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Short-Term Consumption of Freeze-Thawed Microwaved Food Impaired Blood Electrolytes and Lipids In Female Rats

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ABSTRACT

Background and Objective: Dual application of freezing and microwave heating is the most common form of food processing for food with a pleasant taste and texture in commercial and domestic settings. The effect of these processing methods on food was usually investigated in male laboratory animals, while such effects on female rats are sparse data. This study was poised to investigate the effects of various processing methods of foods on the cardiovascular risk and oxidative parameters in female Wistar rats. **Materials and Methods:** Four groups of female rats were employed in the study. Groups 1 to 4 were fed standard rat-chow (CG), freeze-thawed rat-chow (FT), microwaved rat-chow (MO) and freeze-microwaved rat-chow (FM), respectively, for 6 weeks. **Results:** The study showed that the plasma HDL-C was not altered ($p>0.05$) compared with the control group, however, the plasma cholesterol, triglycerides, LDL-C and VLDL-C of the rats fed the experimental rat chows were significantly elevated ($p<0.05$) than the CG group. In addition, the experimental foods altered the natremia and oxidative parameters, however, no significant ($p>0.05$) lipid oxidation was recorded. **Conclusion:** This study demonstrated that short-term consumption of freeze-microwaved food might not have a pronounced effect on oxidative parameters. However, freeze-microwaved food is safer than consumption of either freeze-thawed or microwaved foods, however, there is a need to investigate the long-term effect of this claim.

KEYWORDS

Atherogenicity, female, freeze-thawed, freeze-microwaved, hypernatremia, oxidative-stress

INTRODUCTION

Microwave is one of the most explored frequency-based electromagnetic energy-generating technologies, which has become indispensable in every human endeavour[1] with telecommunication and medical industries' applications. The advent and incorporation of microwave technology into agricultural, food and beverages industries are evolutionarily dynamic[1,2]. Its application in heating foods and food products preservation and sometimes food processing such as baking, drying, pasteurisation, thawing, tempering and sterilisation are enormous and highly rewarding due to its convenience, efficiency and prudence.

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Amid overwhelming popularity and acceptability, scepticism persists with microwaved oven usage and its adverse health effect being a severe concern[3,4]. There are claims that extreme direct exposure to microwave radiation is lethal[4]. However, most such reports are associated with job hazards, primarily via telecommunications devices. The recent advancements seem to be aggressively mediating its effect to be more eco-friendly[5]. Also, microwaved foods are rarely consumed without an initial application or introduction of such materials to other food preservation or processing methods, especially freezing and conventional heating and heat radiation[6-9].

The increased trend in metabolic disorders, cardiovascular and other atherogenic conditions and stress-induced ageing is gaining unprecedented attention from public health organisations and their affiliates[7,8]. Several factors, like oxidative stress indices, remain the major causes either from a sedentary lifestyle, hereditary and epigenetic mechanisms and consumption of foods that enhance free-radicals generation, thus, microwaved oven-heated foods are not exempted[8,9].

To date, microwaved food consumption is sparingly evaluated as a co-application processing method, especially in female subjects. There is a shortage of information on the comparative effect of microwaved foods with freeze-thawed food-one of the most combined foods preserving and heating methods. This study aimed to evaluate the relative and co-application of freeze-thawing and microwave heating effects on the lipids and oxidative indices in female albino rats fed food exposed to these preserving and heating processes.

MATERIALS AND METHODS

Study area and duration: This study was conducted at the Biochemistry Laboratory, Centre for Chemical and Biochemical Research, Redeemer's University, Ede, Osun State, Nigeria, in August, 2021 and spanned till February, 2022.

Research protocol: Twenty female albino Wistar rats were obtained from the Physiology Department, University of Ibadan, Nigeria and housed in the Redeemer's University Facility to sacclimatise for 10 days. They were fed standard rat chow and accessed clean tap water during acclimatisation.

The standard rat chow was subjected to either freeze-thawing, microwave-heating or both but skipped the thawing phase. The freezing temperature was -20°C for 24 hrs and it thawed at room temperature. The thawing lasted until the feed temperature returned to average room temperature. The microwave heating was applied on the feed for 2.5 min/100 g, while freeze-microwave was done by freezing for 24 hrs and then microwaved for (auto-reheat) 2.5 min. These feed-processing methods gave rise to three feed categories and a control group as follows:

- **Control (CG):** Normal rat-chow
- **Freeze-thawing (FT):** Normal rat-chow+-20°C freezing (12 hrs)+thawing
- **Microwave-heating (MO):** Microwaved (2.5 min)/100 g rat chow
- **Freeze+microwave (FM):** -20°C freezing (12 hrs)+microwaved (auto-reheat) 2.5 min

The rats had access to their feeds and water *ad libitum* and 12 hrs of light and darkness and the feed administration lasted for 6 weeks.

