Peroxidation Study in Selected Foods Treated with Microwave Radiation

[a]Aweda M. A, [b]Osuntoki A. A.
[a]Department of Radiation Biology and Radiotherapy, College of Medicine/Lagos University Teaching Hospital, PMB 12003, Lagos, Nigeria.
[b]Department of Biochemistry, College of Medicine/Lagos University Teaching Hospital, PMB 12003, Lagos, Nigeria.
Corresponding author:
Aweda M. A
Department of Radiation Biology and Radiotherapy, College of Medicine/Lagos University Teaching Hospital, PMB 12003, Lagos, Nigeria.

Abstract

Production of peroxides in food samples treated with microwave radiation was investigated. Beans, Egusi-soup, Jollof-rice, Fish and Meat pie from popular eateries in Isolo Area of Lagos, Nigeria were exposure to microwave radiation for 5, 10 and 15 min while the unexposed samples served as control. Melondialdehyde, an index of lipid peroxidation was determined in both the exposed and control samples. The results indicated production of peroxides in the microwave-treated food samples with statistically significant higher level of melondialdehyde. The degree of peroxidation, and hence the extent of the negative impacts on endogenous antioxidant varied with duration of radiation exposure. Peroxidation was most pronounced in egusi (205.10 MDA mg/100g) exposed for 15 min while it was least in fish (6.10 MDA mg/100g) exposed for 5 min. Melondialdehyde being by-product of polyunsaturated fatty acid peroxidation and being genotoxic, reacts with DNA to form highly mutagenic adducts in cells. Regular and heavy consumption of microwave treated foods may expose the consumers to increased health risks, especially cardiovascular diseases, diabetes, astherosclerosis and cancers.

KEYWORDS: Microwave Radiation, Food, Antioxidants, Peroxidation, Health risks.

Introduction

Microwave radiation (MW) refers to the electromagnetic wave within the frequency range of 300 to 300,000 MHz. The radiation can pass through materials like glass, paper, plastic and ceramic, and can be absorbed by foods and water, but they are reflected by metals. Microwave oven as a domestic appliance, heats food based on dielectric property, resulting in rotation of the polar molecules in the food (Percy Spencer 1958). Although, the domestic MW oven is a convenient, fast, simple and cheap for food preparation, the safety of the foods so prepared has recently aroused public concern.

It has been established that exposure of biological materials cause lipid peroxidation (Aweda et al. 2003), a process in which free radicals dislodge electrons from the lipids in cell membranes. This process leads to a free radical chain reaction mechanism that results in oxidative deterioration of polyunsaturated lipids (Nielson, 1995). The reactions are initiated or enhanced by a number of toxic products including endoperoxides and aldehyes (Rosenblum et al. 1989). It affects polyunsaturated fatty acids because they contain multiple double bonds in

between which lie methylene (-CH_2-) groups that possess especially reactive hydrogen. Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occur in enzymatic or non-enzymatic reactions involving reactive oxygen species (ROS) which are responsible for the toxic effects and tissue damages. These ROS include among others hydroxyl radicals, lipid oxyl or peroxyl radicals, singlet oxygen, and peroxinitrite formed from nitrogen oxide (NO). In water, this process generates the most reactive species, hydroxyl radical (OH^*). Excessive production of ROS results in oxidative stress. In other words, i.e. an increased formation of oxygen-nitrogen derived radicals and reduced antioxidant capacity, thereby causing an imbalance that might result in the attack of cellular components, especially lipids. This has been implicated in the pathogenesis of various diseases including diabetes (Kesavulu et al. 2001), cancer (Cerutti, 1994), and atherosclerosis (Kesavulu et al. 2001, Halliwell, 1993).

In biological systems, oxidation of lipids causes damage to membranes, hormones and vitamins, which are vital components for the normal cell activity (Mc Brien and Slater, 1982). At the nutritional level, oxidation of fatty constituents is the major chemical factor in the loss of food wholesomeness by deterioration of flavour and aroma, as well as in decay of nutritional and food safety qualities (Eriksson, 1982). Recently, biological and nutritional aspects have merged; diets based on food containing peroxidized lipids have been related to far-reaching effects such as carcinogenesis, premature aging and other diseases (AICR, 1982).

