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Effect of ingestion of microwaved foods on serum anti-oxidant enzymes and vitamins of albino rats

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ABSTRACT

The effect of ingestion of microwaved foods on serum antioxidant enzymes and vitamins in albino rats was investigated. In the study, thirty two (32) male wistar albino rats were obtained and grouped into four groups (A, B, C and D) of eight animals each. The animals were acclimatized for 7 days on commercial rat feed. The animals in groups B, C and D were all fed *ad libitum* with porridge yam, porridge beans and jellof rice with meat/fish reheated for 2 min, 4 min and 6 min for groups B, C and D respectively for 42 days. Group A was fed with un-microwaved food and water for the duration of the study (42 days) and served as control. Antioxidant enzymes superoxide dismutase (SOD), Catalase (CAT) activities, vitamins A and E concentrations were determined using standard methods. Result obtained from the study showed that microwaved food consumption resulted in a significant ($P < 0.05$) decrease in SOD and CAT activity in rats fed with the microwaved food. Furthermore, antioxidant enzyme activity were more significantly ($P < 0.05$) reduced in rats exposed to food microwaved for 6 min compared to the control group (A). Also, serum vitamins A and E concentrations were significantly ($P < 0.05$) decreased in rats fed with food exposed to microwaves for 6 min as compared to the control group. Microwaves and increased microwaving time resulted to a significant reduction in SOD, CAT, vitamin A and E in fed rats. Therefore our study demonstrated that consumption of microwaved foods resulted in a significant decrease in antioxidant protection and may be implicated in the pathogenesis of oxidative stress and degenerative diseases.

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1. Introduction

The effects of microwave cooking or heating on the nutrient contents of foods have been a subject of research study. Many of the findings of most researchers show that, there are no significant difference between the nutrient contents of foods prepared by conventional method and that prepared by microwave, even though microwave cooking or reheating may give a higher nutrient

retention (Cross & Fung, 1982; Dahl & Matthews, 1980; Dahl, Matthews, & Marth, 1980; Hoffmann & Zabik, 1985; Klien, 1989). Based on the information available in literature about the nutrient contents of microwaved foods and the associated advantages of microwave cooking over conventional one (energy saving, time saving and convenience), many homes and commercial eateries have resorted to the use of microwave for cooking and reheating of foods (<http://www.health.harvard.edu/>, 2015; <http://www.medicaldaily.com/>, 2015).

However, notwithstanding the aforementioned benefits of microwave cooking, a lot of people still have lingering doubts about the safety of microwaved foods (Steven, 2014; Mike, 2015). It is also known that the alternating microwave electric current generated by the magnetron in every microwave oven forces the food

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molecules to rotate at the frequency of 1–100 billion times per second. The friction from this violent, thrashing motion tears at the food, vitamin and enzyme molecules, destroying, for instance, their cells' walls, while heating them savagely and changing their shape (Schrumph & Charley, 1985). Today, many health articles have shown that microwaved foods can change blood composition, change heart rate and contain carcinogens which have abilities of causing various types of cancer in the body (Willians, 2015; <http://www.medicaldaily.com/>, 2015). The works of Eke, Jibiri, Anusionwu, Orji, and Mbamala (2015), Elghazaly, Kamel, Radwan, Said, and Barakat (2014) and Raghuvanshi, Matheor, Sethi, Kumawat, and Choudhary (2013) showed that ingestion of microwaved food by Albino rats caused significant decrease in red blood cells, white blood cells, hemoglobin concentration and significant increase in neutrophils. With all these information, it becomes clear that microwaved foods alter biochemical parameters. Superoxide dismutase and catalase are important antioxidant enzymes found in nearly all living cells. Superoxide dismutase alternately catalyzes the dismutation of the superoxide radical produced during oxygen metabolism (O_2^-) into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2) (McCord & Fridovich, 1969, 1988). Catalase on the other hand, catalyzes the decomposition of hydrogen peroxide, which is a harmful by-product of normal metabolic processes to water and oxygen (Chelikani, Fita, & Loewen, 2004; Gaotani et al., 1996). Vitamin A and vitamin E are nutritional organic compounds found in living cells which have anti oxidative functions (Briglius-Flohe & Traber, 1999; Sen, Khanna, & Roy, 2007). These enzymes (superoxide dismutase and catalase) and vitamins (vitamin A and vitamin E) are known free radical scavengers in the body (Ananya, Vinaya, & Kavitha, 2013; Thamilselvan, Byer, Hackett, & Khan, 2000; Amani, Somchit, Konting, & Kok, 2010; <http://ods.od.nih.gov/>, 2015) which help to prevent cell damage and its associated ill health.