Rats were fasted overnight and sacrificed by the cervical dislocation method[10], after which blood was collected via cardiac puncture into citrate and heparin bottles for different analyses. The blood collected was centrifuged at 4000 rpm for 5 min and plasma was collected for biochemical studies. All animal care and handling complied with the Redeemer's University Research and Animal Management Committee guidelines and the Guide for the Care and Use of Laboratory Animals, 8th edition[11].

Plasma cholesterol, High-Density Lipoprotein Cholesterol (HDL-C) and plasma triglycerides (TAG) were determined using the enzymatic method using Randox® Kits and the procedure followed was according to the manufacturer's manual. Low-Density Lipoprotein Cholesterol (LDL-C) and Very-Low-density Lipoprotein (VLDL-C) were obtained by deduction using the Friedewald equation. The atherogenic index (AI) was also calculated[12]. The catalase (CAT) and superoxide dismutase (SOD) activities were assayed according to the Sinha[13] and Misra and Fridovich[14,15] protocols, respectively. The procedure was followed by Beutler and Kelly[16] to estimate the plasma reduced-glutathione (GSH) concentration.

Statistical analysis: All data were computed in SPSS version 23.0 and analysed using a One-way Analysis of Variance (ANOVA). The Duncan's Multiple Range Test assessed the significance level at $p<0.05$.

RESULTS

Figure 1 showed the plasma lipid profiles of rats fed processed rat-chows in single and co-application methods of freeze-thawing and microwave. The total plasma cholesterol level in rats fed different processed feed (experimental groups) was significantly higher ($p<0.05$) than in the control group, a similar trend was recorded in the plasma LDL-C of the rats. Although the plasma HDL-C of the experimental and control groups was not significantly altered ($p>0.05$), however, the plasma triglycerides and VLDL in the group fed freeze-thawed rat-chow were significantly higher ($p<0.05$) than in the control group.

The plasma electrolytes (sodium, potassium and calcium) was shown in Fig. 2. The plasma calcium of the experimental groups (FT, MO and FM) was not significantly different ($p>0.05$) from the control group. Also, the plasma potassium of the FT, MO and MF was not significantly different ($p>0.05$) from the control

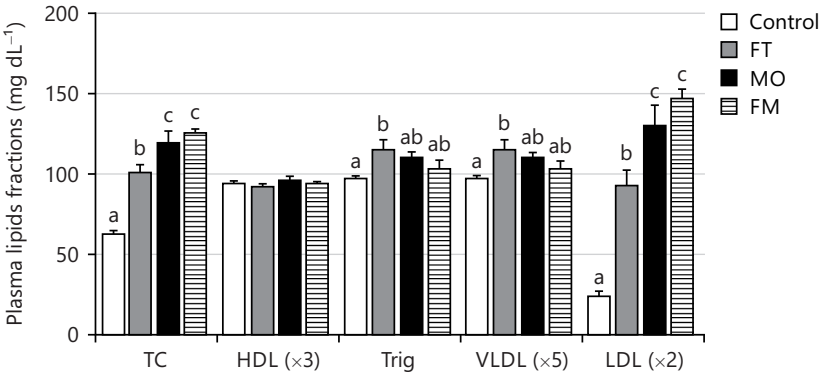

Fig. 1: Lipid profile of rats fed FT, MO, FM and normal rat-chows

[a-c]Same letters on different bars of the same parameter denote no significant difference ($p>0.05$) and [a-c]Different letters on different bars of the same parameter denote significant difference ($p<0.05$)

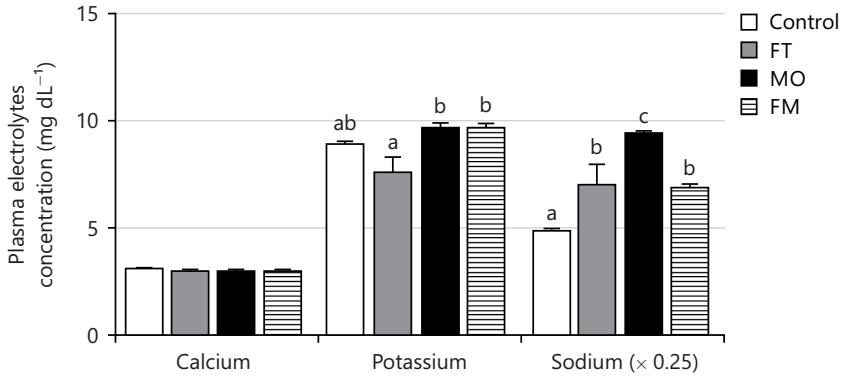

Fig. 2: Plasma calcium, potassium and sodium of rats fed FT, MO, FM and normal rat-chows