Oxidation of lipids can occur in foods containing substantial amounts of fat, like milk and meat products, oils, nuts and also those that contain only minor amounts of lipids, such as vegetable products. Thus, aroma changes may result from new volatile odorous compounds formed flavour modifications caused by hydroxyl acids and the colour darkening as a result of condensation reaction between oxidation products and proteins. Thus, there may be potential harms of MW on food due to peroxides production and its pathogenesis.

As part of our series of recent studies on the safety of MW (Aweda et al., 2010a, 2010b, 2010c, Aweda et al. 2011a, 2011b, 2011c, Usikalu et al.), this work investigates the effects of MW treated food samples most frequently consumed obtained from some public eateries in Lagos, Nigeria. The results hopefully will be an indication of the potential health risks to regular consumers of MW treated foods.

Materials and methods

The five selected commonest food Samples were purchased from fast-food retail outlets centrally located and well patronized in Isolo area of Lagos State, Nigeria. These were Beans, Egusi-soup, Jollof-rice, Fish and Meat pie. Each sample type was divided into four portions. A portion served as control while the other three were exposed to MW for varying time durations of 5 to 15 min. The MW oven used was the model WP700L17, serial number 21200266 from Euroline Rheinland product safety Germany, operating at frequency of 2450 MHz with output power 700 W. After exposure to MW, each sample was subjected to peroxidation analysis. 2 g of each sample was weighed using electronic balance model WH 200-4, serial number 12927 from Wiggen

Hauster Analytical balance, Germany. 20 ml of 0.1 M phosphate buffer product of sigma from Germany, pH value 7.0 as determined using Chemcadet Jr. pH meter model 5982-20 from Cole Parmer instrument Chicago USA were used to extract samples by homogenization using mortar and pestle. The homogenate was centrifuged at 1500 revolution per minute (rpm) for 15 minutes using Power Spin ^{TM} LX table centrifuge, model C856E and serial number L0704012 from UNICO, China. 1 ml of the supernatant was treated with 3 ml of 10 % trichloroacetic acid solution (w/v) to precipitate protein and the precipitate removed by centrifugation at 3500 rpm for 10 min. 1 ml of the supernatant from the previous centrifugation was treated with 2 ml of 0.675 % (w/v) thiobarbituric acid (TBA) product of Sigma from Germany and boiled for 10 min in a water bath. A pink colour developed whose intensity depends on the amount of the Malondialdehyde (MDA) present in the sample. A blank containing 1 ml distilled water and 2 ml of TBA was equally prepared and treated in a similar manner. The absorbance of the sample and the blank were measured using a spectrophotometer model 2100, serial number A0703090, a product of UNICO, China, at 517 nm. Calculation of the lipid peroxidation was determined using the formula (Buege and Aust, 1978):

$$\text{MDA (µmole)} = \frac{A\,(nm) \times V_T \times 10^6 \times \text{Dilution factor}}{\square \times V_S}$$

where Σ = Molar extinction coefficient of MDA (= 1.56×10^5 $M^{-1}cm^{-1}$), A = Absorbance,
V_T = total volume of the sample and reagent and V_S = total volume of sample.

Results

After the exposure of the various food samples to MW as described above, both the samples and the control were analyzed and the level of (µmole) MDA produced determined by TBA method of Buege and Aust, 1978. Table 1 presents the values of peroxidation obtained in the studied food samples as it varies with the duration of MW exposure. Table 2 presents the percentage increase in peroxidation for each of the samples. Intercomparison of peroxidation status in one food sample with another are presented is presented in tables 3 to 12 for easy understanding of the relative level of health risk involved. Table 13 shows the column statistics of the peroxidation level (µmole) MDA in the studied food samples. Mean ± SD, MDA level Jollof-rice 45.73 ± 37.06, beans 75.52 ± 32.87, egusi-soup 126.7 ± 54.83, meat pie 31.73 ± 11.51, and fish 19.12 ± 21.37. Significant differences were determined, using Bonferroni multiple comparison test graph pad version 5.0, at p-value < 0.05. Significant differences were determined using the Bonferroni computer software for multiple comparisons graph pad prism 5.0 version.

