EFFECTS OF FERMENTATION ON THE ANTIOXIDANT PROPERTIES OF FLOUR OF ONE NORMAL ENDOSPERM AND QUALITY PROTEIN MAIZE GROWN IN NIGERIA

*[1]Oladeji, Babatunde Stephen, [2]Akanbi, Charles Taiwo and [2]Gbadamosi Saka Oluwasunkanmi
[1]Department of Food Science and Technology, University of Calabar, Calabar, Nigeria
[2]Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria
E-mail: oladejistephen35@yahoo.com

Abstract
Preservation of foods and supplying essential antioxidants in vivo by natural antioxidants contained in foods is well appreciated. The study aimed at determining the effects of fermentation on the antioxidant properties maize flour. A yellow coloured QPM (TZE-POP-DT-STR-QPM) was compared with yellow coloured normal maize (SUWAN-ISR). The maize sample was divided into two; a portion was fermented and the other portion was decorticated and degermed mechanically without fermentation before milling to flour.Total antioxidant of all the samples was extracted in both aqueous and methanol medium. Total carotenoids and vitamin C contents, total phenol, flavonoids content were determined. DPPH and ABTS and Lipid peroxidation scavenging power of aqueous and methanol extracts were measured. Also, the storage stability of the antioxidant extract was carried out within 72 hour of storage at refrigeration temperature. The result shows that total carotenoid of unfermented samples (35.48 and 46.85 mg/100g for UFNM and UFQPM, respectively is lower than 50.70 and 56.69 mg/100g obtained for fermented samples (FNM and FQPM). DPPH scavenging power of the sample ranged from 20.28-41.79% and 11.38-20.70% for aqueous and methanolic extracts, respectively. DPPH activities of fermented samples were more stable over a three days storage period compared to unfermented samples. The study established increasing effect of fermentation on total carotenoid and vitamin C with a milder reduction effect of some antioxidant capacity in aqueous medium compared to methanol extracts.

Keywords: total carotenoids, ascorbic acid, total phenols, maize, DPPH activities, ABTS activities, lipid peroxidation

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1. INTRODUCTION

The importance of the antioxidants contained in foods is well appreciated for food preservation and supplying of essential antioxidants in vivo. Also, the increasing experimental, clinical and epidemiological data which show the beneficial effects of antioxidants against oxidative stress-induced degenerative and age-related diseases, cancer and ageing, have received renewed attention (Jan *et al*., 2001; Oboh *et al*., 2007). In recent years, cereals and their components have been applied in functional food and nutraceuticals, because they are good sources of dietary fibre, protein, energy, minerals, vitamins and other phytochemicals required for human health (Charalampopoulos *et al.*, 2002). Phenolic acids such as ferulic and its derivatives contribute to the antioxidant properties of cereal grains (Anson *et al*., 2009). To this end, cconsumption of whole grains as part of the diet is now recommended for health reasons because they are rich source of antioxidants.

The fact that there is abundant deposit of nutrients, including antioxidant in quality protein maize (QPM) cannot be overemphasized. Protein, being the primary structural and functional component of every living cell, is one of the most important ingredients that determine the quality of food and feed. (QPM) was developed by lowering the concentration of zein in normal edosperm by 30%. As a result, the concentration of two essential amino acids viz., lysine and tryptophan in grain was increased in QPM genotypes as compared to normal grain maize genotypes. The biological value of QPM is highest among all the food grains for the simple reason that all cereals except QPM are deficient in lysine, and all pulses are deficient in methionine, another essential amino acid (IASRI, 2011).

Maize is prepared and consumed in several ways; it may be sun dried, cooked, fermented (ogi), roasted, pounded or crushed depending on locality or ethnic group (Ayatse *et al.*, 1983). Studies have shown that the effects of processing and method of preparation of maize may increase or decrease its nutritive and health-promoting values depending upon the processing (Ayatse *et al.*, 1983; IITA, 2011; Seema, 2011). Obizoba and Atii, (1991) observed that combination of cooking and fermentation improved the nutrient quality of all cereal and reduced the content of antinutritional factors to a safe level in comparison with other methods of processing. Sprouting or fermentation of maize grains increases the digestibility as well as the content of some of the B vitamins. Milling reduces the concentration of proteins and lipids as well as fiber and cooking improves the antioxidant capacity of corn (Seema, 2011).

QPM has been accepted as a nutritious cereal used in food production, however, the effect of fermentation on its antioxidant have not been extensively studied. The prevalence of protein energy malnutrition among infants and the increasing incidences of degenerative diseases among adult population have continued to attract significant attention. Diets rich in protein and antioxidants are now being advocated and recommended. There is therefore, the need to study the antioxidant properties of fermented and unfermented quality protein maize flour.

2. MATERIAL AND METHODS

Collection of Materials

Two samples of maize varieties were studied; a yellow coloured QPM variety was studied in comparison with yellow colored normal maize variety. The QPM variety: TZE-YPOP-DT-STR-QPM was obtained from IITA, Ibadan while the normal maize; SUWAN-ISR was obtained from IAR and T, Ibadan. Intact whole seeds were picked manually and stored at refrigeration temperature for further investigations.

Samples Preparation

The maize samples were sorted to ensure wholesomeness. Each variety of the sorted grains were divided into two; a batch was fermented whole following the method of Oladeji *et al.*(2014) for traditional preparation of *ogi*. The maize obtained was washed and steeped in clean boiled water in a plastic container with cover. The water was decanted after three days (96 hrs) and the maize wet milled into slurry. The slurry was sieved using muslin cloth, which separated the pomace from the filtrate. The slurry was filtered and oven dried at 50 ^{o}C for 12 hrs. The dried ogi cake was fine milled to flour. The method of Oladeji (2014) was used to prepare the other batch. The grains was sorted, sprinkled with water and allowed to stand for about thirty minutes under ambient temperature to soften the coat. It was then transferred into a locally fabricated decorticating machine which separated the germ and the coat mechanically. The machine is provided with two separate receivers, one for the endosperm and the other for the mixture of germ and seed coat. The clean endosperm was milled into flour using attrition mill. The flour was packaged in cellophane pack prior to analysis

Antioxidant Measurements

Total antioxidant of all the samples was extracted in both aqueous and methanol media for use where applicable. Active antioxidant content of the samples was determined as well as ability of the samples' extract to scavenge free radical using common assays. Also, the storage stability of the antioxidant extract was carried out within 72 hour of storage at refrigeration temperature.