[a-c]Same letters on different bars of the same parameter denote no significant difference ($p>0.05$) and [a-c]Different letters on different bars of the same parameter denote significant difference ($p<0.05$)

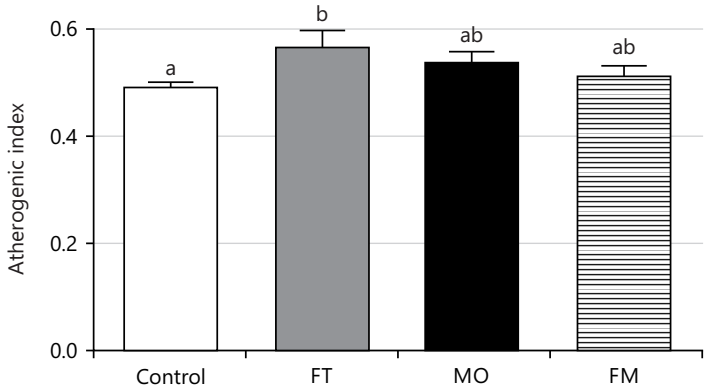

Fig. 3: Atherogenic index of rats fed FT, MO, FM and normal rat-chows

[a-c]Same letters on different bars denote no significant difference (p>0.05) and [a-c]Different letters on different bars denote signi cant difference (p<0.05)

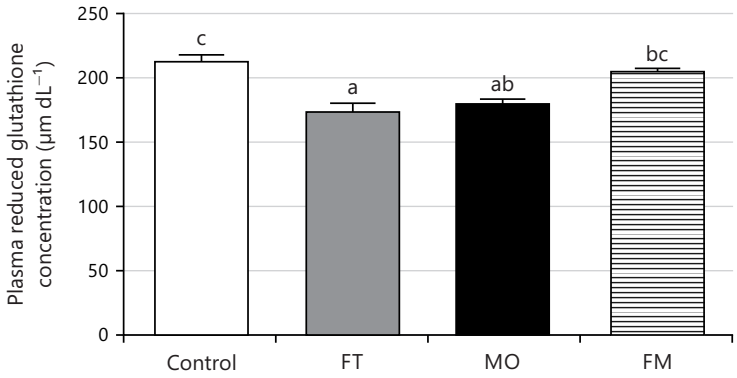

Fig. 4: Plasma reduced glutathione of rats fed FT, MO, FM and normal rat-chows

[a-c]Same letters on different bars denote no significant difference (p>0.05) and [a-c]Different letters on different bars denote significant difference (p<0.05)

group, however, the plasma potassium content of the FT was significantly lower (p<0.0.05) than the MO and MF. The plasma sodium in FT, MO and MF was significantly elevated (p<0.05) compared to the control group. Additionally, the plasma sodium of the MO was significantly higher (p<0.05) than in the FT and MF rats.

The atherogenic index of the control and rats fed experimental rat-chow was depicted in Fig. 3. The AI of the MO and FT was not significantly different (p>0.05) from the control group, however, the FT was significantly higher (p<0.05) than the control rats.

The plasma reduced-glutathione of rats fed freeze-thawed, microwaved, freeze-microwaved rat chows and standard rat-chow was shown in Fig. 4. Compared to the normal rat-chow group, a significant decrease (p<0.05) in GSH was observed in the freeze-thawed and microwaved treated rat-chow-fed groups. No significant difference (p>0.05) was noted between the freeze-microwave and normal rat-chow-fed rat groups. Additionally, no significant (p>0.05) change was recorded between the microwaved and freeze-microwaved groups.

Figure 5 represented that the plasma superoxide dismutase activity of the FT, MO, FM and CG rat groups. The plasma SOD activity of FT, MO and FM was significantly lower (p<0.05) than in the control group. Also, the plasma SOD activity of FT and FM was significantly higher (p<0.05) than in the MO group.