Discussion

The results obtained in this study show that the level of peroxidation produced in Beans as shown in table 1, increased by 14.8 % after 5 min, by 39.0 % after 10 min and by 46.2 % after 15 min of MW exposure respectively. This shows a statistically significant increase in the level of MDA produced compared with control as the exposure time increases. In Egusi-soup, the peroxidation level produced increased by 14.3 % after 5 min, 20.3 % after 10 min and 65.4 % after 15 min exposure time respectively. This shows a statistically significant increase in the level of peroxidation produced as the exposure

time increases compared to control. In fish, the peroxidation increased by 4.4 % after 5 min, 21.9 % after 10 min and 73.7 % after 15 min exposure respectively. Thus showing a statistically significant difference in the level of the peroxidation produced as the exposure time increases compared with control. For Jollof rice, the peroxidation increased by 14.7 % after 5 min, 28.7 % after 10 min and 56.5 % after 15 min exposure respectively. The results show a statistically significant difference as the exposure time increases compared with control. In Meat pie, peroxidation increased by 10.2 % after 5 min, 21.7 % after 10 min and 68.1 % after 15 min exposure time respectively. This also shows a statistically significant difference in the level of peroxidation as the exposure time increases compared with control.

In all cases, the results show that the MDA level increases progressively as the exposure time increases and that there is statistically significant increase in MDA produced in MW treated food samples compared with control. The increase observed is highest in Egusi soup, which is principally made up of melon that is rich in polyunsaturated fatty acid. In addition, it has the highest fluid content compared to the other studied samples. The general observation is the statistically significant increase in the level of MDA in MW treated food samples compared with control. The increase in peroxidation shows a progressive percentage increase in the rate of lipid peroxidation as the exposure time increases (table 1). During MW exposures free radicals (OH*) and reactive oxygen species (ROS) are being generated from MW interactions. These products are highly reactive and polyunsaturated fatty acids are susceptible to their attacks which induce lipid peroxidation. And this reaction leads to formation of MDA (Del Rio et al., 2005), a well known toxic compound which also causes oxidative stress biological systems (MacNee, 2005). Statistical analysis of the results and comparison of MDA produced in the food samples showed 99 % non-significant difference in the levels of peroxidation formed. The comparison of MDA produced in Jollof rice with that of Beans in table 2 shows no statistically significant difference with p-value > 0.05. The comparison of the level of MDA produced in Jollof rice with that of Egusi-soup in table 3 also showed no statistically significant difference as p-value > 0.05. The comparison of the level of MDA produced in Jollof rice with that of Meat pie in table 4 showed no statistically significant difference, the p-value being > 0.05. The comparison of the level of peroxidation produced in Jollof rice with that of Fish in table 5 shows no statistically significant difference since the p-value > 0.05. In comparing the level of MDA produced in the Beans with that of Egusi-soup in table 6, there is no statistically significant difference with p-value > 0.05. The comparison of the level of MDA produced in Beans with that of Meat pie in table 7 as well showed no statistically significant difference as the p-value > 0.05. The comparison of the level of MDA produced in Beans with that of Fish in table 8 shows no statistically significant difference and the p-value > 0.05. The comparison of the level of MDA produced in the Egusi-soup with that of Meat pie in table 9 showed no statistically significant difference as compared since the p-value > 0.05. The comparison of the level of MDA produced in the Egusi-soup with that of Fish shows no statistically significant difference compared with the control for 5 and 10 min exposure times, but there is statistically significant difference in the level MDA

produced in the two samples at 15 min exposure time as shown in table 10. The comparison of the level of peroxidation produced in the Meat pie with that of Fish in table 11 showed no statistically significant difference as compared since the p-value > 0.05. However, there was a statistically significant increase in the level of MDA produced in the MW food samples compared to control. The study also reveals some correlation between the rate of MDA production with the duration of food exposure to MW and fluid content of the food samples. These results and those available in the literatures are in full agreed with Esterbauer, 1993, and Mc Brien and Slater, 1982, who found that the ingestion of foods containing lipid peroxidation products increases the risk of cardiovascular diseases, diabetes, astherosclerosis, and cancer. Hence oxidative deterioration of polyunsaturated lipids could be detrimental to the biological tissues.