Determination of ascorbic acid

An ascorbic acid concentration of the blend was determined using the 2, 6- dichlorophenol-indophenol titration method described in A.O.A.C. (2000). The extracted solution (10 ml) per gram dry sample was mixed with 25 ml of 20% metaphosphoric acid in a 100 ml flask. The resulting solution (10 ml) was pipetted into

a 250 ml flask and titrated against 2, 6-dichlorophenol-indophenol solution.

$$Ascobic\ acid\ \left(\frac{mg}{100\ g}\right) = \frac{0.6 \times litre \times dilution\ factor}{weight\ of\ the\ sample} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (1.0)$$

Total carotenoid determination

Total carotenoid of the samples was determined according to the method of Sallaur *et al.* (1990) by weighing approximately 0.06 g of the sample into 40 ml amber screw top flask that contain 5 ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% ethanol and 10 ml n-hexane. The mixture was stirred on magnetic stirrer for 15 mins after which 3 ml of distilled water was added and then stirred for another 5 mins. Partition to polar and non-polar layer was allowed for 5 mins to separate the top non polar phase before it was filtered with filter paper. The absorbance of the extract at 450 nm was read using n-hexane as blank with UV spectrophotometer.

Total carotenoid was estimated using $£^{1\%}_{1cm}$ = 2,500

$$Total\ carotenoid = \frac{weight\ of\ sample \times dilution\ factor}{Extranction\ coefficient \times absorbance} \ldots\ldots\ldots (2.0)$$

Preparation of aqueous and methanolic extracts

The extraction was done by combination of the methods of Oufnac (2006) and Elekofehinti and Kade (2012) with some modifications. One gram (1 g) each of flour samples was weighed in separate extraction bottle and 10 ml of solvent (either distilled water or 98% metahnol) was added to the bottle containing the flour samples and left to stand for 24 h to allow for extraction at room temperature. Thereafter, the solvent layer from each tube was separated by centrifugation at 2000 rpms for 5 min.

The filtrates (not evaporated) of both aqueous and methanolic extracts were stored air tight in a refrigerator until required for use. The extract was used for total phenol, total flavonoid, and DPPH, ABTS and lipid peroxidation determinations.

Total Phenol content determination

The extractable phenol was determined on the extracts using the method of Singleton *et al.* (1999) as modified by Ogunmoye *et al* (2012). Briefly, 100 µl of each sample extract (aqeous and methanol) was mixed with 800 µl of water and Folin-Ciocalteau reagent (100 µl). This was allowed to wait for 8 mins in the dark. Then 50 µl of 20% sodium carbonate was added, and allowed to stay in the dark for one hour before the absorbance was measured at 760 nm. The amount of total phenols in both extracts was expressed as gallic acid equivalent (GAE). Galic acid was used to prepare five standard solutions to obtain a standard curve used to quantify the total phenol of the flour extract.

Total Flavonoid content determination

The extractable flavonoid of extract was determined using a slightly modified method reported by Meda *et al*. (2005). Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl of 10% $AlCl_3$, 50 µl of 1 M potassium acetate and 1.4 ml water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm in a spectrophotometer. Five different concentration of quercetin in methanol was prepared and used to obtain a standard curve. The total flavonoid was calculated mg/100g QE using quercetin standard curve obtained as standard.

Determination of DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenyl-2 picrylhydrazyl) (DPPH) free radical was evaluated as described by Gyamfi *et al*. (1999) with slight modification. Briefly, 500 µl of the extracts was mixed with 500 µl of 0.4 mm methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance measured at 516 nm in a spectrophotometer. The percentage DPPH free radical scavenging ability was subsequently calculated.

Determination of ABTS radical scavenging activity

The antioxidant ability of extract of the flour samples to trap free radicals of ABTS was evaluated according to the method described by Nenadis *et al.* (2004) and as used by Elekofehinti *et al* (2013). The stock solution included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted by 1ml ABTS solution with 60 ml methanol to obtain an absorbance of 0.706±0.001 at 734 nm using the spectrophotometer. The ABTS scavenging capacity was calculated as;

$$ABTS\ scavenging\ acticity\ (\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times \frac{100}{1} \quad \ldots\ldots\ldots\ldots (3.0)$$

Determination of Lipid peroxidation

Rats were decapitated under mild ether anesthesia and the cerebral (whole brain) tissue was rapidly dissected, placed on ice and weighed. Tissue was immediately homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenates was centrifuged for 10 min at 4000 rpm to yield a pellet that was discarded and a low-speed supernatant (S1). An aliquot of 100 µl of S1 was incubated for 1 h at 37 °C in the presence of extract of the blended flour, with and without the prooxidants, iron (final concentration, 10 µm) and sodium nitroprusside (SNP) (final concentration, 30 µm). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa *et al.* (1979), excepting that the buffer of the colour reaction has a pH of 3.4. The colour reaction was developed by adding 300 µl 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500 µl acetic acid/HCl (pH 3.4) and 500 µl 0.8% thiobarbituric acid (TBARS). This mixture was incubated at 95 °C for 1 h. TBARS produced were measured at 532 nm and the absorbance was compared to that of the controls.

Determination of change in antioxidant capacity of the extract during storage

The stability of the extract was determined by repeating the analysis for the antioxidant properties within 72 hours at 24 hours intervals. Total phenol content was used as indicator for the antioxidant properties while DPPH assay was used as indicator for free radical scavenging ability of the extracts.