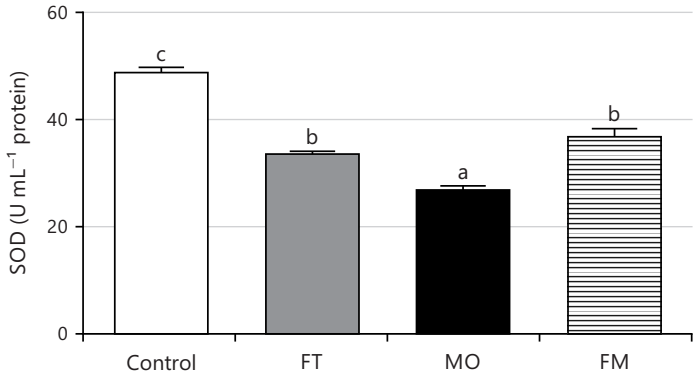

Fig. 5: Plasma superoxide dismutase activity of rats fed FT, MO, FM and normal rat-chows

[a-c]Same letters on different bars of the same parameter denote no signi cant difference (p>0.05) and [a-c]Different letters on different bars of the same parameter denote significant difference (p<0.05)

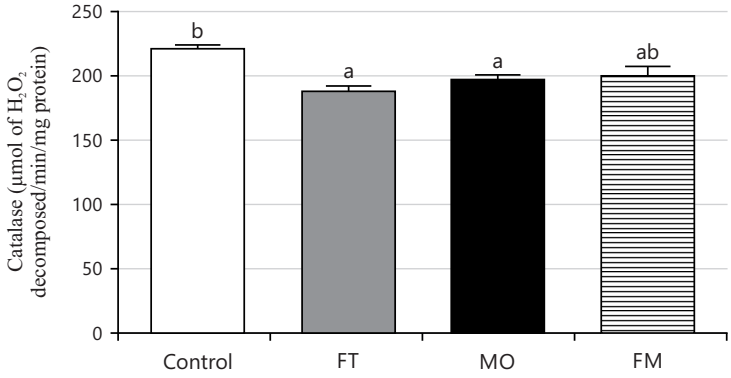

Fig. 6: Plasma catalase activity of rats fed FT, MO, FM and normal rat-chows

[a,b]Same letters on different bars of the same parameter denote no significant difference (p>0.05) and [a,b]Different letters on different bars of the same parameter denote significant difference (p<0.05)

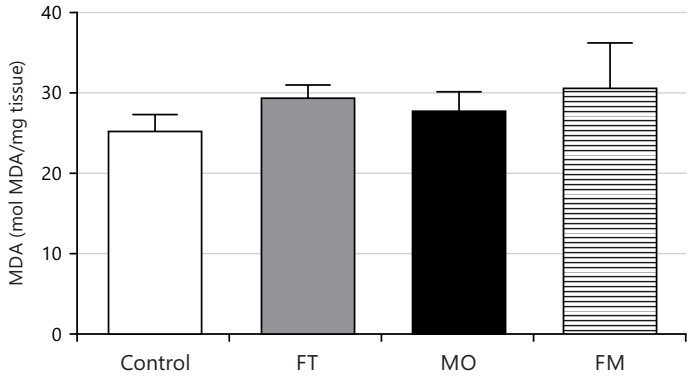

Fig. 7: Plasma lipid peroxidation of rats fed FT, MO, FM and normal rat-chows

The plasma catalase (CAT) activity in CG, FT, MO and FM groups was shown in Fig. 6. A significant decrease (p<0.05) in CAT activity was recorded in the FT and MO compared to the control group. However, the FM was not significantly different (p>0.05) from the CG group. Also, no significant change (p>0.05) was recorded between the FT, MO and FM groups.

The lipids peroxidation (malondialdehyde) level in the plasma of the rats fed microwaved and freeze-thawed rat-chows was shown in Fig. 7. There was no significant (p>0.05) change in the plasma MDA level of the experimental groups (FT, MO and FM) when compared with the control group.

