

# Clove Essential Oil Improves Lipid Peroxidation and Antioxidant Activity in Tilapia Fish Fillet Cooked by Grilling and Microwaving

E. Oskoueian, E. Maroufyan, Y.M. Goh, E. Ramezani-Fard, M. Ebrahimi

**Abstract**—The fish meat plays an important role in the human health as it contains high quality protein. The tilapia fish considered as the third largest group of farmed fish. The oxidative deterioration of fish meat may occur during the cooking process. The proper cooking process and using natural antioxidant to prevent oxidation and enhance the quality of the tilapia fish fillet is necessary. Hence, this research was carried out to evaluate the potential of clove essential oil to prevent lipid peroxidation and enhance the antioxidant activity of tilapia fish fillet cooked using microwaving and grilling methods. The results showed that cooking using microwave significantly ( $p < 0.05$ ) increased the lipid peroxidation and decreased the DPPH and ferric reducing activity power of the fish fillet as compared to grilling method. The fortification of fish fillet using clove essential oil prevented from lipid peroxidation and enhanced the antioxidant activity of the fish fillet significantly ( $p < 0.05$ ). Consequently, fortification of tilapia fish fillet using clove essential oil followed by cooking using griller to have high quality cooked fish meat is recommended.

**Keywords**—Antioxidant activity, fillet, fish, fortification, lipid peroxidation.

## I. INTRODUCTION

THE fish meat plays an important role in the human health as it contains high quality protein and omega-3 fatty acids. The tilapia fish with the annual production growth of 12% can be considered as the third largest group of farmed finfish cultured in the tropical and subtropical area of the world [1]. Oxidation in the meat is one of the primary mechanisms of quality deterioration. The oxidative deterioration of fish meat may occur during the storage and cooking process. The oxidation level depends on the length, type of storage or cooking procedure.

The oxidation starts from unsaturated fatty acids and resulted in production of hydroperoxides. The hydroperoxides are susceptible to further oxidation and may turn to oxygenated compounds, ketones and short-chain aldehydes.

E.O. is with the Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia (phone: +60-129795283; e-mail: ehs424@yahoo.com).

E.M. is with the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia (e-mail: elham\_maroufyan@yahoo.com).

Y.M.G. and M.E. are with the Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia (e-mail: ymgoh@upm.edu.my, mehdiabrahimii@gmail.com).

E.R.F. is with the Department of aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia (e-mail: eramezanifard@gmail.com).

These compounds adversely affect the meat color, nutritive value and shelf life [1].

In the past, in order to prevent the oxidation and maintain the quality of the meat during cooking process the synthetic antioxidant use to be applied. Recent reports showed that the synthetic antioxidants such as butylated hydroxytoluene (BHT) may increase the cancer risk. Hence, the food industries trying to replace the synthetic antioxidants using natural antioxidants such as plant extracts and essential oils. These natural antioxidants improve and maintain the quality of meat during cooking process. Essential oils could be considered as a source of natural antioxidants and recent reports indicated that fortification of food materials using these compounds preserve the food and prevent oxidative deterioration.

The clove essential oil inhibited the lipid peroxidation of linoleic acid in the greater extent as compare to the other source of antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT),  $\alpha$ -tocopherol and trolox [2]. Moreover, the clove oil reported to possess DPPH, ABTS and superoxide anion radicals scavenging activity, ferric ions ( $\text{Fe}^{3+}$ ) reducing power and ferrous ions ( $\text{Fe}^{2+}$ ) chelating activities [2].

With regard to the above mentioned potentials, this research aimed to evaluate the potential of clove essential oil to prevent lipid peroxidation and enhance the antioxidant activity of tilapia fish fillet cooked by microwave and griller.

## II. MATERIAL AND METHODS

### A. Sample Preparation

The tilapia fish were obtained from a local market (Selangor, Malaysia) during summer of 2013 and transferred to the laboratory in an ice containing box. The visceral organs, head, backbone, skin and the tail were removed and the bodies were washed with tap water several times. The fillets ( $54 \pm 12\text{g}$  each) were cut, ground (5mm) using a meat grinder and combined. The fish fillets were divided into 18 homogenous samples of 100g each.

### B. Essential Oil Fortification and Cooking Methods

The 18 homogenous fish fillet samples were divided into 6 treatments and each treatment with three replicates. For fortification, the clove essential oil (Sigma-Aldrich, 8000-34-8, Louis, USA) at the concentration of 0.1% (w/w) was mixed properly with fish fillet. The fish fillets cooked using microwave and griller. The fish fillet samples were grilled in a

griller with the temperature set at 350°C for 10min (each side 5min). The core temperature of the fillets upon cooking was 75±10°C. To cook the fillet samples using microwave, the potency of the machine was adjusted at 10, for 5min. The core temperature of samples upon cooking was 90±5°C. The treatments consisted of raw meat, clove fortified raw meat, microwave cooked meat, clove fortified and microwave cooked meat, grilled meat, clove fortified and grilled meat.

