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Radical Scavenging Properties of a Cultured and Wild Teleost in Ekiti State, Nigeria

Adewumi, A.A.; Ogunlade, I.; Adenegan J. A.

[1]Ekiti State University, Ado Ekiti

Abstract: This project compared the radical scavenging activities (RSA) of *Oreochromis niloticus* harvested from a pond and two reservoirs located in Ekiti state, Nigeria, using different processing techniques. The RSA of the muscles were analyzed using 1,1, diphenyl-1-picrylhydrazyl (DPPH) method. At various concentrations, the RSA of the fresh sample was significantly different ($P>0.05$) from the smoked and oven-dried sample, while the smoked sample and oven-dried samples were not significantly different ($P<0.05$) from each other. The fresh fish proved to be a strong radical scavenger at all DPPH concentrations. The concentration dependent RSA thus followed the order; fresh sample > Oven-dried sample > Smoked sample. The RSA of the fish from the three locations was in the order of pond > reservoir 2 > reservoir 1. The consumption of tilapia should be encouraged to supplement the intake of cereals, which are the main staple foods of developing countries, as they may have significant positive health benefits by inhibiting lipid peroxidation and scavenging free radicals that lead to various diseases and cell degeneration.

Keyword: antioxidant, tilapia, lipid, peroxidation, reservoir, pond

INTRODUCTION

Radical scavenger or antioxidant is a molecule that inhibits the oxidation of other molecules and thus protects the human body against free radicals which are produced through normal metabolic processes *in vivo* and as a result of exposure to radiation or environmental pollution (Bhadra, 2004). Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent thus producing free radicals. In turns, these radicals can start a chain of reactions. These highly reactive species cause damage to cellular components including DNA and produce many pathological and degenerative conditions such as ischemia, anaemia, asthma, arthritis, cancer, heart disease, diabetes, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Polterat 1997). Living tissue can protect itself through different endogenous antioxidants - flavonoids and flavones, which are widely distributed secondary metabolites with antioxidant and anti-radical properties. (Nakayoma and Yamada, 1995). Besides internal defence, the consumption of antioxidants from dietary sources, which is gaining popularity among nutritionists and dieticians, can help in protecting against the oxidative stress, and thus the onset of many degenerative diseases with less potential health hazard compared with synthetic antioxidants.

Lipid oxidation and the radical scavenging processes have been well documented (Pokorný, 1987, Frankel, 1991). A schematic illustration of autoxidation and radical scavenging process is illustrated in Fig. 1 (Frankel, 1991). Autoxidation is the direct reaction of molecular oxygen with organic compounds. Autoxidation of lipids proceeds through a free radical chain mechanism involving initiation, propagation and termination steps (Fig. 1). Lipid oxidation in fish is influenced by several catalytic systems for oxygen activation. To overcome the spin restriction between ground state oxygen and lipids, the reaction requires initiation (or initiator: X•) which may be the activation of ground state oxygen ($^3O_2$) into singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydroxyl radical (HOO•), or peroxides (LOOH), or transformation of unsaturated lipids into lipid radicals (L•). Under most circumstances, autoxidation starts in the presence of initiators with an extraction of H-atom from a fatty acid to produce the free radical. Subsequently the reaction proceeds through propagation reactions, which produce further free radicals. In the terminating reaction two free radicals combine to produce non-radical products.

In biological tissues such as fish muscle, other components such as proteins, amino acids and ascorbate can interact with these free radicals to terminate the reaction. When components other than

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Adewumi, A.A. (Correspondence)
zoewumi@gmail.com
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lipids terminate the reaction, they are often referred to as antioxidants.