Data Analysis

The data was generated in triplicate and the result expressed as mean ± standard deviation. Where applicable data was analyzed statistically using analysis of variance (ANOVA) with $\alpha = 0.05$ (SPSS version 17) to determine statistically the significant difference between the quality attributes and antioxidant activities for different flour samples. Means difference was calculated using Ducan multiple range comparison tests of the same package. Some data were represented on graph and chat where necessary for clearer interpretation.

3. RESULTS AND DISCUSSION

Total Carotenoids and Vitamin C Content of the Flour samples

The result of total carotenoid content of the flour samples in Table 1 showed that there was a significant difference in the total carotenoids contents of all the flour samples at $p \leq 0.05$. The values ranged from 35.48 to 56.69 mg/100g of the samples. This was expected of yellow maize flour as yellow maize had been reported to contain significant amount of carotenoids (Gliszczynska-Swiglo, 2006). The result showed that unfermented decorticated flour samples had the lowest total carotenoid content (35.48 and 46.85 mg/100g for UFQPM and UFNM, respectively) showing that QPM flour had higher carotenoid content than normal maize flour sample (Table 1). Fermentation had a positive impact on the total carotenoid content of the two maize flours in this study. Increased in total carotenoid of fermented samples when compared with unfermented flours is an indication that fermentation had improved availability of total carotenoid in the maize flour samples.

Table 1: Total Carotenoid and Vitamin C contents (mg/100g) of flours

Samples	Total carotenoid	Vitamin C
UFNM	35.48±0.33d	11.33±1.05c
UFQPM	46.85±0.88c	12.67±1.15bc
FNM	50.70±0.70b	14.67±0.58ab
FQPM	56.69±0.27a	15.33±1.10ab

Values reported as means ± standard deviation. Mean values followed by different letter are significantly different ($p \leq 0.05$)
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

Table 2: Total Phenol Content (mg/g TAE) and Total Flavonoids Content mg/g of Quscetin equivalent of flour samples in

	Total Phenol		Total Flavonoids	
Samples	Aqueous Extract	Methanolic Extract	Aqueous Extract	Methanol Extract
UFNM	0.408±0.006a	0.581±0.02d	19.51±1.88b	42.09±3.25c
UFQPM	0.411±0.016a	0.702±0.01abc	31.35±3.59a	47.91±4.06b
FNM	0.275±0.005b	0.564±0.06d	1.72±0.21d	46.06±0.24a
FQPM	0.266±0.023b	0.596±0.01cd	2.32±1.22cd	56.72±0.30a

Values reported as means ± standard deviation. Mean values followed by different letter are significantly different ($p \leq 0.05$)
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

The vitamin C content of the flour samples ranged from 11.33 to 15.33 mg/100g of the flour sample (Table 1). Fermentation increased the vitamin C content of the two maize varieties under study. The result of this study agreed with earlier reports that fermentation had increasing effect on vitamin C content of flour samples (Alexia, 2007; Ademulegun and Koleosho, 2012).

Total Phenol Content of flour samples in Aqueous and Methanol Extracts

The result showed that the range of total phenol of methanolic extract (0.564 to 0.702 mg/100g TAE) was higher than 0.266-0.408 mg/100g TAE obtained for aqueous extract (Table 2). Fermentation had reducing effects on the total phenol content of both the aqueous and methanolic extracts as shown in Table 2. The result also showed that the reducing effect of fermentation was milder on FNM than in the FQPM in aqueous medium; 0.275 and 0.266 mg/100g TAE for FNM and FQPM flour sample, respectively, though no significant difference existed between the two values. Higher value (0.596 mg/100g TAE) was obtained for FQPM than for FNM (0.564 mg/100g TAE) in methanol medium. This means that total phenols are more stable in FNM flour in aqueous medium than in FQPM flour in the same medium. But total phenol is more stable in FQPM flour than in FNM when in methanol medium. Generally, total phenol contents of all the samples in methanolic extract are higher than those of aqueous extract. The result of this study is in agreement with several research reports; Ogunmoyole *et al*. (2011) estimated the phenolic content of *Juglans regia* to be 35.22 ± 0.75 mg/g (GAE) for ethanolic and 20.26 ± 0.55 mg/g (GAE) for aqueous extract. Also, Ogunmoyole *et al.* (2013) estimated the phenolic content of *G. kola* to be 45.2 ± 0.9 mg/g and 30.3 ± 0.4 mg/g gallic acid equivalent (GAE) for the ethanolic and aqueous extracts, respectively.

Flavonoid Content of Maize and Carrot Blends of Aqueous and Methanol Extracts

Just like total phenol content of the maize flour extract (Table 2), fermentation reduced the total flavonoid content of both normal maize and QPM flours. Sample FNM contained 1.72 and 56.06 mg/g QE flavonoid for aqueous and methanolic extract, respectively while FQPM contained 2.32 and 56.72 mg/g QE for aqueous and methanolic extract, respectively (Table 2). The value is close to 58.22 mg/g flavonoid content of the extract in terms of quercetin equivalent earlier reported for Iranian Corn Silk (Ebrahimzadeh *et al*., 2008). The result of

this study showed that total flavonoid content of UFQPM (31.35 and 47.91 mg/g QE) for aqueous and methanolic extract, respectively are higher than the values obtained for UFNM (19.51 and 42.09 mg/g QE) for aqueous and methanolic extract, respectively (Table 2). Furthermore, noteworthy is the fact that the flavonoid content of the methanolic extract was higher than that of the aqueous extract. This may be due to difference in the solubility of phytochemicals depending on the solvent of extraction or/and polarity of the solvent. For instance, quercetin is insoluble in water but soluble in ethanol (Omololu *et al.*, 2011).

