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FERMENTATION ATTENUATES THE FREE RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITIES OF SESAME SEED (*Sesanum indicum*).

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ABSTRACT

Antioxidants work as a major defense against radical-mediated damage by protecting the cells against free radicals, which have been implicated in many oxidative stress related diseases; hypertension, stroke, atherosclerosis, diabetes, cancer and the process of aging, among others. The present study evaluates the effect of traditional fermentation on the antioxidant potential of sesame seeds condiment by determining the total phenolic content, reducing properties, inhibition of lipid peroxidation, free radical scavenging ability against 1,1–diphenyl,2- picrylhydrazyl (DPPH) and vitamin C content, in the raw and fermented sesame seeds. The result showed that fermentation caused a reduction in level of total phenolic compounds of raw sesame seeds from 0.026mg/g to 0.01mg/g after day 7 of fermentation process, this trend was also observed for the level of vitamin C in the fermented sesame seeds with a reduction from 2.01mg/g to 0.93mg/g after fermentation period. The findings were also confirmed using various oxidative assault on hepatic and brain tissues, where ferric reducing antioxidant properties, iron chelation, inhibition of deoxyribose degradation, inhibition of lipid peroxidation in iron and sodium nitroprusside induced oxidative assault established that the raw sesame seeds exhibited better antioxidant ability than the fermented samples irrespective of fermentation days indicating that fermentation significantly reduced the antioxidant potentials of the fermented sesame seeds.

Key word: Fermentation, antioxidants activity, oxidative assault, sesame seeds, vitamin C, phenolic compounds.

INTRODUCTION

The importance of diet with natural source of essential components of a balance diet is assuming an unprecedented status. Foods from plant origin contains most of the useful and health benefiting factors such as antioxidants, minerals, and vitamins. Epidemiological research has shown a positive association between certain diseases and dietary intake of food rich in such essential components [1, 2]. One of the important components found in plant are the natural antioxidants which has the ability to scavenge free radical in the biological system. Antioxidants have been reported to

play an important role in enzymatic and non-enzymatic protection against oxidative stress-induced toxicity [3, 4].

There are evidence which clearly indicated that diets based on fruits and vegetables with a good amount of antioxidant properties contributes to reduced mortality from degenerative diseases such as cardiovascular, cerebrovascular, cancer, atherosclerosis and the process of aging, among others [5]. The phenolic compounds found in plants foods such as anthrocyanides, flavones and flavonols have an antioxidant capacity that is stronger than the vitamins C and E, particularly flavonols and flavones are found to possess antioxidant and free radical scavenging activities in foods [6].

The Sesame seeds (*Sesanum indicum*) are black, brown, or white, 2.5–3 mm long and approx 1.5 mm wide. In Nigeria the white type are commonly seen in the market with relatively cheap price. Local processing involves washing, cooking, fermenting and roasting; these processes generally lead to losses of some nutrients and anti- nutrients (anti nutritional factors). The method of processing and preparing food for human or animal consumption has implication on the nutritional quality of such food [1]. In Africa, the most popular method of preparing this seed is cooking or roasting. The processing of plant originated foods improves their taste and flavour thereby increasing their acceptability [7], but little is known about the effect this could have on the chemistry of these foods. Most foods when subjected to heating processes lose their quality and the usefulness of such item to posses important component necessary to combat and prevent disease conditions are reduced. It has been established by Nishant *et al*., (2003) [8]; Fereidoon *et al*., (2006) [9] that Sesame has a high antioxidant potential activity, free radical scavenging capacity, inhibition of low density lipoprotein (LDL) cholesterol and metal chelating capacity. Bor-Sen *et al*., (2007) [10] also did a similar study on the antioxidant properties of sesame on the incident of oxidative stress. This study is aimed at examining the effects of local preparation and processing methods on the antioxidant and nutritional value of sesame.

MATERIAL AND METHODS

Materials

Sesame seeds were obtained from Oja-oba in Akure, Ondo State, Nigeria in the month of October and identified at the Department of Crop Science and Pest Production, Federal University of Technology, Akure The chemicals used were of analytical grade, and glass distilled water was used.

Animals

Male adult Wistar rats (200–250 g) were used. The animals were used according to the standard guidelines of the Committee on Care and Use of Experimental Animal Resources.

