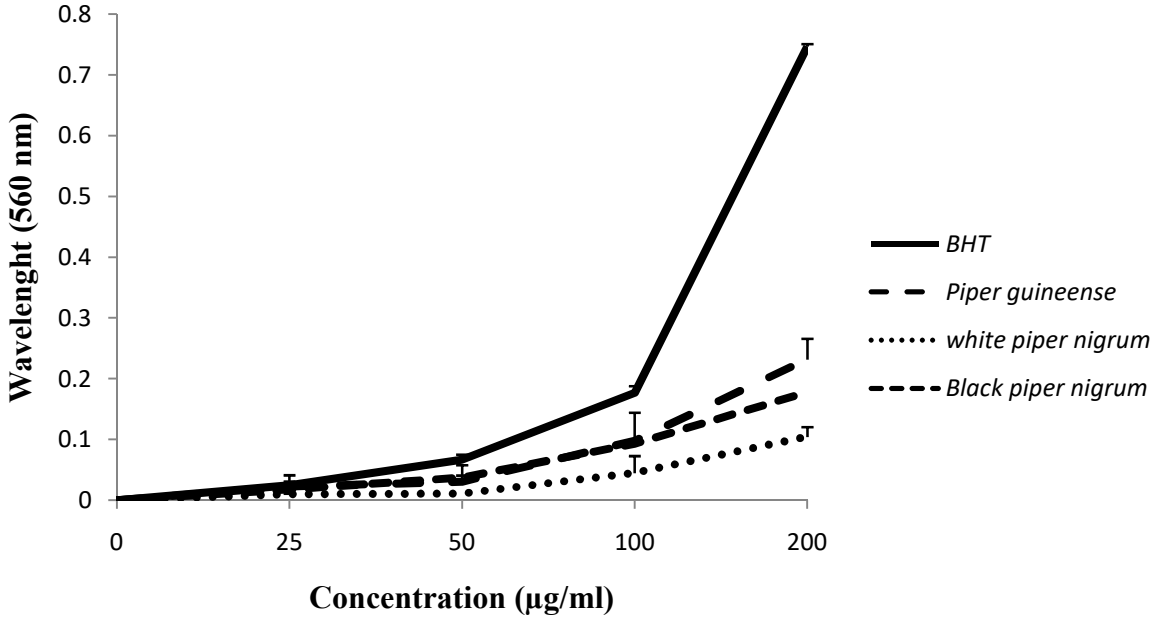
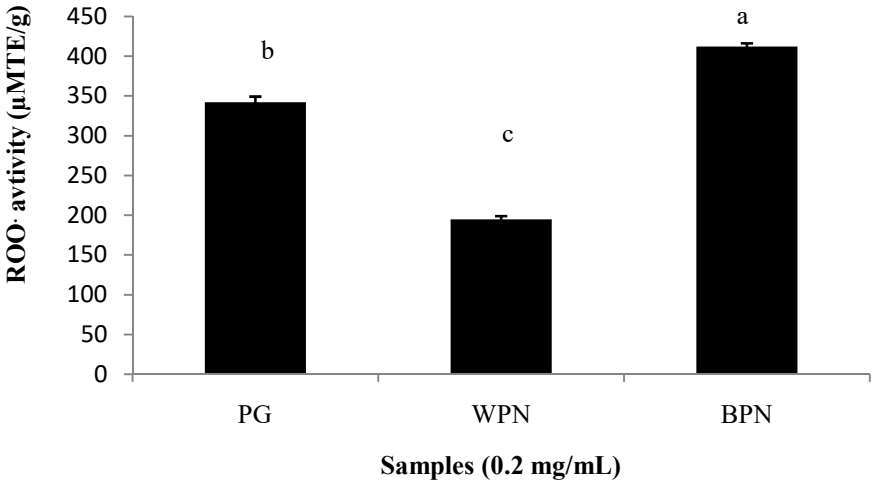

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Wavelenght (560 nm)
0
25
50
100
200
Concentration (µg/ml)
BHT
Piper guineense
white piper nigrum
Black piper nigrum

**Figure 4**


ROO· avtivity (µMTE/g)
450
400
350
300
250
200
150
100
50
0
b
c
a
PG
WPN
BPN
Samples (0.2 mg/mL)