DPPH radical scavenging properties of flour samples

The DPPH radical is widely used to evaluate the free radical scavenging activity of hydrogen donating antioxidants in many plant extracts (Kumar *et al.*, 2011). The ability of the different flour to scavenge DPPH differed significantly from each other as depleted on Figure 1. The ability of the aqueous extract of the flour samples to scavenge DPPH radical is within the range of 20.28 and 41.79% while that of methanol extracts is within the range of 11.38 to 20.70% (Figure 1).

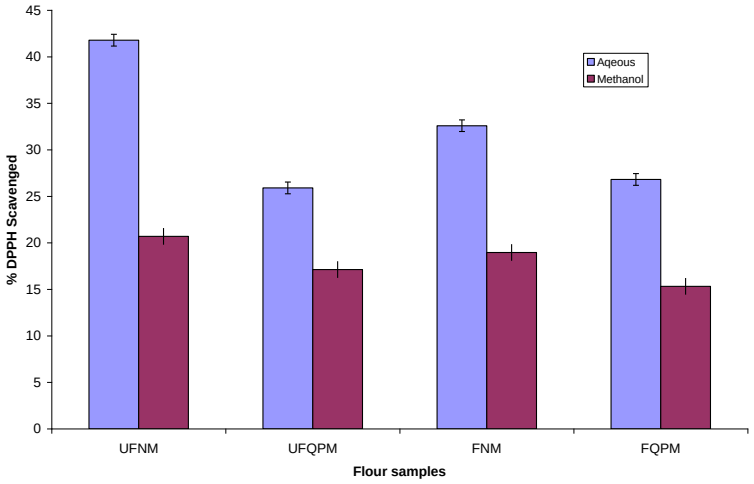

Figure 1: DPPH scavenging ability of aqueous and methanolic extracts of the flour samples
Key: UFNM-Unfermented decorticated normal maize, UFQPM-Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM-Fermented quality protein Maize

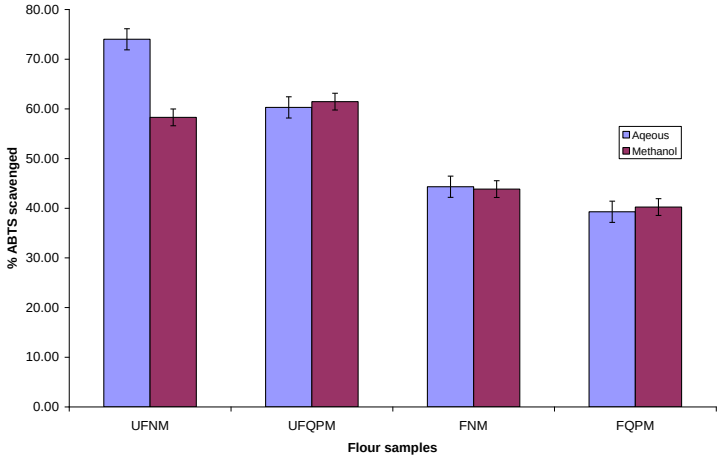

Figure 2: ABTS scavenging ability of aqueous and methanolic extracts of the flour samples
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

The result of the methanolic extract is lower than 16.20 to 32.0% reported for guava fruit by Thaipong *et al.* (2006). This is expected as fruits naturally possess higher antioxidant capacity than grains and had earlier been reported to be one of the fruits which contain exceptionally high antioxidant activity (Thaipong *et al*., 2006). The result is also close to12.85% reported for Kunu without sugar- a Nigerian beverage made from corn (Oboh and Okhai, 2012). Generally, the ability of the aqueous extracts of the maize flour samples to scavenge DPPH radical was higher than those of the methanol extract (Figure 1). Meaning that antioxidant with ability to scavenge DPPH in the flour samples was more soluble in aqueous medium than in methanol. This is good in product development as these flours are meant to be reconstituted with water before consumption; an assurance of higher antioxidant benefit when consumed. The ability of the aqueous extract of the maize flour samples to scavenge DPPH measured in percentage ranged from 20.28 to 40.78% while that of methanol extract ranged between 11.38 and 20.69% (Figure 1). Samples UFNM and UFQPM scavenged DPPH better than fermented samples (FNM and FQPM) meaning that fermentation reduced the DPPPH scavenging ability of flour made from the two maize varieties under study.

ABTS radical scavenging properties of flour samples

Awika *et al*. (2003) reported the superiority of the ABTS assay because it is operable over a wide range of pH, inexpensive and more rapid. The ability of the aqueous and methanolic extracts of the flour blends to decolourise ABTS is similar except for the UFNM flour which is significantly higher than the aqueous (73.99%) and Methanolic extract (58.27%) shown in Figure 2. Higher percentages were obtained for normal maize flour samples in terms of ability of the extract to decolourise ABTS, compared to QPM. In this study, the result of aqueous extract showed that 73.99 and 60.28% of ABTS was decolourised by UFNM and UFQPM flours, respectively (Figure 2). Fermentation reduced the ability of the flour extract to decolourise ABTS radical as 44.23 and 39.28% was obtained for FNM and FQPM flours, respectively.

Change in Total Phenol of Aqueous Extract within 72 Hours

The effect of storage time on the total phenol content of aqueous extract of the flour samples is as depicted on Figure 3. A general reduction was observed in the total phenol content of the aqueous extract as storage time increased from zero to seventy two hours.

