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INFLUENCE OF SMOKING AND NATURAL PRESERVATIVES ON SHELF – LIFE AND MICROBIAL QUALITY OF *Clarias gariepinus* DURING STORAGE

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ABSTRACT

This study investigated the shelf – life and microbial quality of smoked *Clarias gariepinus* using Onion Bulb (OB), Holy Basil (HB) and Turmeric Rhizome (TR) as preservatives during 56 days storage. Sixteen *C. gariepinus* (1-1.5kg) were distributed to four experimental containers: Control, TR2, OB3, and HB4 and the experiment were carried out in triplicates. *Clarias gariepinus* were smoked in a smoking kiln at 40 °C- 60 °C and 120-150 °C for 6 and 18 hours respectively. Biochemical parameters, organoleptic assessment, and microbial analysis were carried out. Data were analyzed using ANOVA at P= 0.05. The result showed that the crude protein of *C. gariepinus* was higher in the treated groups compared to the control. Also, the result shows that the biochemical parameters, organoleptic assessment and the microbial loads in smoked *C. gariepinus* were reduced in the OB, HB, and TR than the control at 1 day, 28 days and 56 days storage respectively. It can be concluded that the natural plants may enhance the shelf life, consumer acceptability, and inhibit the growth of the microbial pathogen in smoked fish.

Keywords: Catfish, Onion bulb, Holy basil, Microbial loads, Turmeric, Preservatives

INTRODUCTION

Clarias gariepinus is a very important freshwater fish in Nigeria (Kumolu-Johnson *et al*., 2010). The quality losses in this fish species can occur very rapidly after catch leading to spoilage due to factors such as moisture, microbial growth, oxygen and temperature (Opara *et al*., 2013). However, interest in bio-preservation of food systems has increased because of the increase in bacterial resistance to synthetic antibiotics (Rahman, 2007). Therefore, it is very important to explore safe and inexpensive alternatives such as natural plants that have antimicrobial and antioxidants properties.

More attention is being given to the presence of phenolic compounds (phenolic acids, polyphenols, and flavonoids) in plants, herbs and spices, because of their antioxidant activity and antimicrobial properties (Sacchetti *et al*., 2005). The health-promoting effect of antioxidants from plants is thought to arise from their potential effects on the reactive oxygen/nitrogen species. Plant-derived essential oils and extracts of various species have long been used as natural agents for food preservation in food and beverages due to the presence of antimicrobial compounds (Nychas *et al*., 2003). Consumption of food containing natural aromatic plant extracts is expected to prevent the risk of many free radical-mediated diseases (Rahman, 2007).

The effect of curing by smoking for quality and shelf life of the product depends on the preparation of raw material, the type of smoking, relative humidity, temperature, density and composition of the smoke and time of smoking (Doe *et al*., 1998). Onion (*Allium cepa*) bulb, holy basil (*Ocimum sanctum)* and turmeric *(Curcuma longa)* rhizome possess natural antioxidant and antimicrobial properties which could be explored as a preservative for several purposes (Omage, 2015). However, there is no adequate information on the antibacterial and antioxidant uses of onion, turmeric and holy basil as fish preservatives. Hence, there is a need to investigate the potential of onion bulb, turmeric rhizome and holy basil as bio-preservatives in the fish processing industry.

MATERIALS AND METHODS

Plant Identification, Treatment and Fish Sample Preparation

Three plants (onion bulb, turmeric rhizome, and holy basil) were used during the experiment and were identified at the Department of Biological Sciences (Botany Programme), Olusegun Agagu University of Science and Technology, Okitipupa. The fresh plants (onion bulb, turmeric rhizome,

and holy basil leaves) were washed with sterile distilled water, ground with hammer mill (4500 g of each plant was homogenized with 4.5 litres of sterile distilled water) and 3500 g of water extract of each of the fresh plants was transferred into a plastic container (45 litres capacity). The *C. gariepinus* were prepared as described by Isaac *et al.*, (2014). The *C. gariepinus* (1.0 – 1.5 kg) were killed by severing the spinal cord with a sterile knife and aseptically eviscerated, washed and rinsed in distilled water and placed on a tray. The fish were bent, hooked together with a wooden stick and were soaked for 2 hours in the water extracts of onion bulb, turmeric rhizome, and holy basil leaves separately per treatment. The fish were cold-smoked between 40 °C- 60 °C for 6 hours and hot smoked between 120 °C-150 °C for 18 hours for proper drying. The fish were removed after 18 hours, allowed to cool and placed in perforated experimental plastic containers for storage.