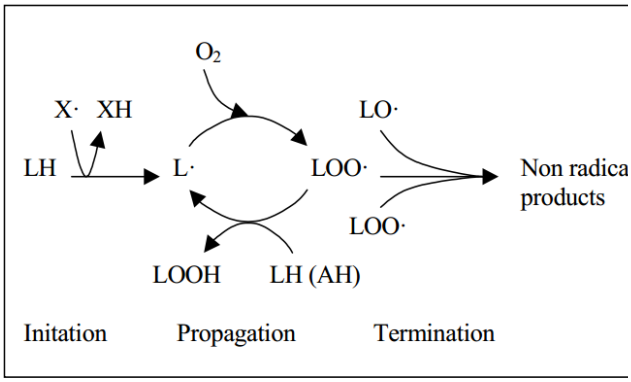

Figure 2.3: Schematic illustration of the lipid oxidation process. *LH = unsaturated lipid, X• = initiator, L• = alkyl radical, LO• = alkoxyl radical, LOO• = peroxy radical, LOOH = hydroperoxide, AH = antioxidant.* (Frankel, 1991)

Halliwell et al. (1995).opined that almost everything found in foods and in living tissues including proteins, lipids, carbohydrates and DNA is able to act as an antioxidant.
1,1, diphenyl-1-picrylhydrazyl (DPPH) free radical is wisely used to assess the antioxidant activity of natural compounds, although it is not a biologically relevant radical (Bougatef et al., 2009). The DPPH scavenging activity shows the ability of the antioxidant compounds to donate hydrogen or electrons, thus converting the radical to more stable species (Prior et al., 2005). Moreover, DPPH has a characteristic absorbance at 517nm, which decreases significantly on exposure to proton radical scavengers (Yamaguchi et al., 1998).

The processing and preservation of fresh fish are of utmost importance since fish is highly susceptible to deterioration immediately after harvest and also to prevent economic losses (Okonta and Ekelemu, 2005). The methods that are commonly employed are the traditional techniques such as salting/brining, sun-drying and smoking, which also increase fish availability to the consumers. The heat and dryness associated with hot smoking, reduces the water activity of the fish, thereby limiting microorganisms, a prerequisite for the spoilage, according to Abolagba and Osifo, (2004). The smoking of fish has the objectives of preservation basically due to dehydration, high temperature of smoking (50-180°C), the preservatives effects of smoke components (phenols, aldehydes, ketones, organic acids etc.) and for purpose of product development due to changes in organoleptic, nutritional, chemical and physical properties during processing (Abraham-Olukayode and Oramadike, 2011).

In developing countries, fish is one of the potential sources of animal protein and essential nutrients for the maintenance of a healthy body (Fawole et al., 2007). . In recent years, fish has become favorite foodstuff for the majority of societies because of several health reasons (Ali and Kiumars, 2010). Fish consumption has been found to be an effective way to regulate the functionality of normal cells. Fuentes (1998) indicated that diet in which unsaturated fatty acids replace the saturated ones, are associated with low incidence of coronary diseases, in order to reduce the risk of cardiovascular diseases. Emphasis has now been placed on the increased consumption of fish and fish products, which are rich in polyunsaturated fatty acids (PUFA) of the omega (w)-3 family, and poor in polyunsaturated fatty acids of w-6 family (Sargent, 1996).

Tlapia, *Oreochromis niloticus* is an easily cultured and commonly consumed and of great economic fish species in Nigeria. They are generally considered to be important tropical fish species for aquaculture. They have an almost Pan African distribution, ranging from the Nile to West Africa and from Algeria to South Africa. This work is designed to detect the RSA of this fish in the various environments; fish pond and reservoirs in Ekiti state, for further knowledge .

MATERIALS AND METHOD

Study Area

Ado –Ekiti Reservoir

Ado–Ekiti Reservoir (Res. 1) was constructed by damming the Ireje River in Ado-Ekiti, Ekiti State, Nigeria in 1958 for the supply of water for domestic uses and production of fish for the people of Ado-Ekiti and its environs (Agbeyo, 1976). At full capacity, the reservoir contains about 47 million gallons of water (Ebisemiju, 1993). The author further reported that the reservoir has a catchment area of 32.50km^2 and total annual discharge of 9.137 millions/m^3 of water. The reservoir is situated on an undulating plane of an average height of 440m above sea level and surrounded by highlands. The reservoir lies between latitude $7^0$35`- $70^0$36 ′North and longitude $5^0$12′– $5^0$13′ East of the Equator as shown in Fig. 2.