In this work therefore, the effect of ingestion of microwaved foods on these important radical scavengers was studied, as this will also go a long way in bringing more understanding to the changes that occur in the body as a result of ingestion of microwaved foods.

2. Materials and methods

2.1. Materials

Thirty two male albino rats (*Rattus norvegicus*) weighing between 180 and 200 g were purchased from rat breeders at Owerri, Nigeria. All animals were 10–11 weeks old and clinically normal on arrival. The animals were kept in a laboratory under controlled light and at the temperature range of 20–24 °C. The microwave oven used in this work was produced by DAEWOO; model KOG – 6C2B with an input power of 1200 W and output power of 800 W. Food items fed to the rats were porridge yam, porridge beans and jellof rice prepared with either meat or fish. The food items were reheated for 2 min, 4 min and 6 min with microwave oven, cooled, and then served to the rats.

3. Methods

3.1. Animal study

The procured albino rats were acclimatized for 7days while being maintained on commercial rat feed (not microwaved) and water. After acclimatization, the rats were grouped into four treatment groups and were housed in similar cages labeled A to D. Group A (rat group fed on foods not microwaved), Group B (rat group fed on foods microwaved for 2 min), Group C (rat group fed

on foods microwaved for 4 min) and Group D (rat group fed on foods microwaved for 6 min). Treatment groups B, C and D were fed with the same food item each day which only varied in time of reheating using microwave. Treatment group A also received the same food, but not microwaved, served to the other groups. The treatment groups were served one food item each day. Foods and water were made available for the different groups *ad libitum*. Throughout the course of the experiment, the animals were attended to in strict adherence to the NIH guide for care and use of laboratory animals. The feeding lasted for six weeks after acclimatization.

At the end of six weeks, animals were anesthetized using chloroform and their blood samples collected by cardiac puncture. The blood samples were stored in plane bottles for the subsequent biochemical analysis.

3.2. Biochemical analysis

The spectrophotometer (life-assistance scientific instrument company, model 721D, China) was used in the estimation of anti-oxidant enzymes and vitamins. The activities of serum superoxide dismutase enzyme was determined using the method described by Freidorich (1999). The ability of the superoxide dismutase to inhibit the autoxidation of adrenalin was the basis of the SOD assay. A 0.2 ml portion of sample was added to 2.5 ml of 0.05 M phosphate buffer (pH 7.8). The mixture was equilibrated in the spectrophotometer before adding adrenaline solution. The reaction started with the addition of 0.3 ml of freshly prepared adrenaline solution (0.059%) to the mixture followed by quick mixing by inversion in the cuvette. The cuvette therefore contained 2.5 ml buffer, 0.3 ml of adrenaline and 0.2 ml of sample. The increase in absorbance was taken at 480 nm for 150 s at 30 s interval. One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline. One unit of enzyme activity was expressed as unit per minute. Catalase activity was assayed by the method of Aebi (1974). A 0.1 ml portion of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by addition of 1.0 ml of freshly prepared 30 mM H_2O_2 . The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm using the equation for a first-order reaction. One unit (U) was defined as the amount of enzyme which decomposed 1 μ mol of H_2O_2 per min at 25 °C and pH 7.0. The serum vitamin A content was determined as described by Dugan, Frigerio, and Siebert (1964) method for the *in-vitro* determination of vitamin A levels in plasma using trifluoroacetic acid (TFA). This method involved the interaction of β -carotene with petroleum ether resulting in complete extraction of carotene and vitamin A which on reaction with the TFA reagent gave blue colour. The reagents used were ethanol (95%), petroleum ether (20%), antimony trichloride ($SbCl_3$), trifluoroacetic acid, acetic anhydride, potassium hydroxide, carotene standard solution and chloroform. Serum vitamin E content was determined by the method of Palan, Mikhail, Basin, and Romney (1973). The method involved the conversion of ferric ions to ferrous ions by α -tocopherol and the formation of red coloured complex with 2,2-dipyridyl. Absorbance of chromophore was measured at 520 nm in the spectrophotometer. The materials used were 2,2-dipyridyl solution (2%), ferric chloride solution (5%), 100 mg of α -tocopherol in 0.1% ethanol and n-butanol.