Experimental Design and System

The experiment was a completely randomized design and was carried out in triplicates with 16 pieces of fish per replicate. The smoked fish was kept at room temperature in 12 plastic containers at the Fisheries and Aquaculture Technology Laboratory, Olusegun Agagu University of Science and Technology, Okitipupa. Fish were allocated randomly into each treatment as follows: Control, Turmeric Rhizome (TR2), Onion Bulb (OB3), and Holy Basil (HB4). The experiment lasted for eight weeks.

Isolation of Microorganism/Counts

One gram (1 g) representative tissue of each sample was obtained aseptically from the smoked catfish (*C. gariepinus*). They were separately macerated and put into the sterile capped bottle containing sterilized peptone water (10 ml) and homogenized (Shalaby *et al.*, 2006). Serial dilution was carried out and 1 ml each from 10^{1} to 10^{-5} dilution factors were dispensed into Petri dishes that were appropriately labelled. For total viable counts and Enterobacteriaceae counts, molten sterile medium (MacConkey agar and Nutrient agar) were allowed to cool after sterilization to about 45 °C before they were poured aseptically into each labelled Petri dishes and were swirled gently for even distribution of inocula and allowed to gel and then incubated at 37 °C for 24 hrs. The organisms grew into visible different colonies after 24 hours. Visible colonies were counted and recorded as total viable counts and Enterobacteriaceae counts, the result was expressed as $\log_{10}$ Colony Forming Unit, CFU/g.

Biochemical Assessment

Total Volatile Base (TVB), Free Fatty Acid (FFA), Peroxide Value (PV) and Thiobarbituric Acid Level (TBA) were determined as described by Conway (1968), Pearson (1968), AOAC (2005), and Antonia da Silva (2002) respectively.

Organoleptic Assessment

Eight (8) trained panelists were constituted for the organoleptic test by using the hedonic scale of 1-7. Score was given to OB, HB, TR and the control with fish samples scoring less than 2 regarded as unacceptable. An evaluation was done at 1 day, 28 days and 56 days storage on taste, flavour, odour, texture, appearance and overall acceptability. The mean of taste, flavour, odour, texture, appearance and overall acceptability during this period was calculated and recorded.

pH Determination

Five grams of each sample was homogenized in 30 ml of sterile distilled water by autoclaving at 121 °C for 15 minutes, and the sample homogenate was allowed to stay for 30 min before the reading was taken. The pH of each sample was measured at 1 day, 28 days and 56 days storage.

Analytical Method

One smoked fish from each treatment was taken at 1 day, 28 days and 56 days storage and analyzed for their proximate composition using the methods of Association of Official Analytical Chemists (AOAC, 2005).

Statistical Analysis

Data that resulted from the experiments were subjected to a one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Science 2006 version 20.0). Duncan's multiple range tests were used to compare the differences among individual means at $P = 0.05$.

RESULTS

Proximate Composition of Smoked *C. gariepinus* Treated with Turmeric, Onion Bulb, and Holy Basil

The highest crude protein was recorded in treated groups while the lowest was in control. The values of crude protein decreased as the days of storage increased. The highest moisture content was recorded in control at 1 day, 28 days and 56 days storage and lowest recorded in OB 3 at 56 days storage. There was a general increase in the ash content of all the treatments at 56 days compared to 1-day storage and there were significant differences ($p < 0.05$) among the treatments. The values of Nitrogen Free Extract (NFE) were increased at 56 days storage compared to the value obtained at 1- day storage and they were significantly different ($p < 0.05$) among the treatments (Table 1).

