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ANTIOXIDANT EFFECT OF *AFRAMOMUM ANGUSTIFOLIUM* SEED ESSENTIAL OIL IN FREEZE STORAGE OF LEAN MEAT

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Abstract - Essential oils are gaining attention as natural substitutes for synthetic antioxidant compounds due to uncertainties about their toxicity. In this study, lean meat (liver and kidney tissues) from beef, were freeze preserved after treatment with *Aframomum angustifolium* seed essential oil and butylated hydroxytoluene dissolved in soya bean oil. Three sample groups were used for the study; essential oil treated group (test), butylated hydroxytoluene treated group (positive control) and soya bean oil group (normal control). At an interval of three days, malondialdehyde concentration was measured by spectrophotometry in the three groups for twenty-four days to ascertain the level of lipid peroxidation. The result of the study indicated a steady increase in malondialdehyde concentration in normal control and test groups of kidney tissue from day 3-15. The concentration peaked at 3.136 ng/g and 3.046 ng/g respectively. Positive control group peaked on day 9 at 3.115 ng/g. A decline was observed in all kidney tissue groups in the remaining days of the experiment. A similar trend was observed in the liver tissue. The essential oil demonstrated antioxidant capacity significantly higher ($p < 0.05$) than butylated hydroxytoluene from day 3-9. Malondialdehyde concentration in the test and positive control groups peaked at 3.061 ng/g and 3.102 ng/g respectively on day 12. Normal control group peaked earlier at 3.37 ng/g on day 9. The results of this study indicate that *Aframomum angustifolium* seed essential oil has capacity to inhibit lipid peroxidation in freeze-stored lean meat.

Keywords: *Aframomum angustifolium*, essential oils, butylated hydroxytoluene, malondialdehyde, lipid peroxidation.

INTRODUCTION

From 1950 to 2017, about 5559 articles were published on the use of essential oils (EOs) in food preservation. Of the number, about 86% were published in the last decade, an indication of the current research interest. Essential oils have been widely studied for bactericidal, virucidal, fungicidal, anti-parasitidal, insecticidal, medicinal and cosmetic applications. Their application for shelf-life extension in foods is mainly due to their antioxidant and antimicrobial properties. Although EOs are promising alternatives to chemical preservatives, high volatility, strong odour and varying compositions are limitations to their use (Fernandez-Lopes and Viuda-Mantos, 2018). The alteration in organoleptic parameters are more obvious when the essential oils are added directly to the food matrix. Their volatile nature may also affect their bioavailability. Encapsulation and incorporation into edible films with controlled release are recent advances to maximize application in food systems (Asbahani *et al.*, 2015; Atares and Chiralt, 2016; Ribeiro-Santos *et al.*, 2017).

Lipid component of lean beef makes it tasteful and acceptable to consumers (Devi and Khatkar, 2016). Freezing remains an important technique for preserving beef and beef products all over the world. This preservation technique inhibits microbial and enzyme action during preservation but not oxidative processes. Thus, physicochemical deterioration in lean meat is not stopped completely. The lipid content undergoes oxidation reactions which surges to a greater extent following repeated freeze-thaw cycles (Amit *et al.*, 2017). The deterioration in quality is a product of the accumulation of primary and secondary oxidation products. This necessitates the treatment with antioxidants to delay lipid oxidation thus extending shelf life. Uncertainties about the safety of synthetic antioxidants have fueled research into the capacity of natural agents.

EOs have gained research attention because of reported bioactivity. EOs make up a small percentage of the entire plant's composition. They are soluble in alcohol and partly soluble in water. The oils are usually a mixture of esters, aldehydes, ketones and terpenes (Falowo *et al.*, 2019; Miguel, 2010). They are aromatic oily liquids mostly used in perfumery and production of scents. The aroma as well as biochemical properties of each oil is a product of the combination of all the constituent phytochemicals (Ebrahimzadeh *et al.*, 2009; Sangwan *et al.*, 2001). Current research interest is motivated by the fact that EOs are composed of organic and biochemically active molecules with diverse nutraceutical, industrial and pharmaceutical applications (Patra *et al.*, 2015). They have gained attention as food preservation materials (Fernandez-Lopez and Viuda-Martos, 2018).

The results obtained from previous studies are promising since very low concentrations of EOs are required ($\leq 0.5\%$). Toxicological reports at this concentration do not raise any concern. The bio-preservative effect of EOs have been studied in virtually all food types but application in the storage of fruits and vegetables have been most reported (Fernandez-Lopes and Viuda-Mantos, 2018). The aim of this study was to determine if the essential oil from *Aframomum angustifolium* seeds can delay lipid oxidation in freeze stored lean meat.