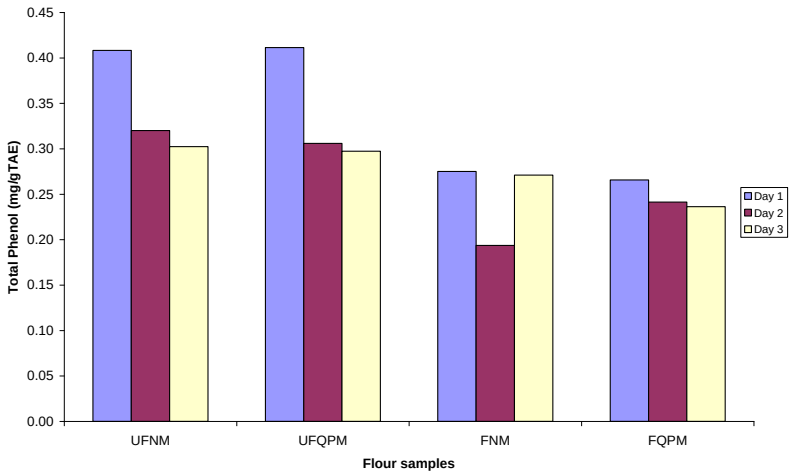

Figure 3: Change in Total Phenol of aqueous extract within 72 hours

Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

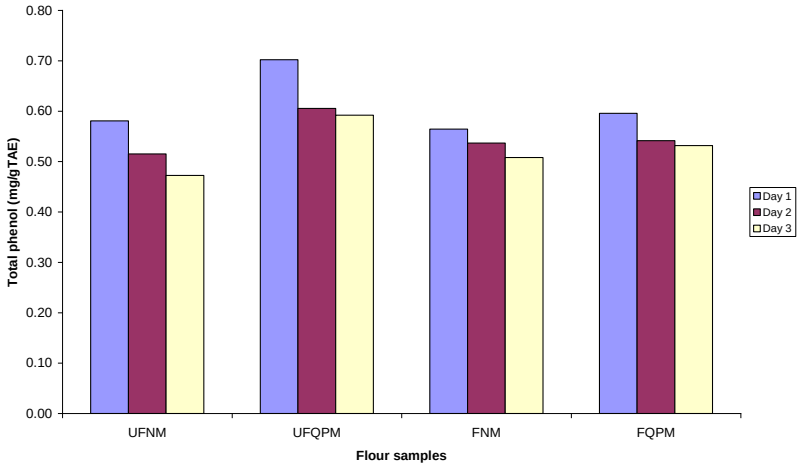

Figure 4: Change in Total Phenol of methanol extract within 72 hours
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

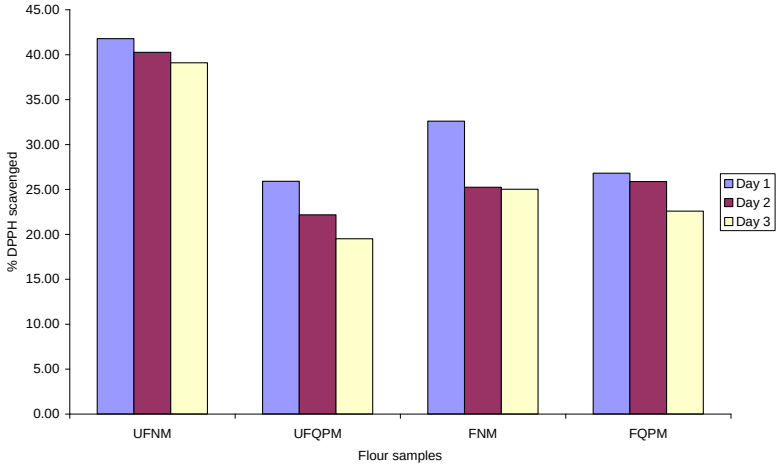

Figure 5: Change in DPPH Scavenging ability of aqueous extract within 72 hours
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

This result is similar to the observation of Bolbol *et al* (2012), who observed a decrease in phenolic content of corn after 24, 36, and 48 hour of germination compared to the control value. Several reasons had been suggested for decrease in phenolic compounds in aqueous medium. Losses may be attributed to decreases in extractability, as lower molecular weight phenolic compounds polymerize, thus becoming insoluble in water (Deshpande *et al.*, 1982). Alternatively, during the period of storage, the enzyme polyphenol oxidase activated during soaking may have multiplied, resulting in degradation and consequent loss of polyphenols (Jood *et al.*, 1998; Saxena *et al.*, 2003). The phenolic content of UFNM reduced from 0.41to 0.32 and 0.30 mg/gTAE first to second day and second to third day, respectively which account for 21.9 to 26.9% decrease. Similarly, total phenol content of UFQPM extract reduced from 0.41 to 0.31 and 0.29 mg/gTAE from first day to second and second day to third day, respectively accounting for 24.3 and 29.3% reduction in total phenol content upon storage (Figure 3). Afify *et al.* (2012) reported a similar range of between 21.97 and 28.30% total phenol losses in sorghum after soaking.

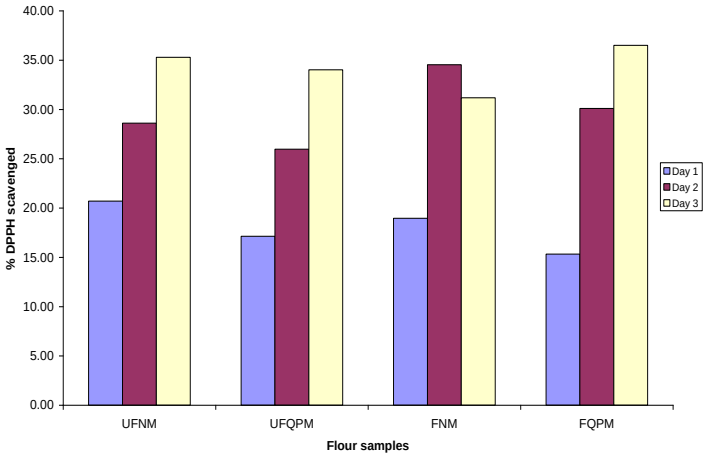

Figure 6: Change in DPPH Scavenging ability of methanol extract within 72 hours
Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