Table 1: Proximate Composition of Smoked *C. gariepinus* Treated with Turmeric, Onion Bulb, and Holy Basil for 56 Days.

DURATION	TREATMENT	MOISTURE	CRUDE PROTEIN	ASH	ETHER EXTRACT	NFE
1 day	Control	4.23±0.01^{c}	73.63±0.05^{d}	11.36±0.05^{c}	8.44±0.01^{d}	2.30±0.01^{a}
	TR2	3.21±0.02^{a}	72.21±0.01^{b}	7.55±0.02^{a}	8.24±0.01^{a}	8.79±0.02^{d}
	OB3	3.20±0.02^{a}	72.32±0.01^{c}	13.17±0.01^{d}	8.31±0.01^{b}	3.0±0.03^{b}
	HB4	3.82±0.05^{b}	71.41±0.05^{a}	9.86±0.02^{b}	8.38±0.03^{c}	6.52±0.04^{c}
28 days	Control	6.32±0.02^{c}	65.73±0.01^{b}	11.52±0.04^{b}	6.89±0.03^{d}	9.54±0.03^{a}
	TR2	4.27±0.02^{a}	64..61±0.06^{a}	13.85±0.01^{d}	6.42±0.05^{c}	10.85±0.04^{b}
	OB3	4.31±0.02^{a}	65.23±0.03^{b}	12.96±0.07^{c}	5.92±0.00^{a}	11.58±0.03^{c}
	HB4	4.42±0.01^{b}	63.41±0.02^{a}	9.86±0.09^{a}	6.18±0.03^{b}	16.13±0.03^{d}
56 days	Control	9.39±0.01^{d}	40.59±0.01^{a}	11.8±.0.00^{a}	3.56±0.04^{b}	34.56±0.01^{c}
	TR2	7.31±0.01^{b}	42.33±0.01^{b}	15.98±0.01^{d}	3.72±0.01^{d}	30.87±0.02^{a}
	OB3	6.51±0.01^{a}	43.31±0.02^{c}	15.54±0.01^{c}	3.51±0.01^{a}	31.12±0.01^{a}
	HB4	6.51±0.01^{a}	43.35±0.0^{c}	12.52±0.002^{b}	3.66±0.01^{c}	33.95±0.04^{b}

Key: The mean values in the column with the same superscripts are significantly different ($p < 0.05$). TR= Turmeric Rhizome, OB= Onion Bulb, HB= Holy Basil

Biochemical Composition of *C. gariepinus* Treated with Turmeric, Onion Bulb, and Holy Basil

The value of thiobarbituric acid, free fatty acid, peroxide value and total volatile base recorded during the study decreased in treated groups compared to the values obtained in the control at 1- day storage and 56 days storage (Table 2).

Table 2: Biochemical Composition of Smoked *C. gariepinus* Treated with Turmeric, Onion Bulb, and Holy Basil Extract

Duration	Parameters	Control	TR2	OB3	HB4
1 day	PV	31.68±0.01^{d}	30.82±0.01^{c}	20.45±0.05^{a}	29.83±0.02^{b}
	TVB	13.21±0.01^{a}	21.86±0.01^{b}	22.56±0.02^{b}	22.78±0.01^{b}
	FFA	0.72±0.01^{d}	0.62±0.015^{b}	0.53±0.00^{a}	0.70±0.01^{c}
	TBA	1.47±0.01^{c}	1.35±0.01^{b}	1.27±0.015^{a}	1.24±0.01^{a}
28 days	PV	32.87±0.02^{d}	29.18±0.01^{c}	21.58±0.01^{a}	27.44±0.02^{b}
	TVB	15.71±0.08^{a}	21.69±0.01^{c}	20.65±0.02^{b}	26.00±0.01^{d}
	FFA	0.82±0.00^{b}	0.52±0.01^{a}	0.48±0.00^{a}	0.50±0.01^{a}
	TBA	1.64±0.02^{c}	1.35±0.01^{b}	1.15±0.02^{a}	1.14±0.01^{a}
56 days	PV	34.13±0.015^{d}	20.06±0.015^{c}	18.33±0.015^{a}	18.68±0.02^{b}
	TVB	22.87±0.01^{c}	13.61±0.02^{bc}	12.46±0.012^{a}	13.40±0.02^{b}
	FFA	0.88±0.01^{d}	0.32±0.005^{b}	0.46±0.015^{c}	0.14±0.01a
	TBA	1.79±0.01^{d}	0.95±0.005a	1.09±0.01^{b}	1.11±0.00c