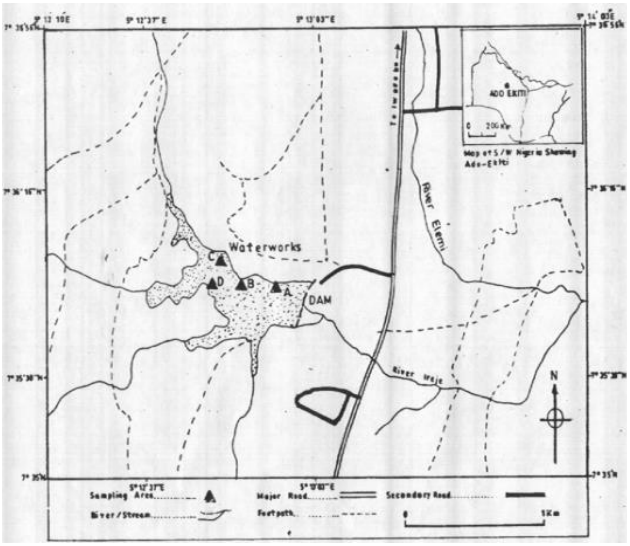

Fig. 2: Ado-Ekiti Reservoir Region

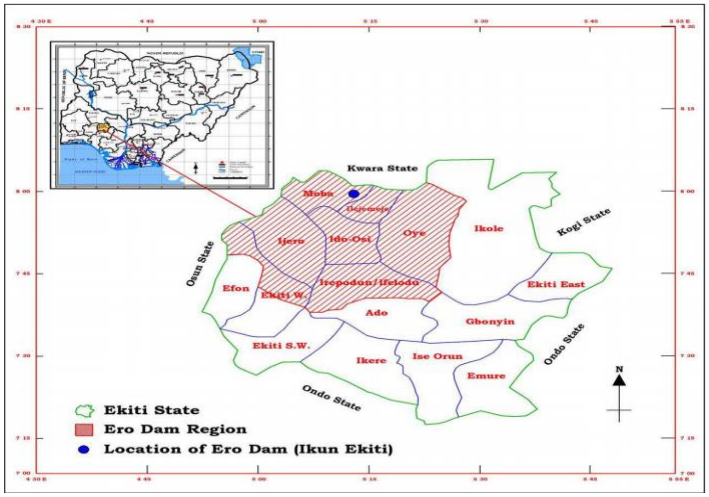

Fig.3: Ero Dam Region. (Source: Ministry of Lands and Housing Ado, Ekiti State, Nigeria, 2013)

Ero Reservoir

Ero reservoir (Res. 2) (Fig. 3) dam is located at Ikun Ekiti in Moba Local Government Area of Ekiti State. The dam is constructed on Ero River which takes its source from the highland region of Orin-Ekiti in Ido-Osi Local Government. The reservoir is located on intersect of latitude $7^{0}35^{1}$N of the equator and on longitude $5^{0}31^{1}$E of the Greenwich meridian. The dam site at Ikun-Ekiti is bounded in the North by Kwara state, in the West by Ikosu-Ekiti, in the South by Ijesamodu-Ekiti and in the East by Ilejemeje Local Government Area. Ikun –Ekiti is a border town between Ekiti state and Kwara state and it is located at about 70km from Ado, the Ekiti state capital. The enlargement of the dam water as it flows is as a result of the contributions of the river tributaries which include Afintoto, Ayo, Igo, Igbegbe, Ipu, Irara, Ilogbe eran and Ofu rivers, as it is usually small at its source.

Procurement of Fish Samples

Fresh samples of adult tilapia fish; (av. 300g) were obtained from fishermen in Ado-Ekiti and Ero reservoir in Ekiti State and the government fishpond, Isinla, Ado Ekiti, Nigeria respectively. The samples were taken twice a month for 3months of February, March and April. The fish were transported immediately to the laboratory. On arrival at the laboratory, the fish were washed immediately and the fillets were separated from the bone. The fish fillets were then washed until it was free from blood. Triplicates of the fresh samples were taken, immediately to Afe Babalola University Laboratory for RSA analysis. Another triplicate set were oven dried at 60^{0}C for 24 hours while another triplicate set was smoke dried in a Chokor Smoking Kiln until about 40% moisture content was obtained.

Radical Scavenging Analysis

The free radical scavenging activity (RSA) of the hydrolysate antioxidants was assessed using the scavenging effect on 1,1, diphenyl-1-picrylhydrazyl (DPPH) free radical. Although there are numerous ways to study antioxidative activity, the use of DPPH is common due to its simplicity and the minimal time required compared to other methods (Wang *et al.*, 2004).