Sample Preparation

The seeds of sesame were sorted to remove grit, dirt and decomposing seeds, the whole seeds were divided into two portions; one part for fermentation and the other part were used as unfermented (raw) sample. The fermented and the unfermented portions were milled respectively using magic blender (SHB-515 model made by sorex company limited, Seoul, Japan) to obtain very fine particles prior to analysis.

Production of fermented samples

A modified method of Omafuvbe et al., (2004) [11] was used. The seeds were rinsed with water and boiled for 6 hours to softness. The seeds thereafter were transferred into a pot, wrapped with banana leaves and allowed to ferment. The fermentation products were taken at the interval of 24 hours, 96 hours and 168 hours for analysis.

Sample Analysis

Determination of total phenol contents

The total phenol contents of the extracts were estimated by the modified method of Siddhuraju and Becker (2003) [12], briefly

(0 - 1.0ml) of each extracts was mixed with equal volume of water; 2.5 ml Folin-Ciocalteau's reagent and 2ml of 7.5% sodium carbonate were subsequently added, the absorbance was measured at 765nm after incubating at 45^0C for 40 min. The amount of phenols in the extracts was expressed as tannic acid equivalent (TAE).

Reducing property

The reducing properties of the two samples extracts were determined by assessing the ability of each extracts to reduce $FeCl_3$ solution as described by Pulido, *et al*., 2000 [13]. Briefly, samples extracts (0-250µl of stock) were mixed with 250µl, 200mM Sodium phosphate buffer (pH 6.6) and 250µl of 1% Potassium ferrocyanide, the mixture were incubated at 50^0C for 20 min, later, 250µl, of 10% trichloroacetic acid was added, and subsequently centrifuged at 650 rpm for 10 min, 1000µl of the supernatant was mixed with equal volume of water and 100µl of 0.1g/100 ml ferric chloride (w/v), the absorbance was measured at 700 nm, a higher absorbance indicates a higher reducing power.

Free radical scavenging ability

The free radical scavenging ability of the sample extracts against DPPH (1, 1 – diphenyl -2 picrylhydrazyl) were evaluated according to Gyamfi, *et al*., 1999[14]; 600µl of extracts (0-100µM) was mixed with 600µl, 0.3mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm.

Fe^{2+} Chelating assay

The Fe^{2+} chelating ability of the sample extracts were determined using a modified method described by Puntel *et al*. 2005[15]. Freshly prepared 500µmol/L $FeSO_4$ (150µL) was added to a reaction mixture containing 168µL of 0.1 mol/L Tris-HCl (pH 7.4), 218µL saline and extracts (0-100µM). The reaction mixture was incubated for 5 min, before the addition of 13µL of 0.25% 1, 10-phenanthroline (w/v). The absorbance was measured at 510nm. The Fe (II) chelating ability was calculated with respect to the reference (which contains all the reagents without sample extract.

Deoxyribose degradation

Deoxyribose degradation was determined according to the methods of Halliwell *et al*. (1987) . Deoxyribose is degraded by hydroxyl radicals with the release of thiobarbituric acid (TBA) reactive materials. Deoxyribose (6mM) was incubated at 37^0C for 30 min with 50mM potassium phosphate pH 7.4 plus Fe^{2+} (0.1mM) and/or H_2O_2 (1mM) to induce deoxyribose degradation, and extracts (0- 50µl of stock). After incubation, 0.8 ml of 2.8% TCA and 0.4 ml of 0.8% TBA were added, and the tubes were heated for 20 min at 100^0C and spectrophotometrically measured at 532 nm.

Lipid peroxidation

Rats were decapitated under mild ether anesthesia and the hepatic (liver) and brain tissues were rapidly dissected, placed on ice and weighed. Tissues were immediately homogenized in cold 50mM Tris–HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged for 10min at 4000g to yield a pellet that was discarded and a low-speed supernatant (S1). An aliquot of 100 ml of S1 was incubated for 1 h at 37^0C in the presence of extracts, with and without the prooxidants, iron (final concentration (10 mM)) and sodium nitroprusside (SNP) (final concentration 3 mM). This was then used for lipid peroxidation determination. Production of thiobarbituric acid reactive species (TBARS) was determined as described by Ohkawa *et al*. (1979) [16], except that the buffer of the color reaction has a pH of 3.4. The color reaction was developed by adding 300 ml 8.1% sodium dodecyl sulfate (SDS) to S1, followed by sequential addition of 500ml acetic acid/HCl (pH 3.4) and 500ml 0.8% thiobarbituric acid (TBA). This mixture was incubated at 95^0C for 1h. TBARS produced were measured at 532nm and the absorbance was compared to that of the controls.