MATERIALS AND METHODS

Sample collection

Dry *Aframomum angustifolium* pods were purchased from a local dealer at Ebelle Market, Igueben Local Government Area, Edo state, Nigeria. Identification was done in the department of Biological Sciences, Samuel Adegboyega University, Ogwa, Edo State, Nigeria.

Extraction of Essential Oil

Aframomum angustifolium seeds were obtained by opening the dry pods and separated from the chaff. The seeds were air dried for seven days at 30°C and thereafter pulverized using a mechanical blender into fine powder. Extraction of the essential oil was done by steam distillation using a Clevenger apparatus. The distilled oil was collected over condensed steam in a graduated funnel and was dried over sodium carbonate in a desiccator.

Beef Offal (Kidney and Liver Sample) preparation

Samples were obtained from an abattoir in Ebelle, Igueben Local Government Area, Edo state, Nigeria and transported to the laboratory immediately. Blood was drained using dry tissue paper and 1 gram portions were obtained. The 1 g portions were mashed to increase oxidative activity and were preserved separately in 1 ml, 0.5% soya bean oil (SBO) solutions of BHT and EO respectively. A separate portion was stored in 1 ml SBO to serve as control.

Thiobarbituric Acid Reactive Substance (Malondialdehyde) Assay

Malondialdehyde (MDA) concentration was determined colorimetrically using thiobarbituric acid according to the method of Buege and Aust (1978). 1 g of sample was finely ground in a hand mortar with acid-washed sand and homogenized with 50 ml of physiological saline for 15 min. The homogenate was centrifuged at 8,000 g for 5 min. 1 ml of the supernatant was added to 2.0 ml of trichloroacetic acid-thiobarbituric acid- HCl reagent, and the solution was mixed thoroughly. The mixture was then placed in a boiling water bath for 15 min. On cooling, the protein precipitate was removed by centrifuging at 10,000 g for 5 min, and the absorbance of the clear supernatant fraction was read at 532 nm against reagent blank. MDA values were calculated using molar extinction coefficient and expressed as ng/g of fresh weight.

Chemicals and Reagents

All chemicals and reagents used for this study were of analytical grade.

Statistical Analysis

The experimental results obtained from triplicate analysis were reported as means. Statistical analysis was done using ANOVA and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The capacity of *Aframomum angustifolium* seeds essential oil to inhibit lipid peroxidation in freeze-stored kidney and liver tissues are presented in figures 1 and 2.

