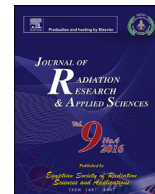


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Effect of ingestion of microwaved foods on serum anti-oxidant enzymes and vitamins of albino rats

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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 (Schrumf & 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

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