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ANTIOXIDANT EFFECT OF *CITRULLUS VULGARIS* (WATERMELON) EXTRACT AGAINST LIPID OXIDATION IN FISH DURING COOKING

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ABSTRACT
The Antioxidant effect of Citrullus Vulgaris (Watermelon) extract was evaluated against lipid oxidation in freshly caught fishes during cooking. GC-MS analysis of Hexane and total phenolic extract of Citrullus Vulgaris flesh reveals that the extracts contain 55 compounds which includes 5-hydroxymethyl furfural, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Hexadecanoic acid, ethyl ester and Palmitic acid ester that are reported as antioxidants. Muscle flesh of fifteen (15) samples of freshly caught fish comprising Group I (Tilapia zilli; n=5), Group II (Clarias hollandis; n=5) and Group III (Clarias gariepinus; n=5) were prepared and Sub Grouped each as Control, Treatment 1, Treatment 2, Treatment 3 and Treatment 4 which receives 0, 50, 150, 250 and 450mg of Citrullus Vulgaris extract per 100g fresh fish muscle weight respectively. Treatment 4 gave the highest protection against lipid oxidation in all groups, however the concentration of MDA in Treatment 3 of Group I and III were not significantly ($p>0.05$) higher as compared to Treatment 4 of same Groups. The results clearly suggested that the extract effectively prevented lipid oxidation in fish and that the rate of prevention is a factor of fish species and extract concentration.
Keywords: Fish, citrullus vulgaris, lipid oxidation, Antioxidants, Malondialdehyde (MDA)

INTRODUCTION

Cooking induced a generalised increase in oxidation (Badiani *et al.,* 2002), and the formation of hydroxyl radicals during meat cooking has been demonstrated (Kanner, 1994). Cooking process affects lipid compounds in meat, especially the fatty acids component, changing the nutritional value of cooked products with respect to raw sample (Badiani *et al.,* 2002). Tichivangana and Morrissey, (1982) demonstrated that cooked fish muscle is very susceptible to warmed over flavour (WOF) development which is a result of lipid oxidation. Haywood *et al*., (1985) have shown that heating of culinary oils to 180°C generated a variety of peroxidation products such as aldehydes and their conjugated hydroperoxy diene precursors.

El- Alim *et al.* (1999) demonstrated that the level of free Fe^{2+} greatly increased during cooking, and accelerated lipid oxidation in cooked meat. This indicates that myoglobin serves as source of Fe^{2+}, being readily broken down during cooking process and catalysing autoxidation. Tichivangana and Morrissey (1984) confirmed that the activities of metal ions and heme pigments in catalysis of lipid oxidation in heated fish muscle system indicated that both types of catalysis accelerated lipid oxidation; however, non heme iron is the major pro-oxidant

Functionally, myoglobin is well accepted as an O_2-storage protein in muscle, capable of releasing O_2 during periods of hypoxia or anoxia (George and Daniel, 2004). During cooking, the oxygen binded to myoglobin is released which promotes lipid oxidation (Chan *et al.,* 1997). The pro-oxidant effect of oxymyoglobin towards lipid oxidation is concentration-dependent (Chan *et al.,* 1997).

Practically all quality attributes of food can be affected by the process of lipid oxidation. Thus, aroma changes result from new volatile odorous compounds formed, flavor modifications are caused by hydroxyl acids, the colour darkens as a result of condensation reaction between oxidation products and proteins, and finally, a new texture might be attributed to the oxidative induction of protein cross links (Kanner and Rosenthal, 1992). Not unexpectedly, the nutritive value and safety of food are impaired (Kanner and Rosenthal, 1992). Various processed foods have been reformulated with oils rich in long-chain polyunsaturated fatty acids because of their recognized nutritional benefits, but lipid oxidation has seriously limited their utilization (Frankel, 1996). Lipid peroxidation is a major problem in the food industry. It leads to quality deterioration, rancidity and accumulation of potentially toxic compounds in foods (Paniangvait *et al*., 1995; Gorelik *et al*., 2008).

At the biological level, the oxidation of lipids means damage to membranes, hormones, enzymes and vitamins, which are vital components for the normal cell activity (McBrein and Slater, 1982). At the nutritional level, the oxidation of fatty constituents is the major chemical factor in the loss of food wholesomeness by deterioration of flavor and aroma, as well as in decay of nutritional and food safety qualities (Frankel, 1998).