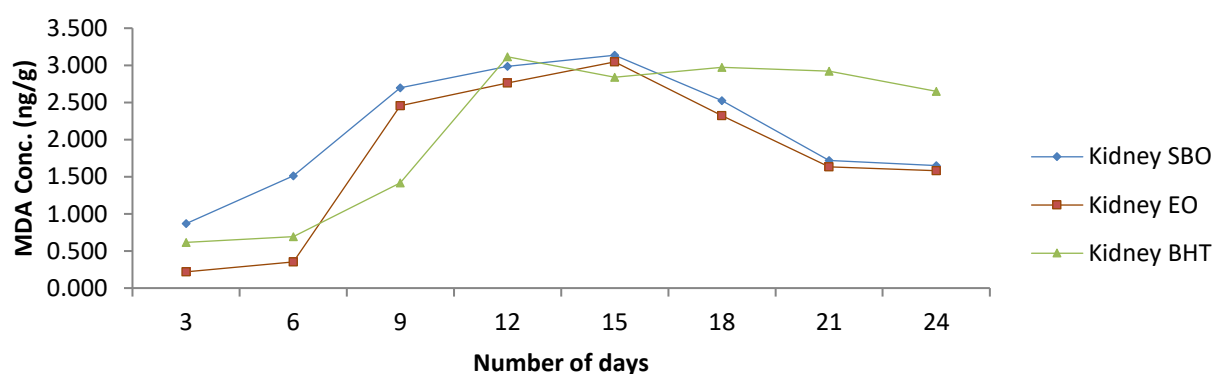


Figure 1: MDA concentration in kidney tissue

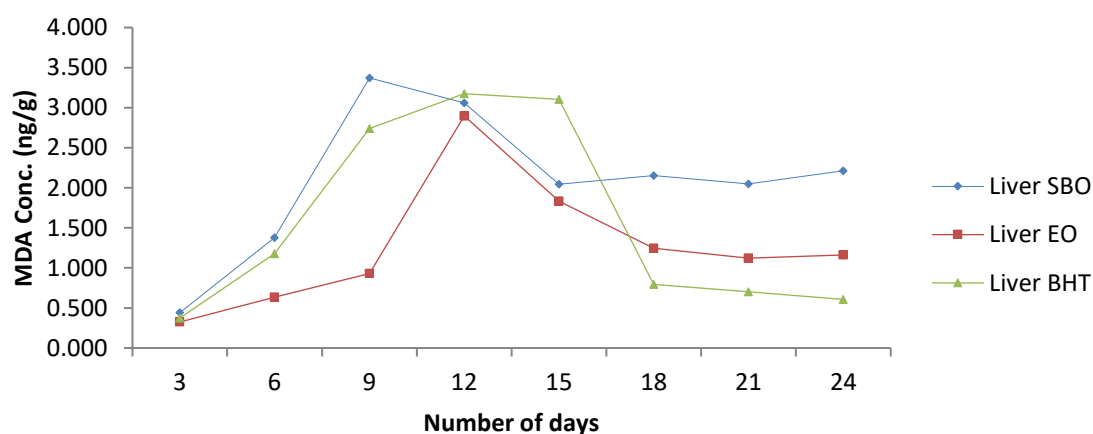


Figure 2: MDA concentration in liver tissue

Aframomum angustifolium seeds contain essential oil and was selected for this study based on the established nutritional and ethnomedicinal use as well as well as availability and reported non-toxicity from oral consumption reports (Etti *et al.*, 2012; Crook, 2013; Anywar and Kirimuhuzya, 2015). The

essential oil composition of *Aframomum* species vary and are not evenly distributed in all parts (Diomandé, Koffi, Tonzibo, Bedi and Figueredo, 2012).

The antioxidant capacity of *Aframomum angustifolium* seed essential oil was measured by its capacity to inhibit lipid peroxidation during freeze storage.

The results show that MDA concentration increased in kidney tissue across the groups from Day 3-12. The normal control group had a higher MDA concentration within the same period. Essential oil was more efficient in the first 6 days and was significantly different ($p < 0.05$) from normal and positive control groups. A downward trend was observed across the groups after peak concentrations of MDA were observed on day 12 and 15.

MDA concentration also increased in liver tissue across all study groups from day 3. The control groups were not significantly different on days 3 and 6. MDA concentration in the test group was significantly lower compared to the controls on days 3, 6 and 9. Across the three groups, MDA concentration was not significantly different on day 12. A similar downward trend as seen in the kidney study groups was also observed from day 12 to the end of the experimental period.

The results obtained from this study are in line with previous reports by other authors on the antioxidant property of essential oils. Oskoueian *et al.* (2013) reported that clove essential oil inhibited peroxidation of linoleic acid to a greater extent compared to synthetic antioxidants. It demonstrated a higher radical scavenging activity measured as 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ABTS, and superoxide anion *in vitro*. It also reduced ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and displayed metal chelating activity. Manjamalai and Berlin-Grace (2012) measured the antioxidant activity of essential oils from *Wedelia chinensis* (Osbeck) *in vitro* and *in vivo* in lung cancer bearing C57BL/6 Mice. The essential oil showed a significant antioxidant activity both *in vitro* and *in vivo* by scavenging free radicals and increasing the level of endogenous antioxidants. *Lavandula angustifolia* essential oil was also significantly effective against lipid peroxidation (Yang *et al.*, 2010), cinnamon essential considerably inhibited MDA production in fresh and heated oil (Keshvari *et al.*, 2013) and positive correlation co-existed between essential oil concentrations and percentage inhibition of free radicals (Manjamalai and Berlin-Grace, 2012).

The observed reduction in the MDA concentration can be attributed to the possibility of secondary peroxidation products to form stable adducts with proteins to form new compounds that cannot be detected by the TBARS assay (Tuma *et al.*, 1996). MDA can bind with lysine residues of protein to form an enamine-type MDA-lysine adduct; N^ε-(2-propenal) lysine (Uchida *et al.*, 1997). This reaction of MDA with proteins is time and concentration dependent and the carbonyl compounds formed can be measured by spectrophotometric or immunochemical methods after derivatization with 2,4-dinitrophenylhydrazine (Burcham and Kuhan, 1996).

CONCLUSION

The observed antioxidant capacity of *Aframomun angustifolium* seed essential oil suggests possible application in chilled storage of meat. A study on the effect the essential oil on the organoleptic parameters, effective concentration and method of application to improve retention during storage is suggested.

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