Minerals are generally not destroyed during normal cooking processes, including MW cooking. However, there might be losses in cooking water or meat drippings. Nevertheless, a study comparing MW and conventional braised beef showed that significantly more phosphorus and potassium were retained in MW cooking (Hill and Ilsi, 1998). On the contrary, during MW exposure of food, free radicals and reactive oxygen species are generated, and as it has also been demonstrated in this study. These radicals and ROS are highly reactive, and polyunsaturated fatty acids are susceptible to their attacks which induce lipid peroxidation MDA, a known highly toxic aldehyde that reacts with DNA to form highly mutagenic adducts in human cells. MDA is the major and perhaps the most studied toxic by-product of polyunsaturated fatty acid peroxidation (Del-Rio et al., 2005). Being genotoxic, it reacts with DNA to form highly mutagenic adducts in human cells (Del-Rio et al., 2005; Cline et al., 2004; Riggins and Marnett, 2001, Imlay, 2003). Lipid peroxidation is a major problem in the food industry. It leads to quality deterioration, rancidity and accumulation of potentially toxic compounds in foods (Gorelik et al., 2008; Paniangvait et al., 1995; Ahn et al., 1993; Ladikos and Lougovois, 1990). The extent of lipid peroxidation produced in MW treated foods is highly significant in the pathogenicity and toxicology associated with oxidative stress. Therefore, the findings from this study show how oxidative injury is derived from lipid peroxidation, likewise lipid peroxidation participates in the pathogenesis of many diseases that afflict millions of unsuspecting members of the public

Microwave radiation treated foods contain free radicals produced as a result of the radiation interactions with the food materials. Lipid peroxidation is a free-radical-mediated chain of reactions that once initiated, results in an oxidative deterioration of polyunsaturated lipids. The most common targets are components of biological membranes. When propagated in biological membranes, these reactions can be initiated or enhanced by a number of toxic products, including endoperoxides and aldehydes.

Since peroxidation of lipid is associated with MDA accumulation, it is important to ascertain the MDA contents of the varieties of food products sold in eateries and likewise prepared at homes with microwave oven. The results obtained from this study provide indication as to the safety of the MW treated foods, a question that has aroused some public concerns. The results from this study

provide strong indication that the longer the time of irradiation and the higher the fluid content of the food, the higher the amount of peroxidation produced. In view of the toxic effects of MDA and its implications on the health of the consumers, this study underscores the need for public enlightenment on the domestic and industrial uses of microwave oven to cook and thaw foods. Campaigns through media to let the public know about the potential health hazard associated with consumption of MW treated foods both in eateries and at their individual homes will go a long way to reduce the health risks associated with significant and/or regular consumption.

References

1. Aweda M. A. Gbenebitse S. O. and Meindinyo R. O. (2003). Effects of 2.45 GHz Microwave exposures on the peroxidation status in Wistar rats. Nig. Postgrad. Med. Journ. 10(4), pp. 243-246.

2. Nielson Suzanne S. (1995), Introduction to chemical analysis of foods. 1st edition Aspen publishers. USA.

3. Rosenblum E. R., Gavaler J. S. and Van Thiel D. H. (1989), Free Radical Biology and Medicine. 7(5), pp. 569-577.

4. Kesavulu M. N., Rao B. K., Giri R., Vijaya J., Subramanyam G. and Apparao C. (2001), Lipid peroxidation and antioxidant enzyme status in Type II diabetics with coronary heart disease Diabetes Research and clinical practice. 53(1), pp. 33-39.