Biological and nutritional aspects of food lipid oxidation effects have been recently merged; diets based on food containing peroxidized lipids have been related to far-reaching effects such as carcinogenesis, premature aging and other diseases. The physical state of a certain food determines the route, rate and final effect of lipid deterioration (Annunziata, 2005).

Malondialdehyde (MDA) is the major and perhaps the most studied toxic by-product of polyunsaturated fatty acid (PUFA) peroxidation (Del-Rio *et al.*, 2005). Several deleterious effects of MDA have been reported (Riggins and Marnett, 2001; Tesoriere *et al.*, 2002; Cline, *et al.*, 2004; Del-Rio *et al.*, 2005). Exposure to MDA induces intracellular oxidative stress leading to membrane lesions in erythrocytes (Tesoriere *et al.*, 2002). MDA is also genotoxic, reacting with DNA to form highly mutagenic adducts in human cells (Riggins and Marnett, 2001; Cline *et al.*, 2004; Del-Rio *et al.*, 2005). Although MDA is a highly toxic aldehyde, there appears to be no direct data in the literature on its oral LD50 values in humans or experimental animals. However studies have shown that exposure of human erythrocytes to a very low concentration (50 µmolar) of MDA brought about early redox impairment, leading to depletion of reduced glutathione, glucose-6-phosphate dehydrogenase and oxygenated hemoglobin (Tesoriere *et al.*, 2002). MDA values range from 101 – 142 mmoles MDA/g fresh weight for fish, which is far more than the estimated LD50 (Okolie *et al.*, 2009). Results from animal investigations and biochemical studies indicate that ingestion of lipid peroxidation products increases frequencies of tumor and atherosclerosis (Esterbauer, 1993). Thus consumption of MDA-tainted foods may pose serious health risks. Hence, the need to prevent and/or reverse lipid oxidation in foods so as to prolong shelf life of food products and attract consumers appetite, more importantly ensure healthiness for consumption.

The research is aimed at characterising and evaluating the Antioxidant effect of *Citrullus Vulgaris* (Watermelon) extract against lipid oxidation in freshly caught fishes during cooking.

MATERIALS AND METHODS

Sample Collection and Preparation

Citrullus Vulgaris (Watermelon)

The ***Citrullus v.*** used were obtained from Kwanan Sabo fruits and vegetable market in march 2012 at Kano, Nigeria. The samples were washed, deseeded and rind removed, the flesh was homogenized and used for experiment.

n-Hexane Extract of *Citrullus Vulgaris* flesh (Extract A)

The homogenized flesh was then put into n-Hexane following the method of Sadler *et al.* (1990) in an Erlenmeyer flask. The Erlenmeyer flask was then swirled until the colour was completely removed. One extraction was sufficient to completely remove colour. The solution was then filtered and evaporated at 40-60^{0}C to obtain dry extract.

Folin-ciocalteau solution extract of *Citrullus vulgaris* flesh (Extract B)

Total phenols content was obtained using the Folin-ciocalteau procedure of Slinkard and Singleton (1977). Phenols were extracted from the homoge
nized *Citrullus v.* flesh by adding it to a universal solvent (4:4:2:0.01) of acetone, methanol, water and formic acid respectively in the ratio 1g/ml. The mixture was swirled in an Erlenmeyer flask. The mixture was then filtered and the filtrate evaporated at 40-60^{0}C to obtain the dry extract.

Sample Preparation for GC-MS analysis

Extract A and B were simply dissolved using hexane in the ratio 1g of extract to 10ml of hexane respectively.

GC-MS Characterization of the *Citrullus vulgaris* flesh extracts

Characterization of the extracts was carried out by Gas chromatography-Mass spectrometry (GC-MS) analysis using a GCMS-QP2010 PLUS SHIMADZU, JAPAN. The extracts were used separately for the experiment. Hexane was used as the mobile phase and silica capillary GC column (SLB-IL82) as the stationary phase. Each extracts were allowed to flow and elute for 30 minuites.

Fish Samples Collection

Freshly-caught fish samples, 5 each, from three different species were taken, which included *Tilapia zilli* (Tilapia), wild *Clarias gariepinus* and *Clarias hollandis i.e* dotch clarias at New Bussa, Niger State.

