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LIPID PEROXIDATION IN SMOKE-DRIED AFRICAN CATFISH TREATED WITH *Moringa oleifera* MARINADE, SALT OR BUTYLATED HYDROXYL ANISOLE

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ABSTRACT

Smoke-dried fish is vulnerable to lipid peroxidation, which can reduce product quality and pose health risks to consumers. The study examined the antioxidant potency of *Moringa oleifera* marinade on oxidative stability of smoke-dried catfish in comparison with salt and Butylated hydroxyl anisole (BHA), a synthetic antioxidant. Seventy-two catfish (208±6 g) were processed, randomly assigned to six antioxidant treatment groups and hot smoked. The treatments are the control (0%), 1%, 2% and 3% (w/v) *Moringa oleifera* marinade (MOM), 5% Brine (w/v) and 0.2% BHA (w/v). The smoke-dried fish were stored at room temperature (35±1^{0}C) for 8 weeks. Lipid peroxidation was monitored weekly using Thiobarbituric acid (TBA) assay. The results showed that *Moringa oleifera* marinade and BHA decreased lipid peroxidation more than ($p<0.05$) the control (0.94 mg/MDA/kg) and salt (0.92 mg/MDA/kg) treated samples. This was shown by the lower Thiobarbituric acid reactive substance (TBARS) values of 1% MOM (0.84 mg/MDA/kg), 2% MOM (0.88 mg/MDA/kg), 3% MOM (0.85 mg/MDA/kg) and BHA (0.80 mg/MDA/kg) treated samples. A general increase in oxidative spoilage was observed for all treatments as storage time progressed. However, the increment was more ($p<0.05$) intense in control and salt treated samples. No significant ($p>0.05$) difference was observed among all Moringa treated samples and BHA. *Moringa oleifera* marinade could be used as an alternative to BHA in suppressing lipid peroxidation in smoke-dried African catfish stored for 8 weeks.

INTRODUCTION

Fish is an important source of food in many regions of the world. It is a good source of animal protein, which is indispensable to a balanced diet, and it is valued in many cultural culinary traditions. Fish is rich in beneficial unsaturated fats, particularly omega-3 fatty acids, that help protect human against diabetes, atherosclerosis and other cardiovascular diseases (Root et al., 2013). However, unsaturated fats are readily susceptible to lipid peroxidation. Peroxidation of lipids can instigate loss of nutritional and quality attributes of foods (Adeyemi et al., 2012) and could predispose consumers to various diseases such as cancer and atherosclerosis (Martin et al., 2013). Due to the susceptibility of fresh fish to spoilage, various processing techniques are employed to curb deterioration, add value and maintain product quality. Some of the processing techniques are smoking, chilling, freezing, salting, canning and drying (Kumolu-Johnson et al., 2010). Among the aforementioned processing methods, smoking is the most common in many developing countries (Bako, 2005). Smoking enhances fish flavor, increases utilization, reduces waste, prolongs the shelf life of fish and increases protein availability (Jallow, 1995; Olley et al., 2000). Nonetheless, despite the effectiveness and the numerous

advantages derived from smoking, smoke-dried fish is still liable to oxidative damage resulting in partial or total loss of nutrients, oxidative rancidity, off-color and warmed-over flavor (Yasemen, 2007). Synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) are effective in curbing lipid peroxidation but are expensive and scarce, especially in developing countries (Adeyemi and Olorunsanya, 2012), and are carcinogenic (Nabavi et al., 2010; Dolatabadi and Kashanian, 2010). There are concerns about the toxicity and safety of synthetic antioxidants in relation to their accumulation and metabolism in the body tissues and organs (Nabavi et al., 2010; Barku et al., 2013). The need to find a lasting solution to the aforementioned problem necessitates the use of natural antioxidants. Phenolic extracts from legumes (Adeyemi et al., 2011), cereals, spices and herbs have been reported to retard lipid peroxidation in oils and fatty foods (Velioglu et al., 1998). *Moringa oleifera* is a natural antioxidant being researched into recently. *M. oleifera* contains myriad phytochemicals that are antioxidants. Kaempferol and Quercetin are recognized as the most effective antioxidants in Moringa leaves (Fahey, 2005). Siddhuraju and Becker (2003) reported that antioxidant properties of Kaempferol and Quercetin are higher than that of conventional antioxidants like Vitamin C, which is also abundant in Moringa leaves. *M. oleifera* leaf is cheap and readily available in many homes in Nigeria. Given the antioxidant properties of *M. oleifera*, it was hypothesized that *M. oleifera* marinade could reduce oxidative spoilage in smoke-dried catfish. The objective of the study was to determine the antioxidant effect of *M. oleifera* marinade on oxidative stability of smoke-dried catfish in comparison with salt, a common preservative used in fish smoking, and butylated hydroxyl anisole, a synthetic antioxidant.