5. Cerutti P. A. (1994) Oxy-radical and cancers. The Lancet, 344 (8926), pp. 862-863.

6. Halliwell B. (1993), free radicals and vascular diseases: how much do we know? British Medical journal. 307, pp. 885.

7. Mc Brien D. C. H. and Slater T. F. (1982), Free Radicals, Lipid Peroxidation and Cancer, Academic Press Incorporated., New York

8. Eriksson C. E. (1982), Oxidation of lipids. Journal of food chemistry 9, pp. 3-20.

9. American Institute for Cancer Research (1982), Diet, Nutrition and Cancer. National Academy Press, Washington, D.C.

10. Buege J. A. and Aust S. D. (1978) Microsomal lipid peroxidation. In methods of enzymology. Fleischer S., Packer L. (editors), Academic press New York, pp. 302-310.

11. Aweda M. A., Gbenebitse S. and Meindinyo R. O. (2010a). Microwave radiation exposures affect the LDL, HDL TCL and TRG status in rats. Int'l J Phys Sci 5(7), pp. 1015-1022.

12. Aweda M. A., Usikalu R. M., Wan J. H., Ding N., Zhu J. Y. (2010b). Genotoxic effects of low 2.45 GHz microwave radiation exposures on Sprague Dawley rats. Int'l Journ. Genetics and Mol. Biol. 2(8), pp. 176 - 181.

13. Aweda M. A., Usikalu M. R., Adeyemi O. O. and Yemitan O. K. (2010c). Anxiolytic and explorative behavioral effects of low SAR microwave radiation exposures on *Sprague Dawley* rats. Archives of Applied Science Research. 2(6), pp. 37-46.

14. Aweda M. A., Usikalu R. M., Awobajo F. O. (2011a). Effects of

exposure to 2.45 GHz microwave radiation on male rat reproductive system. Int'l. Journ. Current Research. 2(1), pp. 120-128.

15. Aweda M. A., Meindinyo R. O. K., Gbenebitse S. O. and Ibitoye A. Z. (2011b). Microwave radiation exposures affect cardiovascular system and antioxidants modify the effects**.** Advances in Applied Science Research. 2(2), pp. 246-251.

16. Aweda M. A., Meindinyo R. O. K., and Ibitoye A. Z. (2011c). Microwave radiation exposures affect hematological parameters and antioxidants modify the effects in rats. Int'l. J. Current Research. 3(4), pp. 140-145

17. Usikalu M. R., Aweda M. A. Babatunde E. B., Awobajo F. O. (2010). Low level microwave exposure decreases the number of male germ cells and affect vital organs of Sprague Dawley rats. Am. J. Sci. Ind. Res., 1(3), pp. 410-420.

18. Del-Rio D., Stewart A. J. and Pellegrini N. (2005), A review of recent studies on malondialdehyde as toxic molecule and biological markers of oxidative stress. Nutrition Metabolism Cardiovascular Disease. Elsevier publishers. 15(4), pp. 316-328.

19. MacNee W. (2005), Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. Proceedings of the American thoracic society. 2, pp. 50-60

20. Esterbauer H. (1993), Cytotoxicity and genotoxicity of lipid oxidation products. American Journal of Clinical Nutrition. 57, pp. 7795-7855.

21. Hill Allison and Ilsi Europe (1998), Microwave Ovens. Europe Microwave Oven Task Force. Ilsi press Brussels Belgium.

22. Cline S. D., Riggins J. N., Tornaletti S., Marnett L. J. and Hanawalt P. C. (2004), Malondialdehyde adducts in DNA arrest transcription by T7 RNA polymerase and mammalian RNA polymerase II. Proceedings of National Academic Science. 101, pp. 7275-7280.

23. Riggins J. N. and Marnett L. J. (2001). Mutagenicity of the malondialdehyde oligomerization products 2-(3-oxo-1 propenyl)-malondialdehyde and 2, 4-dihydroxymethylene -3- (2,2 – dimethoxyethly) glutaraldelyde in Salmonella. Mutation Research. 497, pp. 153-157.