Experimental Design

Fish samples were Grouped as Group I (*Tilapia zilli;* n=5), Group II (*Clarias hollandis;* n=5) and Group III (*Clarias gariepinus;* n=5). Each group was sub grouped as Control, Treatment 1, Treatment 2, Treatment 3 and Treatment 4 which receives 0, 50, 150, 250 and 450mg of the mixed (Extract A and B) *Citrullus vulgaris* extract per 100g fresh fish muscle weight respectively.

Effect of *Citrullus v.* in Cooking Fish

Each group of freshly caught fish samples treated with different concentrations of *Citrullus v.* extract were microwave cooked for 30 minutes in the sequence Control, Treatment 1, Treatment 2, Treatment 3 and Treatment 4 and were analysed for Malondialdehyde (MDA) concentration to ascertain the Antioxidant effect of the extract.

Determination of Fish Tissue MDA

Malondialdehyde (MDA) was measured in fish tissue by Pasha and Sadasivadu's method (1984). MDA reacts with thiobarbituric acid (TBA) to generate a pink colored product, which was measured spectrophotometrically. In acidic solution, the product absorbs light at 532nm. TBA test detects only free MDA and measures the amount of free MDA in peroxidising lipid system. The molar extinction coefficient of MDA-TBA product is 1.54×10^5 at 532nm and it was used to calculate the amount of MDA formed.

Statistical Analysis

Comparism between groups was performed using student's paired t-test. Data are given as the mean ± standard error. Statistical significance was accepted at a level of $p<0.05$.

RESULTS

Various chemical constituent of the Extract A and B of *Citrullus Vulgaris* were obtained and results summarised in tables 1 and 2, Peaks are the graphical apex point of eluted constituent in sample whose area is proportional to the quantity of elute, R_t (retention time) is the time taken for the constituent of a peak to be eluted, Hits are number of times the elutes in a peak are released normally as a defined constituent.

Table 3 shows the effect of the extracts on fish lipid oxidation during cooking.

Table 1: Chemical components of Extract A of *Citrullus vulgaris*.

Peaks	R_t	Hits	Formula	Weight	Name
1	5.042	1, 2 & 5	$C_{10}H_{22}$	142	n-Decane
		3 & 4	$C_{11}H_{24}$	156	Undecane
2	5.692	1, 2 & 3	$C_{10}H_{16}O$	152	Perillol
		4	$C_{10}H_{18}O$	154	Nerol
		5	$C_{10}H_{16}O$	152	4-isopropyl-1- cyclohexenyl methanol
3	6.633	1, 2 & 4	$C_{11}H_{24}$	156	Undecane
		3	$C_{10}H_{22}$	142	n-Decane
		5	$C_{13}H_{28}$	184	n-Tridecane
4	8.250	1, 2 & 4	$C_{12}H_{26}$	170	n-Dodecane
		3	$C_{13}H_{28}$	184	n-Tridecane
		5	$C_{16}H_{34}$	226	n- Hexadecane
5	9.833	1, 3 & 5	$C_{13}H_{28}$	184	n-Tridecane
		2 & 4	$C_{12}H_{26}$	170	n-Dodecane
6	11.342	1 & 5	$C_{14}H_{30}$	198	n-Tetradecane
		2	$C_{12}H_{26}$	170	n-Dodecane
		3	$C_{13}H_{28}$	184	n-Tridecane
		4	$C_{16}H_{34}$	226	n- Hexadecane
7	12.750	1, 2 & 3	$C_{15}H_{32}$	212	n-Pentadecane
		4	$C_{16}H_{34}$	226	n- Hexadecane
		5	$C_{13}H_{28}$	184	n-Tridecane
8	14.092	1	$C_{16}H_{34}$	226	n- Hexadecane
		2&4	$C_{14}H_{30}$	198	n-Tetradecane
		3	$C_{13}H_{28}$	184	n-Tridecane
		5	$C_{15}H_{32}$	212	n-Pentadecane
9	16.708	1	C18H38	228	n-Octadecane
		2	$C_{14}H_{30}$	198	n-Tetradecane
		3&5	$C_{15}H_{32}$	212	n-Pentadecane
		4	$C_{17}H_{36}$	240	n-Heptadecane
10	20.400	1	$C_{19}H_{40}$	254	n Nonadecane
		2	$C_{16}H_{34}$	226	n- Hexadecane
		3	$C_{14}H_{30}$	198	n-Tetradecane
		4	$C_{26}H_{54}$	366	7-n Hexyleicosane
		5	$C_{15}H_{32}$	212	n-Pentadecane
11	23.775	1	$C_{18}H_{34}O_2$	282	Oleic acid
		2	$C_{16}H_{30}O_2$	254	9-hexadecanoic acid
		3	$C_{18}H_{34}O_2$	282	6-Octadecanoic acid
		4	$C_{22}H_{42}O_2$	338	Eruric acid
		5	$C_{15}H_{28}O_2$	240	4- Hydroxy-Cyclopentadecanoic
12	25.775	1	$C_{24}H_{50}$	338	Tetracosane
		2	$C_{27}H_{56}$	380	Heptacosane
		3	$C_{34}H_{70}$	478	Tetratriacontane
		4&5	$C_{44}H_{90}$	618	Tetratetracontane
13	27.458	1&2	$C_{24}H_{38}O_4$	390	n-Octylphthalate
		3, 4&5	$C_{24}H_{38}O_4$	390	Isooctylphthalate