Determination of Vitamin C

The vitamin C content of the samples was determined using Ascorbic acid as a standard compound prepared by dissolving 2mg/ml of ascorbic acid in water. 300µl of the sample preparation was mixed with 100µl of 13% TCA and 75µl of DNPH (dinitrophenylhydrazine), the resultant solution were incubated in a water bath at 37^{o}C for 3 hours, then 500µl of 65% H_2SO_4 was added. The absorption was read at 520nm (Benderitter *et al*., 1998) [17].

Result and Discussion

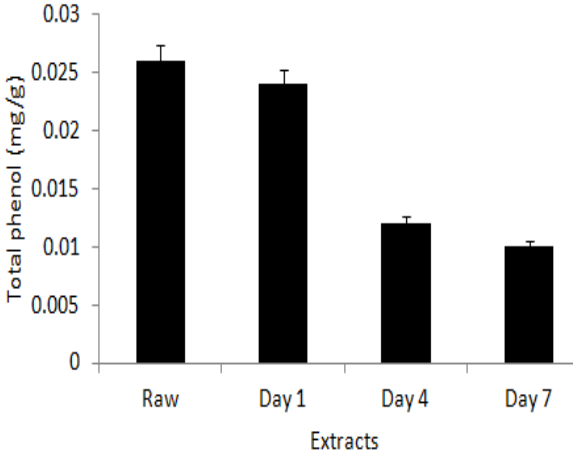

Fig. 1: Total phenollic levels of raw and fermented sesame. Values are expressed as mean±SD of 3 independent experiment performed in triplicate.

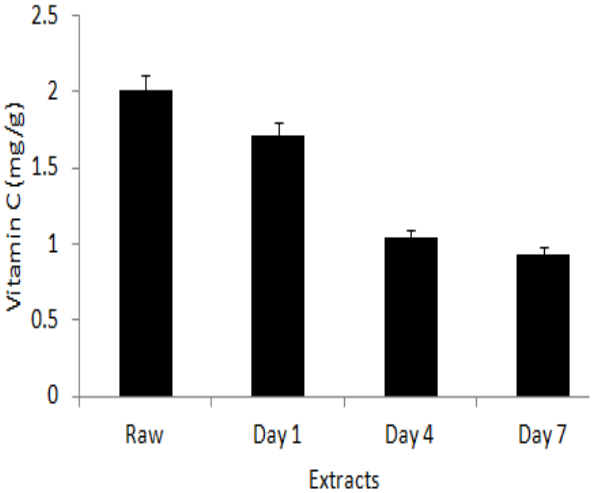

Fig. 2: Vitamin C levels of raw and fermented sesame. Values are expressed as mean±SD of 3 independent experiments performed in triplicate.

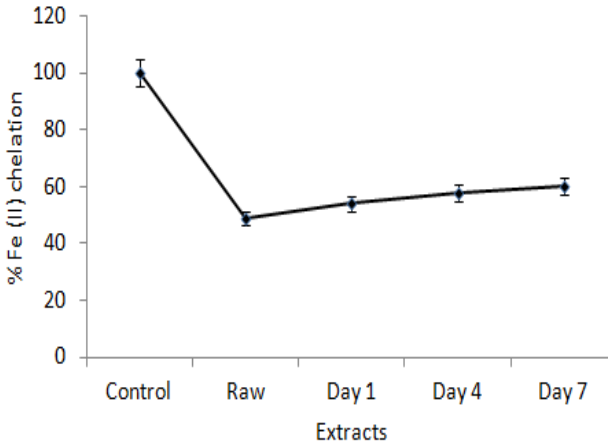

Fig. 3: The Fe (II) chelation ability of raw and fermented sesame, muskmelon and white melon. Values are expressed as mean±SD of 3 independent experiments performed in triplicate

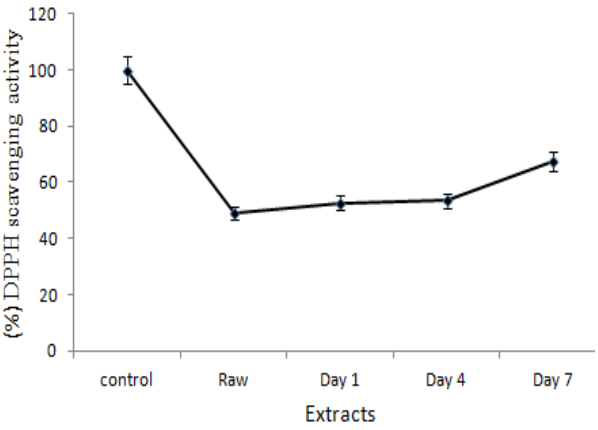

Fig. 4: DPPH free radical scavenging activity of the extracts of raw (unfermented) and fermented sesame seeds. Values are expressed as mean±SD of 3 independent experiment performed in triplicate. (DPPH: 1,1-diphenyl-2-picryl-hydrazyl free radical).