24. Imlay J. A. (2003). Pathways of oxidative damage. Annu Rev. Microbiology. 57, pp. 395-418

25. Gorelik S., Ligumsky M., Kohen R. and Kanner J. (2008), A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. Federation of American Societies for Experimental Biology Journal. 22, pp. 41-46.

26. Paniangvait P., King A. J., Jones A. D. and German B. G. (1995), Cholesterol oxides in foods of animal origin. Journal of Food Science. 60, pp. 1159-1174.

27. Ladikos D. and Lougovois V. (1990), Lipid oxidation in muscle foods: A Review Food Chemistry.Elsevier publishers. 35, pp. 295-314.

28. Ahn D. U., Wolfe F. U., Sim J. S., and Kim D. H. (1993), Packaging cooked turkey meat patties while hot reduces lipid peroxidation. Journal of Food Science. 57, pp. 1075-1078.

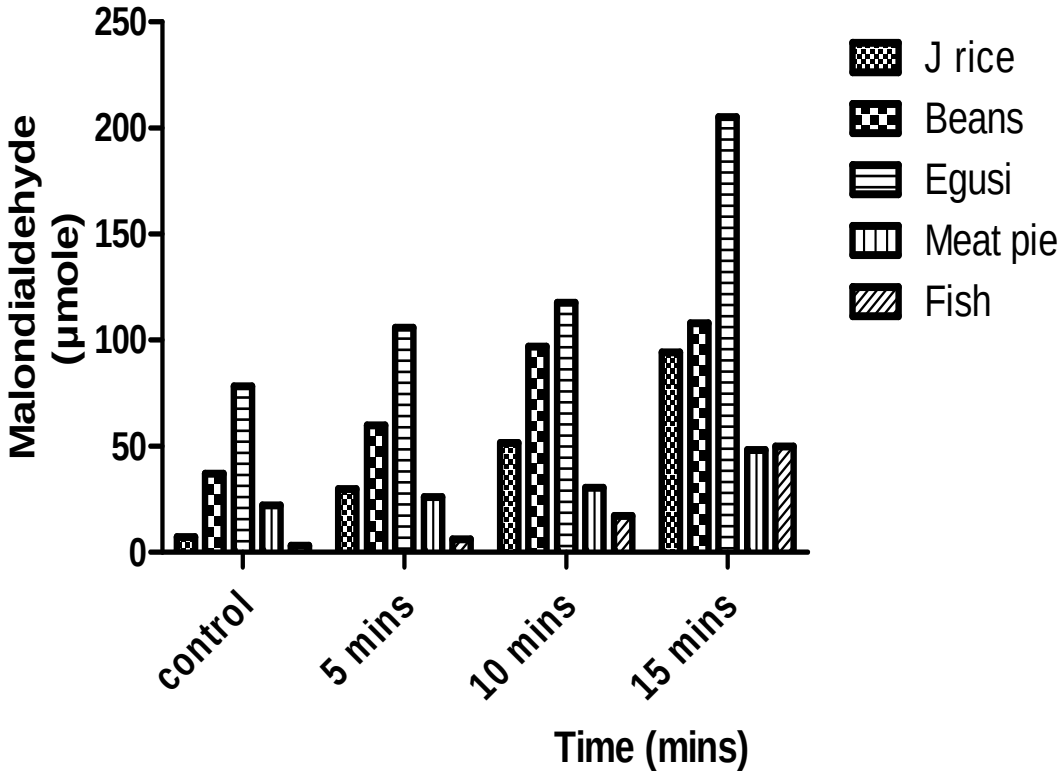

Figure 1: Bar Charts showing the statistical analysis of MDA (µmole) variation in the studied food samples with exposure time.

Table 1: Peroxidation (MDA mg/100g) variation produced in food samples with time.