Table 2: Chemical components of Extract B of *Citrulus vulgaris*.

Peaks	R_t	Hits	Formula	Weight	Name
1	4.517	1	$C_5H_6O_2$	98	6-oxabicycloHexan-3-one
		2	$C_6H_{10}O$	98	2-Methylcyclopentanone
		3	$C_{10}H_{22}N_2O$	186	Isopentylnitrosopentyl- amine
		4	$C_6H_{10}O$	98	Cyclohexanone
		5	$C_8H_{16}O_2$	144	Hexylacetate
2	8.200	1&2	$C_6H_8O_4$	144	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one
		3	$C_6H_{12}O_2$	116	2,4,5-Trimethyl-1,3-dioxolane
		4	$C_5H_{10}O_2$	102	4-Methyldioxane
		5	$C_6H_{16}N_4$	144	1,2,4,5-tetramethyl-1,2,4,5-Tetraazinane

Table 2 continue

Peaks	R_t	Hits	Formula	Weight	Name
3	9.833	1&2	$C_6H_6O_3$	126	5-hydroxymethyl furfural
		3	$C_8H_{14}O$	126	4-Methyl-4-heptene-3-one
		4	$C_8H_{14}O$	126	4,5-dimethyl-4-hexen-3-one
		5	$C_8H_{14}O$	126	5-methyl-4-hepten-3-one
4	11.050	1	$C_6H_{11}BO_3$	142	1,3,2-Trioaborolate-4,4-dimethyl -5-oxo-2-ethyl
		2	$C_4H_6N_2O$	98	4,5-dihydro-2-methylimidazol -4-one
		3	$C_5H_{11}NO_2$	117	4-methylmorpholine-4-oxide
		4	$C_{15}H_{24}N_6O_6$	348	4,7,11,14,18,21-Hexaaza-1,3,8,10,15,17-hexaoxacyclobeneicosane-4,6,11,13,18,20-hexaene
		5	$C_6H_{13}NO$	115	2,6-Dimethylmorpholine
5	20.317	1&2	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
		3	$C_{14}H_{28}O_2$	228	Tetradecanoic acid
		4	$C_{20}H_{40}O_2$	312	Arachidonic acid
		5	$C_{15}H_{30}O_2$	242	Pentadecanoic acid
6	23.275	1	$C_{18}H_{34}O_2$	282	Oleic acid
		2	$C_{16}H_{30}O_2$	254	9-Hexadecanoic acid
		3	$C_{22}H_{42}O_2$	338	Eruric acid
		4	$C_{15}H_{28}O_2$	240	2-Hydroxycyclopentadecane
		5	$C_{18}H_{34}O_2$	284	6-Octadecenoic acid
7	27.433	1&2	$C_{24}H_{38}O_4$	390	Dioctylphthalate
		3&4	$C_{24}H_{38}O_4$	390	Isooctylphthalate
		5	$C_{24}H_{38}O_4$	390	phthalic acid, bis(2-ethylhexyl)este

Table 3: Effect of *Citrullus vulgaris* extracts on MDA values in freshly caught fish during microwave cooking.