MATERIALS AND METHODS

Preparation of Moringa oleifera Marinade

Fresh Moringa leaves were obtained from Olayinka Village Igbaja Oke-ode, Kwara state, Nigeria. The leaves were air-dried for 4 days and ground into powder using a food blender (Starlite, Model No: SL-999 CHINA). *Moringa oleifera* marinade (MOM) was prepared by adding separately specific quantity (10 g, 20 g or 30 g) of *M. oleifera* leaf powder to 1000 ml of water. 50 g salt or 2 g BHA were also added to a separate 1000 ml of water. The percentage concentration of the marinade was estimated using the formulae:

$$\% \text{ Concentration} = \frac{\text{Weight of additive (g)}}{\text{Volume of water (ml)}} \times 100$$

Salt was used as a treatment in this trial because it is a common preservative for smoke-dried fish.

Preparation and smoking of fish

The processing and smoking of the fish were carried out at Godbet Homestead Fish Farm, Basin road Ilorin, Nigeria. The total and average weights of the fish were 15 kg and 208±6 g, respectively. The fish were gutted using a sharp knife by cutting laterally from the end of the gill cover through the belly portion to the anus. Thereafter, they were washed and rinsed. The total and average weights of the fish after gutting were 13.3 kg and 200±7 g, respectively. The fish were treated and smoked in a metallic smoking chamber.

Experimental design

The fish were randomly assigned to six antioxidant treatments. The treatments were the control (0%), 1%, 2% and 3% *M. oleifera* marinade (MOM), 5% Brine and 0.2% BHA. Each treatment was replicated thrice with 4 fish/replicate. The fish were soaked in the marinade for 2 h. Thereafter, the fish were set in the smoking chamber consisting of five-twin tiers and subjected to hot smoking for 18 h with charcoal as the heat source. The tiers were interchanged every 6 h to ensure uniform heat distribution and drying. After smoking, the fish were stored in air-free netted boxes to prevent contamination by flies and to enhance flow-through ventilation throughout the storage period on laboratory shelves at room temperature (35±1^{0}C) for 8 weeks.

Lipid Peroxidation in Smoked Catfish

Lipid peroxidation in the smoke-dried catfish samples was evaluated by the 2-thiobarbituric acid (TBA) test. The Thiobarbituric acid reactive substance (TBARS) values were measured in triplicate 10 g samples using the distillation method of Tarladgris et al. (1964) as described by Adeyemi and Olorunsanya (2012). 10 g of the smoked catfish sample were homogenized with 47.5 ml of distilled water in a specimen bottle using a glass pestle. The homogenized mixture was rinsed with 50 ml of distilled water into a round bottom flask and, thereafter, 2.5 ml of dilute Hydrochloric acid (1:2 solutions) were added and the mixture was distilled through a condensing assembly to collect about 20 ml of the distillate. 5 ml of the distillate were mixed with 5 ml of the Thiobarbituric (TBA) acid (0.02 M) and boiled for 35 min in water. Afterwards, the mixture was cooled for 10 min in a water bath for color development. The absorbance readings were measured at a wavelength of 538 nm against a blank of 5 ml of Thiobarbituric acid reagent and 5 ml of Hydrochloric acid using a spectrophotometer (CECILL-2000). The absorbance values were multiplied by factor 7.8 (Tarladgris et al., 1964) to obtain the TBARS value in milligram per malondialdehyde per kilogram sample (mg/MDA/kg).

Table 1. Main effect of antioxidant and storage time on oxidative stability of smoke-dried catfish

Factor	TBARS (mg/MDA/Kg)							
Antioxidant	control	1% MOM		2% MOM	3% MOM	5% Brine		0.2% BHA
	0.94[b]	0.84[a]		0.88[a]	0.85[a]	0.92[b]		0.80[a]
Storage weeks	1	2	3	4	5	6	7	8
	0.74[a]	0.82[a]	0.82[a]	0.90[b]	0.90[b]	0.92[b]	0.93[b]	1.25[c]

a, b, c means having different superscript along the same row are significantly different ($p<0.05$);
MOM = *Moringa oleifera* marinade

Statistical Analysis

The experiment followed a completely randomized design (CRD) in a 6 (antioxidant treatments) × 8 (storage weeks) factorial arrangement. The data obtained were analyzed by the Generalized Linear Model procedures of SAS Version 9.2 software (Statistical Analysis System, SAS Institute Inc, Cary, NC, USA). Mean differences were considered significant at $p<0.05$ and separated by Duncan multiple range test.