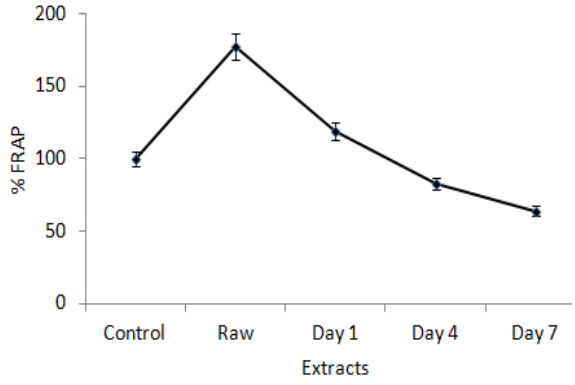

Fig. 5: Ferric reducing power (FRAP) of the extracts of raw (unfermented) and fermented Sesame seeds. Values are expressed as mean±SD of 3 independent experiments performed in triplicate.

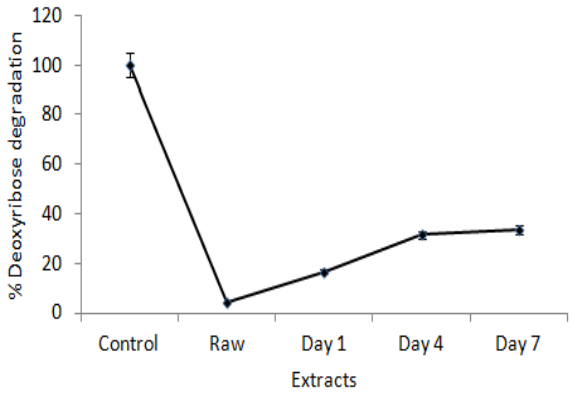

Fig. 6: The inhibition of deoxyribose degradation by raw (unfermented) and fermented sesame. Values are expressed as mean±SD of 3 independent experiments performed in triplicate.

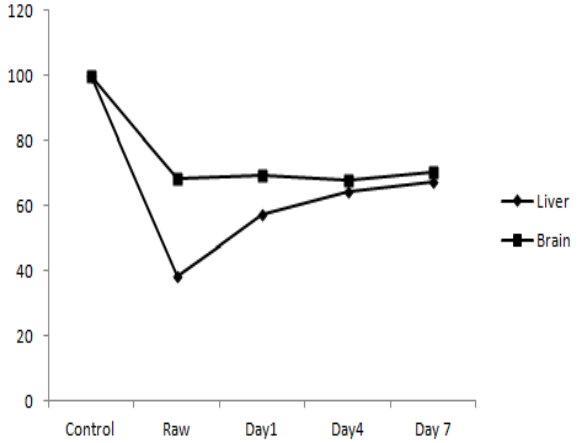

Fig. 7: Effect of the extracts of raw (unfermented) and fermented sesame seeds on hepatic and brain lipid peroxidation under Fe^{2+} oxidative assaults. Values are expressed as mean±SD of 3 independent experiment performed in triplicate.

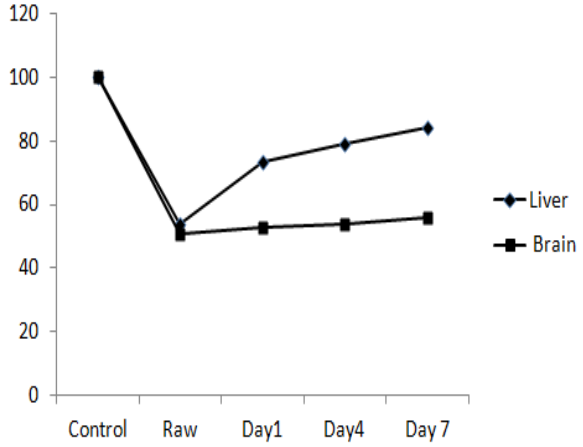

Fig. 8: Effect of the extracts of raw (unfermented) and fermented sesame seeds on hepatic and brain lipid peroxidation under SNP oxidative assaults. Values are expressed as mean±SD of 3 independent experiments performed in triplicate.