Change in Total Phenol of Methanol Extract of the Flour Samples within 72 Hours

Also, a general reduction in total phenolic content was observed in all the samples from 24 hours to 48 hours and to 72 hours of storage. Total phenolic content of UFNM reduced from 0.58 to 0.47 mg/gTAE from 24 hour to 72 hours of storage, respectively, accounting for 10.34 to 18.97% reduction. Maximum of 18.97% losses in total phenolic content observed in UFNM flour is higher than 14.29% obtained for its UFQPM (Figure 4). The result implies that the phenolic content of methanol extract is more stable than aqueous extracts over the same storage condition and time. The range of losses obtained in this study is lower than 15.52 to 44.82% reported for methanol extract of corn over germination period of 12 hours to 48 hours by Boldol *et al.* (2012). Rate of loss in total phenol content of FNM extract reduced from 0.56 to 0.54 and to 0.51 mg/gTAE from 24 hours to 48 hour and to 72 hours, respectively. A reduction of 0.60 to 0.54 and 0.53 mg/gTAE was observed in FQPM from 24 hour to 48 hour and 72 hours, respectively (Figure 4). The rate of losses in total phenol content of the fermented samples is lower than the unfermented samples. This is expected as fermentation is an efficient method of preserving food and food components (Jenny, 2009).

Change in DPPH Scavenging Power of Aqueous Extract of the Flour Samples within 72 Hours

The effect of storage time on the DPPH scavenging power of aqueous extracts of the flour samples within 72 hours at refrigeration temperature is presented in figure 5. DPPH scavenging capacity of all the samples decreased steadily with increasing storage period (Figure 5). In unfermented samples, the rate of decrease in scavenging ability of DPPH of UFQPM extract (14.44 and 10.23% for 48 and 72 hours, respectively) is higher than 3.7 and 2.7% obtained for UFNM at 48 and 72 hours, respectively (Figure 5). The range (19.52 to 25.91% scavenging ability obtained for UFQPM is in line with 21.72 to 27.69% scavenging ability reported for raw sorghum by Afify *et al*. (2012) and 33.05% reported for corn (Boldol *et al*,. 2013). However, in fermented samples, the rate of decrease in scavenging ability of DPPH of FQPM extract (22.55 and 23.25% for 48 and 72 hours, respectively) is lower than 3.46 and 3.47% obtained for FNM at 48 and 72 hours, respectively (Figure 5).

Change in DPPH Scavenging Power of Methanolic Extract of the Flour Samples within 72 Hours

The effect of storage period on DPPH scavenging ability of methanolic extract of the

flour samples is shown on Figure 6. Unlike in the result of DPPH scavenging ability of the methanolic extracts of the flour showed an uncommon pattern; the scavenging ability of all the flour extract increased with increasing storage time. The scavenging ability at 48 hours is greater than at 24 hours and that of 72 hours is greater than that of 48 hours (Fig. 6).
At initial stage (24 hours of storage) the scavenging ability of the aqueous extract of the flour are higher than that of the methanolic extract but after storage when the aqueous extract lost their scavenging power the methanolic extracts increased in strength (Figures 5 and 6). The explanation for increase in DPPH scavenging ability of the methanolic extracts of the flour may be due to influence of organic solvent on DPPH radical. There is possibility that while water dissolve the extract readily in methanol medium, the extract's availability increased with storage period. This will enhance the activity of DPPH of which it reaction had been reported to take much longer time than ABTS and FRAP; extract reacted rapidly with ABTS (2 hours) or ferric ion (30 mins) than DPPH which is 24 hours (Thaipong *et al.,* 2006). DPPH scavenging ability of UFNM flour sample increased from 20.70 to 28.61 and to 35.29% from 24 to 48 and 72 the aqueous medium, hours, respectively. While UFQM flour sample increased from 17.13 to 25.97 and 34.02% at 24, to 48 and 72 hours, respectively (Figure 6). This result agree with the findings of Lopez-Amoros *et al*. (2006) that methanolic extracts of peas and beans undergo a significant increase in antioxidant activity after germination. In contrary, Bolbol *et al*. (2012) observed a reduction (30.11 to 26.40%) in DPPH scavenging ability of methanolic extract of corn from 12 hours to 48hour germination period.

Lipid Peroxidation

Two proxidants (iron (Fe) and sodium nitroprusside (SNP)) were used in this study to investigate the antioxidant potentials of the flour extracts to offer protective benefits to lipids subjected to oxidative assaults.

Inhibitory effects of the flour extracts on Fe^{2+}-induced cerebral lipid peroxidation

The inhibitory effects of aqueous and methanolic extracts of the flour blends on Fe^{2+} - induced cerebral lipid peroxidation is shown in Figure 7. Aqueous extract exhibited a significantly higher inhibitory effect than the methanolic extract (Figure 7).

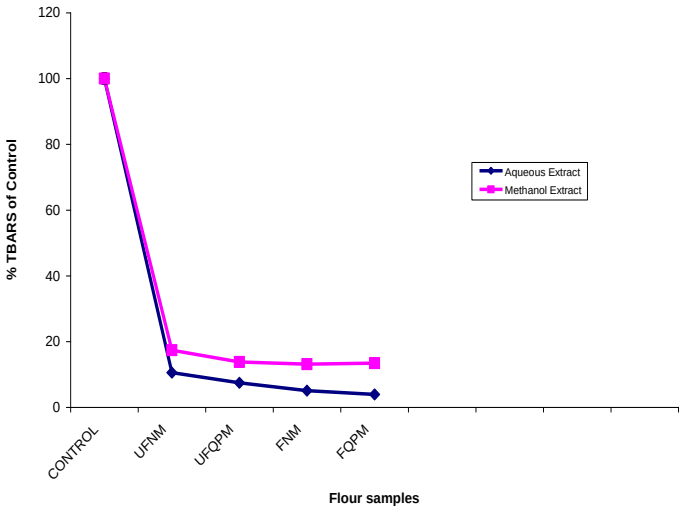

Figure 7: Inhibitory effects of aqueous and methanolic extracts of the flour samples on Fe^{2+} - induced cerebral lipid peroxidation

Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

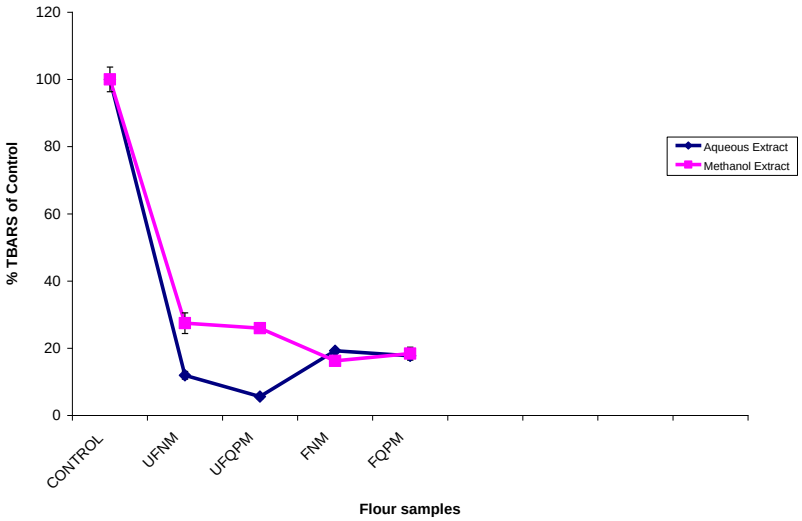

Figure 8: Inhibitory effects of aqueous and methanolic extracts of the flour samples on SNP - induced cerebral lipid peroxidation

Key: UFNM-Unfermented decorticated normal maize, UFQPM- Unfermented decorticated quality protein maize, FNM-Fermented normal maize, FQPM- Fermented quality protein Maize

The effect of UFNM on lipid peroxidation subjected to oxidative assaults induced by iron is 10.58% and 17.37% for aqueous and methanolic extracts, respectively. While that of UFQPM is 7.50% and 13.82% of the control for aqueous and methanolic extracts, respectively (Figure 7). This is to show that UFQPM flour has a higher inhibitory ability than UFNM extract. Similar trend was observed for fermented flour samples. Ogunmoyole *et al*. (2011) reported that ethanolic extracts showed a stronger inhibitory effect on peroxidation than the aqueous extracts. However, this development of aqueous extract showing a better inhibitory potential would be advantageous since maize flour is prepared with water before consumption. Fermentation favoured inhibitory potential of both aqueous and methanolic extract of the flour on Fe^{+2} induced brain assault (Figure 7). The values for fermented flour samples (5.08, 3.94% and 13.12, 13.44%; FNM, FQPM for aqueous and methanolic extracts, respectively) were farther to the control than the unfermented flour samples (Figure 6).

Inhibitory effects of the flour extracts on SNP-induced cerebral lipid peroxidation

The results on figure 8 show that when brain lipids were subjected to stress-induced peroxidation caused by SNP in the presence of the flour extracts, the extract exerted a significant inhibitory effect on the peroxidation processes. Following the same trend as in the case of Fe^{2+} induced brain assault (Figure 8), aqueous extracts exhibited a significantly higher inhibitory effect than the methanolic extract. The inhibitory effect of extract of UFNM on lipid peroxidation subjected to oxidative assaults induced by SNP is 11.95% and 27.46% for aqueous and methanolic extracts, respectively. While that of UFQPM is 5.62% and 25.95% of the control for aqueous and methanolic extracts, respectively (Figure 8). This shows that UFQPM flour has a higher inhibitory ability than UFNM extract. The result of methanolic extracts (27.46 and 25.95%) obtained in this study (Figure 8) is similar to earlier report of inhibitory effect for ethanolic extract of wall nut (Ogunmoyole *et al*., 2011).

4. CONCLUSIONS

The study established that flour of fermented complementary food could be produced from both normal maize and quality protein maize. It is obvious from the study that the flour is rich

in antioxidant capacity. Though, fermentation caused a reduction in some of the antioxidant capacity of the flour samples, a significant increased was established in total carotenoids and vitamin C contents of the flour as a result of fermentation. The increasing effect of fermentation on total carotenoids and vitamins C contents is a breakthrough considering the role of carotenoids in diet as in combating free radical induced degenerative diseases and ageing. Also, the reduction effect of fermentation on some antioxidant capacities is more pronounced in methanolic medium than aqueous medium, giving information on the design of processing method for optimum nutritional and health promoting usage in maintaining adequate diet to reduce the risk degenerative and ageing related diseases was also established.

5. REFERENCES

[1] Afify, A. M. R.; El-Beltagi, H. S.; Samiha, M. A. and Azza, A. O. (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, ß-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pacific Journal Tropical Biomedicine: 203-209

[2] Alexia, J. M. (2007): Effect of sprouting on nutrient composition and some anti-nutritional factor of sorghum and soybean. Journal of Food Chemistry, Vol. 101: 1202 – 2111.

[3] Anson, N.M., Van Den Berg, R., Havenaar, R., Bast, A. and Haenen, G.R.M.M. (2009): Bioavailability of ferulic acid is determined by its bioaccesbility. Journal of Cereal Science, 49, 296-300

[4] AOAC (2000) Analysis of the Association of Official Analytical Chemists (AOAC) International, William, H. (ed). 17th Ed., Gaithersburg, M. D, U.S.A.: Official Method. 923, 03, 923.05, 962.09, 979.09.

[5] Awika, J.M., Rooney, L.W., Wu, X., Prior, R.L., Cisneros-Zevallos, L., (2003). Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products. Journal of Agricultural and Food Chemistry 51, 6657–6662.

[6] Ayatse, J.O., Eka, O.U. and Ifon, E.T. (1983). Chemical evaluation of the effect of roasting on the nutritive value of maize. Food Chemistry, 12, 135–147.