3.3. Statistical analysis

Means and standard deviations of all data collected for all groups, per parameter, were calculated and differences between means separated by one way ANOVA test, with the least significant

difference fixed at 0.05. All data analyses were done using the statistical software, Gen Stat for windows version, developed by VSN international.

4. Results and discussion

The result of the mean serum activity of the superoxide dismutase enzyme for all the different groups is shown in Fig. 1. The mean value of the superoxide dismutase activity for group A (group that received un-microwaved foods) was significantly higher ($P < 0.05$) than those of groups B, C and D that received different levels of microwaved foods. However, among the groups (B, C, D) that received microwaved foods, group D (group fed with food microwaved for 6 min) showed a significantly lower ($P < 0.05$) value of superoxide dismutase, indicating that serum superoxide dismutase activity decreased with increase in length of microwave heating.

Fig. 2 shows the mean serum activity of the catalase enzymes of the different groups A, B, C and D. The groups that received different levels of microwaved foods showed significantly lower activity when compared with that of group A that received un-microwaved foods. This also showed that, microwave heating caused significant reduction ($P < 0.05$) of serum catalase enzyme activity comparing the mean activity of group A (254.1 ± 7.04 u/mg) to those of groups B (237.6 ± 7.48 u/mg), C (223.4 ± 12.52 u/mg) and D (179.9 ± 13.3 u/mg).

Result of this study (Fig. 3 and Fig. 4) showed that microwaving of food caused a significant decrease in serum vitamin A and vitamin E of rats that consumed the microwaved foods. There was no significant variations ($P < 0.05$) in vitamin A and vitamin E concentrations in the groups B and C that consumed food microwaved for 2 min and 4 min respectively. Group D that consumed foods microwaved for 6 min had significant decrease in serum antioxidant vitamins A and E concentrations. Vitamin A and vitamin E are important lipid soluble antioxidants. Vitamin E is particularly very important in its protection of membranes from oxidation by reacting with lipid radicals produced in lipid peroxidation chain reaction (Traber & Atkinson, 2007). A reduction of vitamin E concentration as a result of ingestion of microwaved food could be detrimental to membrane integrity and cell health. γ -tocopherol is a nucleophile that reacts with electrophilic mutagens, while tocotrienole may protect neurons from damage. Conditions that result to their depletion are not advantageous to health.

It can be deduced from the above results that as the time of exposure of foods to microwave radiation increases, the activities of the serum antioxidant enzymes and concentrations of vitamins decrease. This could be as a result of formation of more free radicals in the foods at longer time of exposure of the foods to microwave

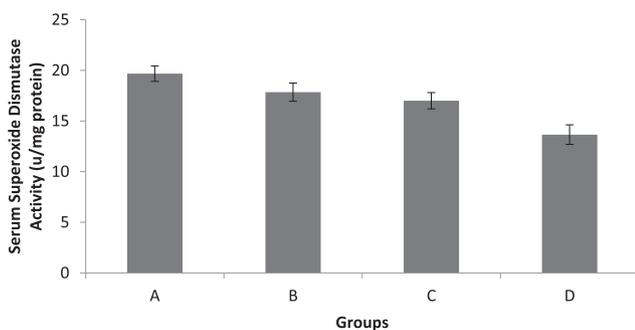


Fig. 1. Mean serum superoxide dismutase activity of the different groups. A (Group fed with un-microwaved food). B (Group fed with food microwaved for 2 min). C (Group fed with food microwaved for 4 min). D (Group fed with food microwaved for 6 min).