Discussion

Determination of the total phenolic contents of the raw (unfermented) and fermented extracts of sesame seed (Figure 1), showed that fermentation of the sesame seeds caused a reduction in the level of phenolics from (0.026mg/g) for the raw (unfermented) to 0.01mg/g after day 7 of the fermentation. This result falls in the range of phenolics found in commonly consuming fruits, vegetables and grains (Hertog *et al*., 1992) [18]. Hence, the consumption of raw seeds may be of beneficial effect than the fermented sesame seeds. Hertog *et al*., 1992 [18] and Wang *et al*., 1999 [19] reported that the antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, particularly the flavanoids, which are known to be potent antioxidants. There was also a reduction in the level of Vitamin C (fig.2), from 2.01mg/g(unfermented) to 0.93mg/g after day 7 of fermentation.

Vitamin C is important dietary antioxidants [20, 21]. Considering the 40mg daily recommended intake of ascorbic acid, consumption of unfermented sesame seeds may provide the day's requirement of vitamin C. There was significant difference ($p<0.05$) in vitamin C content among the days of fermentation.

Free radical scavenging ability

The effect of fermentation on the ferric reducing antioxidant properties of raw (unfermented) and fermented sesame seeds are shown in figure 3, the raw sesame seeds exhibit highest antioxidant reducing properties than the fermented seeds. It has been reported that the reducing power was associated with the antioxidant activity and its relationship with phenolic constituents have been well established in several plant sources including vegetables [12]. Reducing power is considered a defense mechanism which is related to the ability of the antioxidant agents to transfer electron or hydrogen atom to oxidants or free radicals [22]. The ferric reducing antioxidant

properties of the raw (unfermented) sesame seeds are significantly ($p<0.05$) greater than the fermented seeds.

Fe^{2+} chelating assay

The ability of the raw and fermented sesame to chelate and deactivate transition metals is shown in figure 4, fermented sesame do not have any significant transition metal (Fe) chelating ability irrespective of the days of fermentation compare to the raw seeds. Generally, the chelating ability is regarded as an antioxidant mechanism to prevent oxidative assault on biological macromolecules such as lipids, proteins and nucleic acids. Free iron is a potential enhancer of ROS formation as it leads to reduction of H_2O_2 and generation of the highly aggressive hydroxyl radical [23]. Due to the inefficiency of our endogenous defense systems as well as the existence of some physiopathological situations, such as; cigarette smoke, air pollutants, UV radiation, inflammation, ischaemia/reperfusion [24].

Deoxyribose degradation

The ability of raw (unfermented) and fermented sesame seeds *to* inhibit the OH generated during deoxyribose degradation are shown in figure 5. The inhibitory ability of the raw (unfermented) sesame seeds extract was significantly ($p<0.05$) higher than the ability of the fermented extracts different fermentation days. The hydroxyl radical (•OH) in the cells can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing •OH is very important for the protection of living systems [22]. The ability of the extracts to prevent assault on deoxyribose degradation by hydroxyl radical can be related in part to their antioxidant properties.

Lipid peroxidation

Figs. 7 and 8, show the results of iron and sodium nitroprusside induced oxidative assault on hepatic and brain tissues. The inhibition of lipid peroxidation by the extracts of raw (unfermented) and fermented sesame seeds were measured in male adult Wistar rats liver and brain homogenates in vitro; this was expressed as percentage inhibition of Malonialdehyde production. In both figures, the raw and fermented sesame seeds exerted inhibition of lipid peroxidation, it was however, observed that the raw sesame seeds exerted the most significant ($p< 0.05$) inhibition on the both cases of iron and sodium nitroprusside induced oxidative assault. Lipid peroxidation contains a series of free radical-mediated chain reaction processes and is also associated with several types of biological damage [25]. The role of free radicals and ROS is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis [26, 27].

Conclusion

A substantial literature documents the successful fermentation of plant materials for condiment production. Both microbial and biochemical changes involved therein have received much attention. From this present study, the fermentation of sesame seeds for condiment did not significantly improve the antioxidant status when compare to the raw (unfermented) sample. It could therefore be of more health benefiting to consume the raw sesame seeds than when fermented, since the fermented samples exhibited a lesser antioxidant potentials.

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