Key: The mean values in the column with the same superscripts are significantly different ($p < 0.05$). TR= Turmeric Rhizome, OB= Onion Bulb, HB= Holy Basil, PV= peroxide value, TVB=total volatile base, FFA= free fatty acid, TBA= thiobarbituric acid

Organoleptic Assessment of Smoked *C. gariepinus* of Turmeric Rhizome, Holy Basil and Onion Bulb

The results of the organoleptic assessment of *C. gariepinus* at 1- day, 28 days and 56 days storage revealed that treated groups had better overall acceptability when compared to the control. There was a general decrease in appearance, quality, texture, odour, flavour, taste, and overall acceptability as the storage period increased. Turmeric rhizome and onion bulb treatments had the best overall acceptability at 1- day, 28 days and 56 days storage. However, there were no significant differences (P>0.05) among the treatments (Table 3).

Table 3: Organoleptic Assessment of Smoked *C. gariepinus* Treated with Turmeric Rhizome, Holy Basil and Onion Bulb

DURATION	TREATMENTS	ODOUR	FLAVOUR	TEXTURE	TASTE	APPEARANCE	OVERALL ACCEPTABILITY
1 day	CONTROL	5.8±0.5^{a}	6.3±1.5^{a}	6.7±1.50^{a}	7.4±2.00^{a}	ND	6.5±1.35^{a}
	TR2	8.5±0.85^{a}	9.3±0.00^{a}	7.7±0.30^{a}	9.2±0.15^{a}	ND	8.7±0.6^{a}
	OB3	9.5±0.5^{a}	9.7±1.35^{a}	8.2±1.15^{a}	8.7±1.05^{a}	ND	8.6±1.00^{a}
	HB4	8.1±0.5^{a}	8.3±0.7^{a}	7.3±0.3a	8.7±1.05^{a}	ND	8.1±0.6^{a}
28 days	CONTROL	5.6±0.5^{a}	5.3±1.30^{a}	5.5±0.65^{a}	6.0±0.00^{a}	7.8±1.00^{b}	5.8±1.35^{a}
	TR2	7.3±0.8^{a}	7.1±1.00^{a}	8.8±0.85^{a}	8.0±0.35^{b}	9.0 ±0.64^{b}	8.5±0.6^{a}
	OB3	6.8±0.8^{a}	8.3±0.00^{a}	9.2±0.15^{a}	8.3±0.5^{b}	8.0±0.35^{ab}	8.4±1.00^{a}
	HB4	6.6±1.00^{a}	5.7±0.35^{a}	7.2±0.15^{a}	6.o±0.00^{a}	6.5±0.5^{a}	8.3±0.05^{a}
56 days	CONTROL	2.8±0.5^{a}	1.5±0.9^{a}	2.8±2.25^{a}	0.3±0.00^{a}	4.8±0.5^{a}	3.0±0.2^{a}
	TR2	5.8±0.2^{b}	5.2±0.2^{ab}	6.9±1.35^{ab}	4.7±0.35^{b}	6.9 ±0.65^{a}	5.9±0.4^{a}
	OB3	5.8±0.2^{b}	5.9±1.7^{b}	6.3±1.3^{b}	5.5±1.85^{b}	6.0±1.25^{a}	6.0±1.3^{a}
	HB4	4.3±0.9^{a}	3.3±0.7^{ab}	5.8±0.8^{ab}	2.3±0.3^{ab}	6.5±0.85^{a}	4.7±0.9^{a}