Extraction Procedure

2g of the sample was weighed and macerated in a blender using 50ml of methanol. Then the sample suspension was filtered using Whatman filter paper and resultant filtrate was used for analysis. After

filtration, the filtrate was made to 50ml to make 2g/50ml. 40mg ml^{-1}., 30mg ml^{-1}, 20mg ml^{-1} and 10mg ml^{-1} of the filtrate were prepared from the stock.

RESULTS AND DISCUSSION

The radical scavenging abilities of variously processed *O. niloticus* fish from the various sources, at different concentrations of DPPH are displayed in Table 1. In the control, there was no significant difference (P<0.05) between the three samples (fresh, smoked and oven-dried). At concentration of 10%, the RSA of the fresh sample was significantly different (P>0.05) from the smoked and oven-dried sample, while the smoked sample and oven-dried samples were not significantly different (P<0.05) from each other. The same applies for concentrations 50%, 40%, 30%, and 20% respectively. The fresh sample showed the highest antioxidant properties at all concentrations. The concentration dependent RSA followed the order, Fresh sample>Oven-dried sample>Smoked sample. The fresh sample was a strong radical scavenger with 0.61 mg/mL at concentration 10%.

Table 1: The radical scavenging abilities of variously processed *O. niloticus* fish from the various sources, at different concentrations of DPPH

	FRESH			OVEN DRIED			SMOKED		
Conc. (mg ml^{-1})	**Pond**	**Res. 1**	**Res. 2**	**Pond**	**Res. 1**	**Res. 2**	**Pond**	**Res. 1**	**Res. 2**
Control	0.65^{a}	0. 58^{a}	0.59^{a}	0.63^{a}	0.62^{a}	0.58^{a}	0.58^{a}	0.60^{a}	0.62^{a}
10	0.64^{a}	0.47^{b}	0.57^{a}	0.62^{a}	0.46^{b}	0.54^{ab}	0.56^{b}	0.43^{c}	0.50^{b}
20	0.60^{a}	0.40^{c}	0.56^{a}	0.60^{b}	0.42^{c}	0.52^{b}	0.53^{b}	0.34^{d}	0.42^{c}
30	0.59^{a}	0.35^{d}	0.56^{ab}	0.54^{ab}	0.31^{d}	0.45^{a}	0.49^{b}	0.26^{d}	0.35^{d}
40	0.54^{a}	0.31^{d}	0.55^{ab}	0.48^{b}	0.29^{d}	0.44^{c}	0.45^{c}	0.19^{e}	0.27^{d}

Values with the same subscript within a row are not significantly different at P<0.05. Res. 1= Ado Ekiti reservoir; Res. 2= Ero reservoir

Judging from the trend of the decrease of the RSA as the free radical (DPPH) concentration increases (Table 1), it could be observed that the RSA of the fish from the pond, reservoir 1 (Ado) and reservoir 2 (Ero) was in the order of pond>reservoir 2>reservoir 1.

The scavenging ability obtained in the current study (average of 0.64; 0.45 and 0.57 for the fresh fish from pond, reservoirs 1 and 2 respectively, at 10% concentration) was better than that reported using protein hydrolysate of mackerel (*Scomber austriasicus*) and Sardinella (*Sardinella aurita*) which had scavenging activity of 0.15 and 0.41%, respectively (Wu et al., 2003 and Souissi et al., 2007). The RSA values observed in this work however, are higher than the values reported for this same fish by Wahidu et al, (2014). Variation of antioxidant composition among fish species are mainly affected by fish diets. Higher content of *n3* fatty acids and higher RSA is found in freshwater fish species that use phytoplankton for nutrition (Vujkovic et al. 1999), as does *O. niloticus*. The cultured (pond) fish was found to contain significantly higher (p>0.05) RSA than the reservoir fish probably because compounded feed used in pond culture were supplemented with vitamin C and/or E (ascorbic acid/tocopherol) and other supplements that have antioxidant ability. The higher RSA observed in fish from reservoir 2 than reservoir 1 could be due to nutrient enrichment from the wild. Reservoir 2 (Ero) is fed by more river tributaries than the Ado Ekiti reservoir.