### C. Determination of Lipid Peroxidation

In order to determine the lipid peroxidation the malondialdehyde (MDA) concentration was determined in the samples as described by Ohkawa et al. [3], with slight modifications. One g sample was homogenized in 4ml of potassium chloride (1.15%). Then, a 200µl homogenized sample was mixed with 300µl DW, 35µl BHT, 165µl sodium dodecyl sulphate (SDS) and 2ml TBA. Next, the mixture was heated for 60min at 90°C. The solution was immediately cooled in running water. After adding 3ml of n-butanol, the solution was centrifuged at 5,000 rpm for 10min. The butanol fraction obtained was used to estimate the absorbance at 532nm by spectrophotometer. The standard curve was prepared with different concentrations of TEP (2.5-50 µM).

### D. DPPH Scavenging Activity

The DPPH scavenging activity was determined as described by Qwele et al. [4]. The methanolic solution of DPPH (0.05mm) was prepared and 800µl of DPPH solution was mixed with 200µl of homogenized sample. After 20min, absorbance was recorded at 517nm in a UV-visible micro plate reader (Molecular Devices, Sunnyvale, CA). The lower absorbance of the reaction mixture indicates higher free radical scavenging activity which calculated by the DPPH radical concentration was calculated using the following equation:

$$\text{Free radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100\%$$

where A<sub>0</sub> was the absorbance of the control (methanol+DPPH) and A<sub>1</sub> was the absorbance of the sample. In this assay, trolox was used as positive control.

### E. Ferric Reducing Activity Power

The ferric reducing activity power (FRAP) was determined based on Benzie and Strain [5]. This method was based on the reduction of 2,4,6-tripyridyl-s-triazine complex with yellow color (Fe<sup>3+</sup>-TPTZ) to the ferrous form (Fe<sup>2+</sup>-TPTZ) with the blue color. The 200 µl of homogenized sample was mixed with 800µl of 10mM ferric-TPTZ reagent and the changes in the absorbance were monitored at 593nm after 20min incubation using a micro plate reader (Molecular Devices, Sunnyvale, CA). Trolox was used as standard and the ferric reducing activity power of the meat extract was reported as trolox equivalent antioxidant capacity.

### F. Statistical Analysis

Data were analyzed using the general linear models (GLM) procedure of SAS (2003) in a completely randomized design

(CRD) and the means were compared with Duncan's Multiple Range test.

## III. RESULT AND DISCUSSIONS

Fig. 1 shows the effect of addition of clove essential oil on lipid peroxidation of the tilapia fish fillet cooked by a different procedure. The result showed the level of lipid peroxidation in the raw meat, clove fortified raw meat, microwave cooked meat, clove fortified and microwave cooked meat, grilled meat, clove fortified and grilled meat with the values of 321.4, 300.1, 587.9, 503.6, 471.0 and 418.4 µM MDA/ g wet wt respectively.

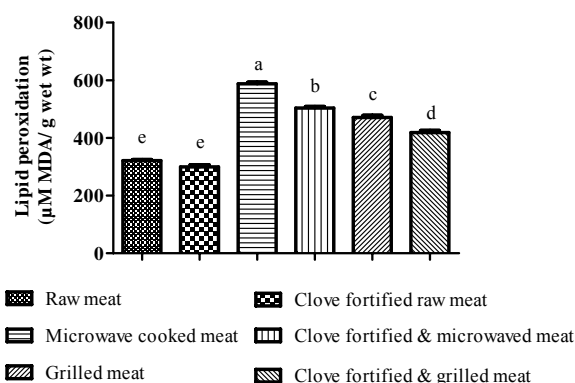


Fig. 1 Effect of addition of clove essential oil on lipid peroxidation of the tilapia with different methods of cooking. Different letters show significant differences among treatments ( $p < 0.05$ )

The microwaving and grilling increased the lipid peroxidation level significantly ( $p < 0.05$ ) as compared to the raw meat. In addition, the fish fillet cooked using microwave indicated the high level of lipid peroxidation as compared to the grilled meat. Addition of essential oil reduced the lipid peroxidation level significantly ( $p < 0.05$ ) in all the treatments.

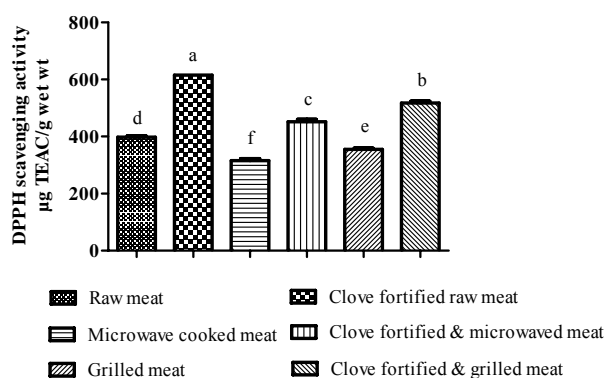


Fig. 2 Effect of addition of clove essential oil on the DPPH scavenging activity of the tilapia meat with different methods of cooking. Different letters show significant differences among treatments ( $p < 0.05$ )