	Group I	Group II	Group III
Control	2.09 ± 0.08	2.43±0.15	2.63±0.15
Treatment 1	0.98 ± 0.11*	1.44±0.12*,[a]	1.22±0.25*
Treatment 2	0.87 ± 0.07*	1.38±0.16*,[a]	0.74±0.21*
Treatment 3	0.82 ± 0.18*	1.33±0.17*,[a]	0.62±0.03*
Treatment 4	0.77 ± 0.04*	0.84± 0.02*	0.54±0.12*

Values are expressed as mean ± standard error (µmol/g fresh weight) of n=5; Values bearing * are significantly different from control at $p<0.001$ in the same Group and values bearing [a] are significantly different from Treatment 4 at $p<0.05$ in the same Group. Group I (*Tilapia Zilli),* Group II (*Clarias hollandis)* and Group III (*Clarias gariepinus),* Treatment 1=50mg/100g, Treatment 2=150mg/100g, Treatment 3=250mg/100g and Treatment 4= 450mg/100g.

DISCUSSION

GC-MS Characterisation of *Citrullus v.* extracts

Table 1 and 2 shows the chemical components of Extract A and B. The results depict that the extracts contain 55 compounds, 25 from extract A and 30 from extract B. Saturated and unsaturated short- and long-chain fatty acids phynolic compounds etc were identified. Among which 5-hydroxymethyl furfural has been reported as an electron acceptor and preventing oxidation (Fredrik and Barbel, 2002). 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl- was also reported to play Antimicrobial, anti-inflammatory and antioxidant activity (Praveen, *et al*., 2010). Hexadecanoic acid, ethyl ester and palmitic acid ester were also reported to play Antioxidant role (Praveen, *et al*., 2010; Kumar, *et al*., 2012).

Study reveals that Nerol (23mmol/kg diet) was fed to male buffalo rats for 14 days before and for 42 days after the transplant of morris 7777 heoatoma. Tumor growth was suppressed at $p<0.001$ (Yu, *et al.,* 1995). Perillol, was shown to have anti-inflamatory, and antioxidant effect, it also subside artheroschelosis (Brenner, 1993).

Effect of *Citrullus v.* extracts on fish lipid oxidation during cooking

Table 3 shows results on cooked fish species treated with different concentrations of *Citrullus v.* extract. The results demonstrate that the MDA concentration was significantly higher ($p<0.001$) in the control samples compared to those treated with the extracts. The formation of MDA is also related to the extract concentration: the higher the concentration of the extract, the lower the MDA formed after cooking.

The whole set of data clearly suggests that the highest concentration of extract (450mg/100g) i.e. Treatment 4 gave the highest protection against lipid oxidation, however the concentration of MDA in treatment 1, 2 and 3 of Group I and Group III were not significantly higher ($p>0.05$) than treatment 4 of the same Groups. From this, it was observed that concentrations below 450mg/100g of the extract down to 50mg/100g concentration did not make any difference and can efficiently retard lipid oxidation in Group I and Group III like the highest concentration used but not in Group II were the values obtained for treatment 1, 2 and 3 are significantly different ($p<0.05$) from treatment 4 suggesting that higher concentrations above 450mg of the extract may prevent oxidation better.

The likely reasons for the variations in pattern of lipid oxidation inhibition among different species using the extracts may be as a result of the susceptibility of meat to lipid peroxidation which is dependent on animal species, muscle type, and anatomical location (Rhee and Ziprin, 1987; Rhee *et. al.,* 1996). The content, composition, and quality of dietary fat in feed and the tendency of animal species to store fatty acids into membrane phospholipids which can affect the fatty acid composition of membrane and its susceptibility to lipid peroxidation (Rhee *et. al*., 1996; Rhee and Ziprin, 1987; Song and Miyazawa, 2001).

It can also be observed that the extract does not completely halt the oxidation process even at the highest concentration used and can therefore be hypothesised that this may be due to some possible oxidation routes that cannot be prevented by the extract.

CONCLUSION

From the results, it may be concluded that the extract significantly retarded or prevented oxidation in fish samples and the pattern of antioxidation depend on fish species. The MDA values in treated freshly caught fish decreases with increase in concentration of extract. The extract may be more efficient if in synergy with other antioxidant extracts from different sources. The mechanism with which the constituent antioxidants function in preventing the lipid oxidation process should be elucidated to enhance utilisation.

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