RESULTS AND DISCUSSION

There were significant differences ($p<0.05$) among the antioxidant treatments (Table 1). The control samples had the highest TBARS value that was not significantly different ($p>0.05$) from salt treated samples. There was no significant difference among the Moringa and BHA treated samples. The potency of all Moringa treated samples could have resulted from various phytochemicals present in Moringa leaves. Fahey (2005) reported that kaempferol and quercetin were the most effective antioxidants in Moringa leaves in addition to other flavonoid antioxidants such as isoquercitrin, kaempferitrin and rhamnetin. Moringa leaves also contain high amount of vitamin C and E, which are potent antioxidants (Grubben and Denton, 2004). However, this observation was contrary to the report given by Adeyemi et al. (2012) in which *M. oleifera* powder was not effective in cooked broiler meat samples but was effective in raw broiler meat samples. This suggests that antioxidant compounds in *M. oleifera* leaves are heat labile. The discrepancy between the earlier report and the present study could also be due to the nature of heat used. Furthermore, the oxidative stability exhibited by Moringa treated samples in the present study could be due to the synergistic effect of the antioxidant compounds liberated during the smoking process from the charcoal and the inherent antioxidants present in *M. oleifera* leaves. Smoking deposits certain preservative compounds into the smoked products (Ekpenyong and Ibok, 2012). The amount and type of compounds deposited depend on the type of wood or charcoal used (Ekpenyong and Ibok, 2012).

A general increase in TBARS values was observed as storage progressed (Table 1). There were no significant differences among the TBARS values recorded in the first, second and third week of storage. These values were significantly different from TBARS values observed from the fourth to the seventh week. The eighth week incurred the highest TBARS value that was significantly different ($p<0.05$) from those observed for other weeks. This observation was in line with the report given by Yasemen (2007) who observed that peroxide and thiobarbituric acid values of hot smoke-dried catfish stored in refrigerator increased, whilst the sensory scores declined as storage time continued. The author concluded that in order to improve the shelf life of hot smoked catfish, a suitable preservation and packaging technique should be used or developed.

There was no significant difference among the antioxidant treatments in the first, third, fourth and sixth week of storage (Table 2). In the second week, brine (salt) treated samples had the highest TBARS value that was significantly different ($p<0.05$) from other treatments. In the seventh week, there was no significant difference ($p>0.05$) between the control and BHA treated samples. This was unexpected because BHA has been reported to be a potent synthetic antioxidant (Mariod et al., 2012; Laghari et al., 2011). There was no significant difference among all levels of Moringa and salt treated samples in the seventh week. In the eighth week, BHA and all levels of Moringa marinade performed equally, while the control and salt treated samples were not significantly different ($p>0.05$). There was a steady increase in TBARS values of the control samples from week one to eight. The pattern of increase in TBARS values does not differ for 1%, 2%, 3% MOM and BHA. This clearly attests the preservative capability of *M. oleifera* marinade (MOM) which lends credence to our earlier studies (Adeyemi et al., 2013a; Adeyemi et al., 2013b) in which MOM maintained the chemical and microbiological quality of smoke-dried catfish. The antioxidant effect of *M. oleifera* marinade could be attributed to the presence of phenolic compounds, which donate hydrogen atoms from their hydroxyl groups thus reducing the formation of hydroperoxides, the first product of lipid peroxidation (Velasco and Williams, 2011). Phenolic compounds exert their antioxidant properties by three main processes (Velasco and Williams, 2011).

Table 2. Combined effect of antioxidant and storage time on oxidative stability of smoke-dried African catfish

Antioxidant	TBARS (mg/MDA/kg) Storage week							
	1	2	3	4	5	6	7	8
Control	0.53^{a}	0.74_{x}^{b}	0.78^{b}	0.85^{b}	0.99^{c}_{y}	0.99^{c}	1.20^{c}_{y}	1.27^{d}_{y}
1% MOM	0.58^{a}	0.64^{a}_{x}	0.75^{a}	0.82^{b}	0.76^{a}_{x}	0.90^{b}	0.89^{b}_{x}	0.90^{b}_{x}
2% MOM	0.52^{a}	0.70^{a}_{x}	0.66^{a}	0.81^{b}	0.90^{b}_{x}	0.95^{b}	0.80^{b}_{x}	0.98^{b}_{x}
3% MOM	0.54^{a}	0.63^{a}_{x}	0.76^{a}	0.82^{b}	0.86^{b}_{x}	0.81^{b}	0.81^{b}_{x}	0.91^{b}_{x}
5% Salt	0.67^{a}	0.87^{a}_{y}	0.76^{a}	0.78^{a}	0.85^{a}_{x}	0.82^{a}	0.90_{x}	1.145^{b}_{y}
0.2% BHA	0.56^{a}	0.69^{a}_{x}	0.74^{a}	0.82^{b}	0.85^{b}_{x}	0.89^{b}	1.02^{b}_{y}	1.03^{b}_{x}

a, b, c, d means having different superscript along the same row are significantly different ($p<0.05$); x, y means having different subscript along the same column are significantly different ($p<0.05$)