Key: The mean values in the column with the same superscripts are significantly different (p< 0.05). TR= Turmeric Rhizome, OB= Onion Bulb, HB= Holy Basil, ND= Not determined

Microbial Analysis of Smoked *C. gariepinus* Treated with Turmeric Rhizome, Holy Basil and Onion Bulb

The OB, HB and TR treatments recorded lower values of Enterobacteriaceae and total viable counts when compared with the control and there were significant differences (P<0.05) among the treatments. Onion Bulb (OB 3) recorded the lowest Enterobacteriaceae and total viable counts while the highest was recorded in control (Table 4).

Table 4: Microbial Analysis of Smoked C. *gariepinus* Treated with Turmeric, Onion Bulb and Holy Basil (log_{10} Colony Forming Unit, CFU/g)

	1 day		28 days		56 days	
Treatment	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts	Enterobacteriaceae counts	Total viable counts
Control	6.05±0.02^{b}	6.07±0.01^{b}	6.31±0.01^{d}	6.34±0.01^{d}	6.35±0.01^{c}	6.42±0.01^{d}
TR2	5.95±0.02^{a}	5.98±0.01^{a}	5.89±0.01^{a}	6.03±0.05^{b}	6.24±0.01^{ab}	6.29±0.01^{c}
OB3	5.94±0.01^{a}	5.96±0.01^{a}	5.95±0.04^{b}	5.99±0.01^{a}	6.10±0.01^{a}	6.23±0.05^{a}
HB4	5.83±0.01^{a}	5.99±0.01^{a}	6.06±0.03^{c}	6.22±0.01^{c}	6.22±0.01^{ab}	6.28±0.02^{b}

Key: The mean values in the column with the same superscripts are significantly different (p< 0.05). TR= Turmeric Rhizome, OB= Onion Bulb, HB= Holy Basil

The pH of Smoked *C. gariepinus* Treated with Turmeric Rhizome, Onion Bulb, and Holy Basil

The pH of the treated sample was lower than the control at 1 day of storage compared to 28 days and 56 days storage. The highest pH value was recorded in control (6.08±0.01, 6.05±0.01 and 6.06±0.01) at 1 day, 28 days and 56 days storage while the lowest pH was recorded in OB3 (6.04±0.05, 6.02±0.05 and 6.01±0.01) at 1 day, 28 days and 56 days storage (Table 5).

Table 5: pH of smoked *C. gariepinus* Treated with Turmeric, Onion Bulb, and Holy Basil

Treatment	1 day	28 days	56 days
Control	6.08 ± 0.01^{a}	6.05 ± 0.01^{a}	6.06 ± 0.01^{a}
TR2	6.05 ± 0.00^{a}	6.03 ± 0.01^{a}	6.02 ± 0.02^{a}
OB3	6.04 ± 0.05^{a}	6.02 ± 0.05^{a}	6.01 ± 0.01^{a}
HB4	6.04 ± 0.05^{a}	6.03 ± 0.01^{a}	6.03 ± 0.01^{a}

Key: The mean values in the column with the same superscripts are not significantly different ($p< 0.05$). TR= Turmeric Rhizome, OB= Onion Bulb, HB= Holy Basil

DISCUSSION

The proximate composition of smoked catfish may be used in considering the freshness and safety of the fish. The result shows that proximate composition (crude protein, ash content, ether extract, moisture, and nitrogen free extract) were better in onion bulb, holy basil and turmeric rhizome treatments when compared to the control. The results of the crude protein content of this study revealed an increase in the value of the fish at 1- day storage this might be due to an increase in dry matter content per unit of weight following sample dehydration during smoking but the values decreased in all the treatments at 56 days of storage. This result was similar to the work of Antonia da Silva (2002) who reported an increase in the crude protein of smoked blue catfish *(Ictalurus furcatus*) treated with antimicrobial agents and antioxidants during 6-week storage at room temperature. Also, Tao and Linchun (2008) and Isaac *et al*., (2014) recorded an increase in the crude protein of grass Carp (*Ctenopharyngodon idellus*) filets and smoked *C. gariepinus* on different concentration of preservatives during ambient storage which was similar to the present findings. A higher value obtained in the crude protein of the treated groups may be due to preservatives effects of these plants which slow down autolysis in the fish muscles and consequently slow down the protein break down.