[7] Bolbol R.R., Mohamed A.S. and Mohamed A.K. (2012). Changes In Total Phenolics and DPPH· Scavenging Activity During Domestic Processing In Some Cereal Grains. Annals. Food Science and Technology; 13, 2, 190 - 196

[8] Charalampopoulos, D. Wang , R. Randiella, S.I. and Webb, C. (2002). Application of cereals and cereal component in functional foods. A review. International Journal of Microbiology, 79,131-141.

[9] Ebrahimzadeh, M.A., Pourmorad, F., and Hafezi S. (2008). Antioxidant activities of Iranian corn silk. Turk Journal of Biology; 32: 43-49

[10] Elekofehinti O.O, Kamdem J.P., Kade, I.J Adanlawo I. G. and. Rocha J. B. T (2013). Saponins from solanum anguivi lam. Fruit exhibit in vitro and in vivo antioxidant activities in alloxan-induced oxidative stress. Asian Journal of Pharmaceutical and Clinical Research .Vol 6, Suppl 2,

[11] Elekofehinti, O. O and Kade, I. J (2012). Aqueous extract of Solanum anguivi Lam. fruits African Egg Plant) inhibit Fe2+ and SNP induced lipid peroxidation in Rat's brain – In Vitro. Der Pharmacia Lettre; 4 (5):1352-1359.

[12] Gliszczynska-Swiglo, A. (2006). Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferric reducing antioxidant power) assays. Food Chemistry, 96, 131–136.

[13] Gyamfi, M.A., Yonamine, M. and Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana: Thonningia sanguinea on experimentally-induced liver injuries. General Pharmacology, 32, 661–667.

[14] IASRI (2011). Quality Protein Maize for Food and Nutritional Security in India. Indian Agricultural Statistic Research Institute, Library Avenue, New dehli

[15] Ibukun O. E., Kade I. J. Oguntoyinbo T. A. Ogunmoyole T and Johnson O. D (2012). Effect of ripening on the phytochemical constituents and antioxidant properties of plantain (Musa paradisiacal). Journal of Medicinal Plants Research Vol. 6(37), 5077-5085.

[16] IITA (2011): Nutrition and quality of maize. Research Guide 33

[17] Jan Pokorny, Nedyalka Yanishlieva and Michael Gordon (2001): Antioxidants in food; Practical applications Woodhead Publishing Limited, Abington Hall, Abington Cambridge CB1 6AH, England (Edited by)

[18] Kumar, M.; Kumar, S.; Kaur, S. (2011): Investigations on DNA protective and antioxidant potential of chloroform and ethyl acetate fractions of Koelreuteria paniculata Laxm. African Journal of Pharmacy and Pharmacology; 5(3): 421-427.

[19] Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry, 91, 571–577.

[20] Nenadis N, Lan-Fen W, Tsimidou M, Zhang HY (2004). Journal of Agric and Food Chemistry; 52: 4669 - 4674.

[21] Obizoba, J.C. and Atti , J.V. 1991. Effect of soaking, sprouting fermentation and cooking on the nutrient composition and some antinutritional factor of sorghurm (Guinesia) seeds. Plant Food Hum. Nutr. 42: 13- 23.

[22] Oboh, G., Puntel, R.L. and Rocha, J.B.T. (2007). Hot pepper (Capsicum annuum, Tepin and Capsicum Chinese, Habanero) prevents Fe2 + - induced lipid peroxidation in brain: in vitro. Food Chemistry, 102, 178–185.

[23] Oladeji B.S (2014). Physico-chemical and Antioxidant Properties of Quality Protein Maize and Carrot Blends. PhD Thesis, Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Nigeria.

[24] Oladeji, B.S. Akanbi, C. T and. Ibironke, S. I (2014). Physico-chemical and nutritional evaluation of co-processed fermented yellow maize ogi (an infant diet) and carrot blends, Anal;Food Science and Technology ; Volume 15, Issue 1, pp 83-91

[25] Ohkawa H, Ohishi H, Yagi K (1979). Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal Biochemistry, 95: 351-358.

[26] Omololu P.A, Rocha J.B.T, Kade I.J (2011). Attachment of rhamnosyl glucoside on quercetin confers potent iron-chelating ability on its antioxidant properties Exp. Toxicology Pathology; 63(3):249-245.

[27] Oufnac, D. S. (2006): Determination of antioxidant capacity in corn germ, wheat germ and wheat bran using solvent and microwave-assisted solvent extraction, M.Sc. Thesis, Food Science Dept., Fac., Agric. And Mechanical., Louisiana State Univ., USA.

[28] Sallur G., Dans J., and Dezman, D., (1990). Rapid Extraction of Lycopene and β-carotene from reconstituted Tomato paste and Pink Grape Fruit Homogenates. Journal of Food Science. l.55 pp 1460

[29] Seema A. (2011): Health benefits of Corn. Organic facts. Available at www.organicfacts.net/health-benefits/cereal/health-benefits-of-corn.html

[30] Saxena, A. K., Chadha, M. and Sharma, S. (2003): Nutrients and antinutrients in chickpea (Cicer arietinum L.) cultivars after soaking and pressure cooking. Journal of Food Science and Technology, 40 (5): 493–497

[31] Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Cioalteau Reagent. Methods in Enzymology, 299, 152–178.

[32] Thaipong, K.Boonprakoba U, Crosbyb K Crosbyb, Luis Cisneros-Zevallosc, David Hawkins Byrnec (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19: 669–675

[33] Ogunmoyole T., Olalekan O.O., Fatai O., Makun J. O., Kade I.J..(2012). Antioxidant and phytochemical profile of aqueous and ethanolic extract of Garcinia kola. Journal of Pharmacognosy and Phytotherapy Vol. 4(5), pp. 66-74,

[34] Ogunmoyole T., Kade I. J. and Korodele B. (2011**).** In vitro antioxidant properties of aqueous and ethanolic extracts of walnut (Juglans regia)**.** Journal of Medicinal Plants Research Vol. 5(31), pp. 6839-6848