The effect of addition of clove essential oil to the DPPH scavenging activity of the tilapia fish fillet is presented in Fig. 2. The DPPH scavenging activity of the raw meat, clove fortified raw meat, microwave cooked meat, clove fortified and microwave cooked meat, grilled meat, clove fortified and grilled meat values were 397.5, 615.8, 315.5, 451.2, 355.1 and 517.4  $\mu\text{g TEAC/g wet wt}$ , respectively. The cooking reduced the DPPH scavenging activity of the fish fillet significantly ( $p < 0.05$ ). The DPPH scavenging activity of grilled fish fillet with the value of 355.1  $\mu\text{g TEAC/g wet wt}$  was significantly ( $p < 0.05$ ) higher than microwave cooked meat with the value of 315.5  $\mu\text{g TEAC/g wet wt}$ . The addition of clove essential oil significantly ( $p < 0.05$ ) enhanced the DPPH scavenging activity of the fish fillet in all the treatment.

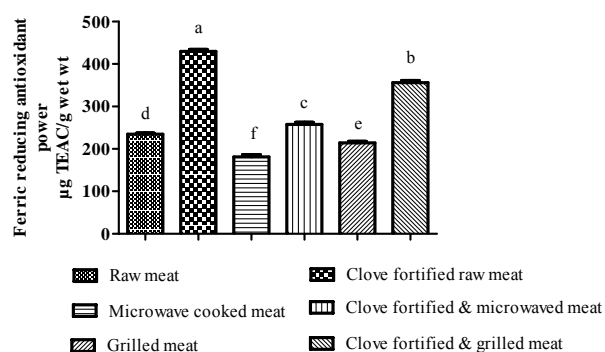


Fig. 3 Effect of addition of clove essential oil on ferric reducing antioxidant power on tilapia meat with different cooking methods. Different letters show significant differences among treatments ( $p < 0.05$ )

The effect of addition of clove essential oil of ferric reducing antioxidant power of the tilapia fish fillet is presented in Fig. 3. The ferric reducing antioxidant power of the raw meat, clove fortified raw meat, microwave cooked meat, clove fortified and microwave cooked meat, grilled meat, clove fortified and grilled meat values were 234.6, 429.5, 181.1, 257.6, 214.3 and 355.9  $\mu\text{g TEAC/g wet wt}$ , respectively.

The ferric reducing antioxidant power of the raw fish fillet with the value of 234.6  $\mu\text{g TEAC/g wet wt}$  was significantly ( $p < 0.05$ ) suppressed to 181.1 and 214.3  $\mu\text{g TEAC/g wet wt}$  in the fish fillets cooked by microwave and griller, respectively. Fortification using clove essential oil significantly ( $p < 0.05$ ) increased the ferric reducing antioxidant power activity of the fish fillet in all the treatments. The highest ferric reducing antioxidant power value was found in the raw fish fillet fortified by clove essential oil while the lowest value recorded in the fortified fish fillet cooked by microwave.

The results obtained in this study were in agreement with Weber et al. [6] who observed the higher lipid peroxidation and lower antioxidant activity in the silver catfish fillet cooked by microwave as compare to grilled fish fillet. Similarly, Erkan and Bilen [7] reported the lower lipid peroxidation and higher antioxidant activity in the chub mackerel fillets treated by black thyme oil.

#### IV. CONCLUSION

The microwave cooking potentially increased the oxidation and decreased the antioxidant activity of the fish fillet as compared to grilling. The fortification of fish fillet using clove essential oil prevented the oxidation and enhanced the antioxidant activity of the fish fillet.

#### ACKNOWLEDGMENT

The authors would like to thank to the department of veterinary preclinical sciences, faculty of veterinary medicine and institute of tropical agriculture, Universiti Putra Malaysia for the laboratory facilities.

#### REFERENCES

- [1] P. E. Simitzis, G.K. Symeon, M.A. Charismiadou, J.A. Bizelis, and S.G. Deligeorgis, "The effects of dietary oregano oil supplementation on pig meat characteristics," *Meat Science*, vol. 84, pp. 670-676, 2010.
- [2] I. Gulcin, M. Elmastas, and H.Y. Aboul-Enein, "Antioxidant activity of clove oil – A powerful antioxidant source," *Arabian Journal of Chemistry*, vol. 5, pp. 489-499, 2012.
- [3] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical biochemistry*, vol. 95, pp. 351-358, 1979.
- [4] K. Qwele, A. Hugo, S.O. Oyedemi, B. Moyo, P.J. Masika, and V. Muchenje, "Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay," *Meat Science*, vol. 93, pp. 455-462, 2013.
- [5] I.F. Benzie, and J. Strain, "Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration," *Methods in Enzymology*, vol. 299, pp. 15-27, 1999.
- [6] J. Weber, V.C. Bochi, C.P. Ribeiro, A.D.M. Victorio, and T. Emanuelli, "Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets," *Food Chemistry*, vol. 106, pp. 140-146, 2008.
- [7] N. Erkan, and G. Bilen, "Effect of essential oils treatment on the frozen storage stability of chub mackerel fillets," *Journal für Verbraucherschutz und Lebensmittelsicherheit*, vol. 5, pp. 101-110, 2010.