The result of the peroxide value revealed that the values increased as the storage period increased. The treated groups recorded lower peroxide values compared to the control and there were significant differences ($P< 0.05$) among the treatments. A similar result was observed by Antonia da Silva (2002) and Isaac *et al.* (2014) who also recorded an increase in peroxide value of smoked *C. gariepinus* as the week of storage increased. The results of thiobarbituric acid revealed that the values increased as the weeks of storage increased. The treated groups recorded lower values of thiobarbituric acid compared to the control and there were significant differences ($p<0.05$) among the treatments. This might be a result of anti-oxidant (vitamin C) and antimicrobial agents (flavonoids, saponins and tannins) present in turmeric, onion bulb, holy basil. A similar result was observed by Isaac *et al.,* (2014) who reported that the thiobarbituric acid of smoked *C. gariepinus* increased as the week of storage increases.

The result of free fatty acid in this present study showed that the values were higher at 1-day storage but the values decreased as the weeks of storage increased. Although the study showed lower values at 56 days storage, the values recorded in the treated groups were better than the control. There were significant differences ($P <0.05$) among the treatments. This might be a result of the action of turmeric, onion bulb and holy basil preservatives. The result of the total volatile base revealed that the values increased as the weeks of storage increased. The treated groups recorded lower values of the total volatile base compared to the control and there were

significant differences (P <0.05) among the treatments. This result was similar to the result of Antonia da Silva (2002) who reported that the total volatile base increased as the week of storage increased in the non-treated group but low in the treated group of smoked *C. gariepinus*.

The result of the study on organoleptic assessment such as taste, flavour, texture, appearance, odour and overall acceptability of the smoked *C. gariepinus* were higher at 1- day storage than the values at 28 days and 56 days storage. The acceptability decreased as weeks of storage increased and this result was in agreement with the report of Omojowo *et al., (*2010) and Isaac *et al.,* (2014) who reported a decrease in acceptability of smoked catfish *C. gariepinus* during storage. The treated groups had better overall acceptability when compared to the control. The results of the study revealed lower microbial loads in treated groups compared to the control. The control recorded higher values in Enterobacteriaceae counts and total viable counts and there were significant differences (P <0.05) among the treatments. The lower microbial loads observed in treatments that had natural plants as compared to the control could be attributed to the antimicrobial properties and anti-oxidant properties present in the turmeric rhizome, onion bulb and holy basil (Omage, 2015). Antimicrobial action and antioxidant properties of these natural plants slow down the growth of bacteria for extended periods, assuring a long shelf life and increased product safety. This result supports the report of Oluborode *et al*., (2010) and Isaac *et al*., (2014) who observed a decrease in the microbial loads of smoked *C. gariepinus* on different concentration of preservatives compared to the untreated group.

The results of the present study show a decrease in pH (hydrogen ion concentration) at 28 days of storage compared to 1 day of storage. There was no significant difference (P > 0.05) between 1 day, 28 days and 56 days storage. This result was similar to the report of Antonia da Silva (2002) who also reported a decrease in the pH of smoked *C. gariepinus* as the week of storage increased.

CONCLUSION

This study revealed that the utilization of natural plants such as onion bulb, holy basil, and turmeric rhizome on *C. gariepinus* can extend the shelf life and flesh quality of the fish for 56 days storage period. It is therefore recommended that research on the combination (synergy) of these plants should be carried out on *C. gariepinus* or other species of fish.

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