Geckil et al, (2005) earlier commented that increased RSA, as found in this fish, is possibly due to increased solubility within the aqueous medium due to increased percentage degree of hydrolysis (DH). A possible mechanism for DPPH scavenging is the protonation of DPPH to its more stable DPPHH form. Because of its unpaired electrons, DPPH has its maximum absorbance at 520nm. As it gets reduced (electrons get paired off in the presence of the radical scavenger) the absorbance stoichiometrically decreases with respect to the number of electrons taken up. Reports by Souissi *et al.*, (2007) showed that protein hydrolysates with good percentage RSA are rich in histidine, leucine, tyrosine, methionine and cysteine which can enhance the activities of antioxidant peptides (Chen et al., 1996). It is

therefore likely that the fresh fish samples contained all this antioxidant-related amino acids, while some form of damage could have occurred in the smoked and oven-dried samples.

In fresh fish, the balance between the prooxidative and antioxidative factors, which control oxidative reactions, is maintained by numerous factors (Hultin, 1992, 1994; Undeland, 1997). With processing and prolonged storage time the control of oxidation is lost and the onset of lipid oxidation can no longer be prevented (Flick et al., 1992). How long this will take is highly dependent on the type of handling the fish is subjected to and the level of antioxidants present in the fish tissue (Decker, 1998; Petillo et al., 1998).

Processing methods also affect RSA of fish. The effect of processing on tocopherol content in fish has been studied. Erickson (1992) measured the effect of cooking on minced channel catfish. About 60% of the α-tocopherol remained after 5 minutes of heating at 177°C, and over 80% of the γ-tocopherol. Other cooking methods like frying in vegetable oil may even increase the tocopherol concentration in fish. Storozhok (1985) determined the tocopherol content of pelleted whitefish. The α-tocopherol content was 11 mg/100 g tissue in freshly caught fish and 37.1 mg/100 g in whitefish fried in vegetable oil. Same author reported that the α-tocopherol level was only 4.3 mg/100 g in dry cured whitefish. Lighter processing like freezing also affects the tocopherol content of fish. Syväoja and Salminen (1985) measured α-tocopherol in blast-frozen herring fillets. The tocopherol content fell from 420 mg/kg lipid to 270 mg/kg during six months frozen storage that is; over 60% of the α-tocopherol remained. Still, processes like hot-smoking of fall Atlantic mackerel (*Scomber scombrus*) left the vitamin E virtually unchanged (Bhuiyan et al., 1993).

The nutritional composition of fish also varies greatly from one species and individual to another, depending on age, feed intake, sex and sexual changes connected with spawning, the environment and season (Silva and Chamul, 2000).

Seasonal variations in tocopherol content of fish species may be considerable. Syväoja and Salminen (1985) measured tocopherols and tocotrienols in finned fish and fish products and found that fish caught in the spring, the spawning season of most species, had a higher tocopherol content and lower fat content, than those caught in the autumn. Hardy and Mackie (1969) found a decrease in fat content, followed by an increase in tocopherol content in the period from October to March in sprats. It is therefore expedient to study the RSA of *O. niloticus* over various seasons.

CONCLUSION

At various concentrations, the RSA of the fresh sample of *O. niloticus* were found to be significantly different ($P>0.05$) from the smoked and oven-dried sample, while the smoked sample and oven-dried samples were not significantly different ($P<0.05$) from each other. The fresh fish thus proved to be a strong radical scavenger at all DPPH concentrations. The concentration dependent RSA thus followed the order; fresh sample > Oven-dried sample > Smoked sample. The RSA of the fish from the three locations was in the order of pond > reservoir 2 > reservoir 1. The consumption of tilapia should be encouraged to supplement the intake of cereals, which are the main staple foods of developing countries, as they may have significant positive health benefits by inhibiting lipid peroxidation and scavenging free radicals that lead to various diseases and cell degeneration.

The consumption may have significant positive health benefits by inhibiting lipid peroxidation and scavenging free radicals that lead to various diseases and cell degeneration. Since the chemical composition of fish meat vary with various preservation methods and geographical locality of catch, it is essential to determine and evaluate the composition of different fishes in relation to these factors.

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