These include one or more transition metal-chelating activities (Andjelković et al., 2006), singlet-oxygen quenching capacity (Mukai et al., 2005) and free radical scavenging activity (Zheng et al., 2009). *M. oleifera* leaves contain ample amount of vitamin C (ascorbic acid) and vitamin E (α-tocopherol) (Grubben and Denton, 2004) which exhibited high radical scavenging properties as reported by Kulisic et al. (2004). In addition, Yeum et al. (2009) reported synergistic properties between α-tocopherol and ascorbic acid against peroxidation.

CONCLUSION

M. oleifera marinade and BHA were more effective than control and salt in protecting smoke-dried catfish against lipid peroxidation. There was an increment in oxidative spoilage as storage time progressed. However, the increment was more intense in the control and salt treated samples. *M. oleifera* marinade and BHA were equally potent as antioxidants. *M. oleifera* marinade could be used to retard lipid peroxidation in smoke-dried catfish for two months. Since salt is a common preservative used in fish smoke drying, further study should be carried out to determine the combined effect of *M. oleifera* marinade and salt on lipid peroxidation in smoke-dried catfish.

Sažetak

LIPIDNA PEROKSIDACIJA KOD DIMLJENOG AFRIČKOG SOMA TRETIRANOG MARINADOM *Moringa oleifera,* SOLJU I BUTIL HIDROKSI ANISOLOM

Sušena dimljena riba izložena je lipidnoj peroksidaciji, što može smanjiti kvalitetu krajnjeg proizvoda, ali i ugroziti zdravlje potrošača. Ovim istraživanjem ispitano je potencijalno antioksidantsko djelovanje marinade *Moringa oleifera* na stabilnost oksidacije kod dimljenog soma te je ono uspoređeno s djelovanjem soli i butil hidroksi anisola (BHA), sintetičkog antioksidanta. Obrađeno je 72 primjeraka soma (208 ± 6 g), a ravnomjerno su raspoređeni u 6 skupina tretiranih antioksidansima te izloženih vrućem dimu. Skupine s obzirom na vrstu tretmana i postotak izloženosti bile su sljedeće: kontrolna skupina (0%), 3 skupine somova tretiranih marinadom *Moringa oleifera* (MOM) (1%, 2% i 3%), somovi u rasolu (w/v) (5%) i somovi u butil hidroksi anisolu (w/v) (0,2%). Dimljena riba bila je pohranjena na sobnoj temperaturi (35 ± 1^{0}C) 8 tjedana. Peroksidacija lipida tjedno je praćena pomoću ispitivanja tiobarbiturnom kiselinom (TBA). Rezultati su pokazali smanjenje lipidne peroksidacije kod skupina somova u marinadi *Moringa oleifera* i u butil hidroksi anisolu ($p<0,05$), za razliku od rezultata povećane lipidne peroksidacije kod somova iz kontrolne skupine (0,94 mg/MDA/kg) i onih tretiranih solju (0,92 mg/MDA/kg). Dakle, reaktivne promjene prilikom ispitivanja tiobarbiturnom kiselinom (TBARS) su kod pojedinih skupina somova bile manje: uzorci tretirani 1% marinadom *Moringa oleifera* (0,44 mg/MDA/kg), uzorci tretirani 2% i 3% istom marinadom (0,88 mg/MDA/kg; 0,85 mg/MDA/kg) i uzorci tretirani BHA-om (0,80 mg/MDA/kg). Tijekom izloženosti riba prije navedenim uvjetima, praćen je proces kvarenja riba uzrokovan oksidacijom. Prirast je bio intenzivniji u kontrolnoj i skupini riba tretiranih solju ($p<0,05$). Nema značajne razlike kod skupina riba tretiranih marinadom *Moringa oleifera* i onih tretiranih butil hidroksi anisolom. Marinada *Moringa oleifera* mogla bi se u osmotjednom periodu izloženosti dimljenog afričkog soma koristiti kao alternativa za BHA u suzbijanju lipidne peroksidacije.

Ključne riječi: antioksidant, BHA, sol, *Moringa*, lipidna peroksidacija

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