Food Sample	Control	Exposure Time		
		5 min	10 min	15 min
Beans	37.1381	59.8644 (14.8%)	97.0025 (39.0%)	108.0885 (46.2%)
Egusi-soup	78.1563	105.8713 (14.3%)	117.5116 (20.3%)	205.0910 (65.4%)
Fish	3.3258	6.0973 (4.4%)	17.1833 (21.9%)	49.8870 (73.7%)
Jollof-rice	7.2059	29.9322 (14.7%)	51.5499 (28.7%)	94.2310 (56.5%)
Meat pie	22.1720	26.0521 (10.2%)	30.4865 (21.7%)	48.2241 (68.1%)

NB: Fig in brackets is the percentage increase in peroxidation in the food sample.

Table 2: Comparison of peroxidation produced in Jollof rice with that of Beans.

	Jollof rice	Beans	Difference	p-value
Control	7.15	22.07	14.92	0.21
5 min	29.92	44.88	14.96	0.36
10 min	51.52	74.26	22.74	0.07
15 min	94.21	101.10	6.94	0.07

Table 3: Comparison of the level of peroxidation produced in Jollof rice with that of Egusi-soup.

	Jollof rice	Egusi-soup	Difference	p-value
Control	7.15	78.15	71.00	0.07
5 min	29.92	105.80	75.92	0.05
10 min	51.52	117.50	65.99	0.10
15 min	94.21	205.10	110.90	0.06

Table 4: Comparison of peroxidation produced in Jollof rice with that of Meat pie.

	Jollof rice	Meat pie	Difference	p-value
Control	7.15	50.15	42.99	0.07
5 min	29.92	65.93	36.00	0.05
10 min	51.52	73.81	22.29	0.07
15 min	94.21	126.60	32.41	0.05

Table 5: Comparison of peroxidation produced in Jollof rice with that of Fish.

	Jollof rice	Fish	Difference	p-value
Control	7.15	3.41	-3.74	0.72
5 min	29.92	6.10	-23.82	0.13
10 min	51.52	17.17	-34.36	0.08
15 min	94.21	49.78	-44.43	0.07

Table 6: Comparison of peroxidation produced in Beans with that of Egusi-soup.

	Beans	Egusi soup	Difference	p-value
Control	22.07	78.15	56.07	0.06
5 min	44.88	105.80	60.96	0.08
10 min	74.26	117.50	43.25	1.01
15 min	101.10	205.10	103.90	0.12

Table 7: Comparison of peroxidation produced in Beans with that of Meat pie.

	Beans	Meat pie	Difference	p-value
Control	22.07	50.15	28.07	0.62
5 min	44.88	65.93	21.04	0.13
10 min	74.26	73.81	-0.45	0.14
15 min	101.10	126.60	25.47	0.07

Table 8: Comparison of the level of peroxidation produced in the Beans with that of Fish.

	Beans	Fish	Difference	p-value
Control	22.07	3.41	-18.66	0.06
5 min	44.88	6.10	-38.78	0.06
10 min	74.26	17.17	-57.09	0.08
15 min	101.10	49.78	-51.37	0.12

Table 9: Comparison of peroxidation produced in Egusi-soup with that of Meat pie.

	Egusi soup	Meat pie	Difference	p-value
Control	78.15	50.15	-28.00	0.07
5 min	105.80	65.93	-39.92	0.06
10 min	117.50	73.81	-43.70	0.10
15 min	205.10	126.60	-78.45	0.12

Table 10: Comparison of peroxidation produced in Egusi-soup with that of Fish.

	Egusi-soup	Fish	Difference	p-value
Control	78.15	3.41	-74.74	0.07
5 min	105.80	6.10	-99.75	0.07
10 min	117.50	17.17	-100.30	0.51
15 min	205.10	49.78	-155.30	0.01

Table 11: Comparison of peroxidation produced in Meat pie with that of Fish.

	Meat pie	Fish	Difference	p-value
Control	50.15	3.41	-46.73	0.11
5 min	65.93	6.10	-59.83	0.07
10 min	73.81	17.17	-56.65	0.06
15 min	126.60	49.78	-76.84	0.05