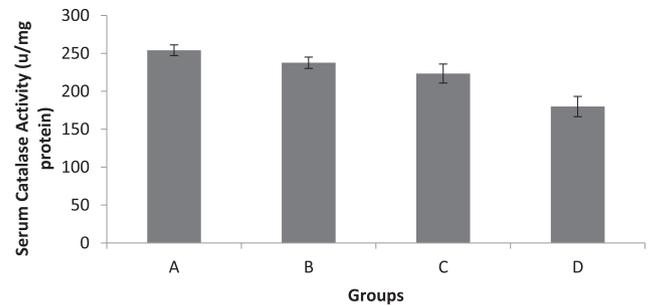


Fig. 2. Mean serum catalase activity of the different groups. A (Group fed with un-microwaved food). B (Group fed with food microwaved for 2 min). C (Group fed with food microwaved for 4 min). D (Group fed with food microwaved for 6 min).

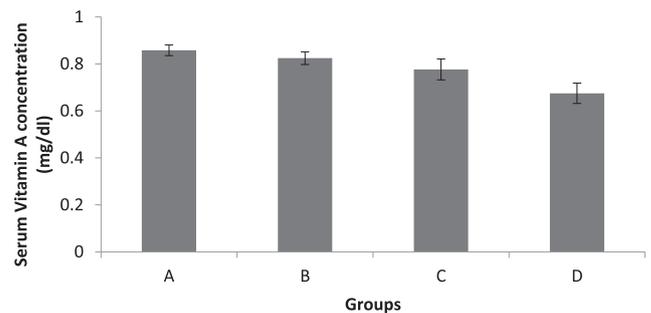


Fig. 3. Mean serum vitamin A concentration of the different groups. A (Group fed with un-microwaved food). B (Group fed with food microwaved for 2 min). C (Group fed with food microwaved for 4 min). D (Group fed with food microwaved for 6 min).

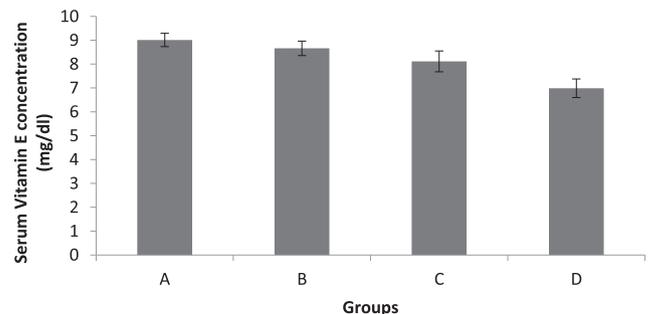


Fig. 4. Mean serum vitamin E concentration of the different groups. A (Group fed with un-microwaved food). B (Group fed with food microwaved for 2 min). C (Group fed with food microwaved for 4 min). D (Group fed with food microwaved for 6 min).

radiation which consequently required more of the serum antioxidant enzymes and vitamins to scavenge them, thus resulting to the lower concentrations of these antioxidants at longer time of microwave heating. This was supported by the work of Alam and Waheed (2012) which showed that increase in the ingestion of oxidized food samples led to decrease in serum radical scavenging activity of lipids extracted from liver, brain and muscles of rabbits.

5. Conclusion

Significant reduction of superoxide dismutase and catalase activities and vitamins A and E concentrations in the body as a result of microwave heating may lead to complete dismutation of superoxide radicals leading to increased possibility of cell damage, reduction of natural immunity of the body and good vision. Hence, exposure of food to longer time of microwave heating as well as

continual ingestion of microwaved food may therefore be detrimental to health.

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