DISCUSSION

The differential effect of various applications on lipid profile, oxidative stress and electrolyte imbalance in female rats were observed in this study. After 6 weeks of feeding groups of rats with microwaved, FT, freeze-microwaved and standard rat-chow, the plasma cholesterol and LDL-C of the experimental groups were significantly elevated ($p<0.05$) than the control group. This significant increase suggests that freezing, microwaving and combining the two methods may induce hypercholesterolemia, consequently forming plaque on the blood vessel as a deposit[17-20]. The increased plasma cholesterol and LDL-C may be ascribed to the ability of microwave heating to increase the free lipids contents, which may enhance the unregulated adsorption of fats in the gastric tract, especially the short-chain fatty acids and aromatic lipids[18-23]. Additionally, microwave heating oxidation or transformation of some aromatic lipids molecules could also impair the hepatic biotransformation of such lipids and result in unregulated concentration in the blood. Previous reports have shown that a few minutes of pulse microwave application on food material caused the transformation of lipids and hydrolysis of triglycerides[23-25], while Khalil *et al*.[24] showed that microwave enhances lipids extraction in flaxseed. Freezing alike has been reported to induce a high alteration of the fat content of fish products[26]. Thus, this may explain why this study's freeze-thawing method induced hypercholesterolemia in female rats.

Although hypercholesterolemia and elevated LDL-C were recorded in the rats fed the experimental rat-chow, the plasma HDL-C of the rats was not altered significantly ($p>0.05$) by any processing methods. Though this seemingly positive effect may appear innocuous, the increase in LDL may protect the LDL-C/HDL-C ratio, because HDL-C has been considered a pivotal chylomicron subunit that can be used in proportion with LDL-C to predict the risk of systemic impaired lipids transportation and deposition[27].

In this study, the plasma calcium and potassium levels in the experimental rat groups were not altered significantly ($p>0.05$) compared with the control group. The role of blood electrolytes in switching between an individual's physiological and pathological states has maintained the frontline in recent studies and the dietary influence on these electrolytes is remarkable[28,29]. Also, the plasma sodium was significantly ($p<0.05$) elevated in the experimental groups and the MO was doubly elevated compared with the control group. This increase may be ascribed to the fact that microwave heating has been reported to increase the mineral bioavailability of some food and edible products like fish meals[30]. Dietary regulation of blood electrolytes such as sodium, calcium and potassium is an effective non-pharmacological approach to managing cardiovascular disease[31]. Furthermore, freezing has been reported to reduce minerals of plant products such as vegetables[32] significantly, this was not the case because hypernatremia was also recorded in the group fed FT rat-chow. Interestingly, the FM had a significantly ($p<0.05$) lower natremia than the MO group.

This investigation revealed that neither microwaved rat-chow nor freeze-microwaved rat-chow significantly ($p>0.05$) poses a threat to the atherogenic index of plasma. However, FT rat chow significantly ($p<0.05$) increased the risk of the atherogenic index of plasma in rats. Plasma atherogenic index is a strong marker to predict the risk of atherosclerosis and coronary heart disease and is associated with the size of pre-and anti-atherogenic lipids constituents[33]. Literature has shown that the number of freeze-thawing cycles is proportional to lipid peroxidation intensity of food materials[33-35] and consequent elevated atherogenic risk in female rats.

The GSH, SOD and CAT play some important enzymatic and non-enzymatic roles in preventing free radicals-mediated cellular stress and injuries. At the same time, lipids peroxidation is an index of the overwhelming effect of reactive oxygen species[35,36].

In this study, the SOD activity and GSH were altered ($p<0.05$) significantly, however, the CAT and LPO level were not significantly ($p<0.05$). Thus, no significant oxidative stress was recorded.

This study demonstrated that the domestic use of microwave ovens for food preservation and quality enhancement might not pose any significant atherogenic risk and oxidative stress in the short term, however, using a microwave oven to defrost frozen food items is relatively safer than room-temperature thawed food. Hence, it is recommended that concurrent application of refrigeration and microwaving-a process that is often applicable in fast-food outlets, should be encouraged to moderate atherogenic risk induced by the consumption of freeze-thawed or microwaved foods.

CONCLUSION

This study revealed that short-term consumption of freeze-thawed food might increase cardiovascular risk, while microwave-heated food induced hypernatremia. However, the sequential application of freezing and microwaved heating (freezing-microwaving) seems not to cause any significant dyslipidemia and oxidative stress in the plasma of female rats.

SIGNIFICANCE STATEMENT

This study revealed that short-term consumption of microwaved food poses no significant health risk via oxidative stress induction, plasma electrolyte distortion and lipidemic status in females. Nonetheless, microwaved heated frozen food was further shown to be safer than consuming each processing method singly. Thus, reports from this work will be beneficial in further substantiating the safety of microwaved food for female health. This study will help the researchers uncover the critical areas of nutritional safety of the combined application of frozen and microwave-heating in females that many researchers have yet to explore. Thus. A new approach to freeze-thawed food safety is improved when microwaved heat thaws frozen food.

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