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Research Paper

Microbial evaluation of *Carica papaya* leaf extract pre-treated smoke cured grass eater (*Distichodus rostratus* Gunther 1864)

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The sensory and microbial evaluation of *Carica papaya* leaf extract pre-treated smoke cured *Distichodus rostratus* (Grass eater) was studied. Twelve (12) fish samples weighing between 0.80-1.80 kg were weighed eviscerated and washed properly with tap water. These were randomly divided into 4 groups of 3 fish each (T_1, T_2,T_3 andT_4). T_1 was immersed into 3% Brine only. T_2 was immersed in a mixture of 3% brine and 2.5% *C. papaya* leaf extract). T_3 was immersed into 3% Brine and 5% *C. papaya* leaf extract. T_4 was immersed into 3% Brine and 8% *C.papaya* leaf extract. These mixtures were thoroughly mixed and allowed to stand for three hours. Soaked samples were poured out on muslin clothing for draining and cured with wood smoke on a brick kiln for four hours at a temperature range of 85-95°C. The cured samples were left overnight to cool and were wrapped in sterile polythene bags and kept in a refrigerator for microbial analysis. The samples were subjected to microbial analysis, culturing, incubating, colony count and statistical analysis. Increasing concentrations of water extract of *C. papaya* leaf inhibited microbial growth at all dilutions (highest T_1 {control} was 2.6 x 10^7 cfu/ml while T_2= 2.2x10^5 cfu/ml, T_3 = 1.5 x 10^5 cfu/ml and T_4= 1.24 x 10^4 cfu/ml ,decreased in that order). Probable organisms in T_1 were *Staphylococcus aures*. T_2 and T_3 had *Proteus* sp. T_4 had *Klebsiella* sp. Biochemical characterisation of pure culture isolates confirmed the presence of these organisms. The fungi found were *Aspergillus* (T_1*), Penicillum* (T_2 andT_3) and Mucor (T_4). These results show that usage *of C. papaya* in processing and preservation of fish will improve the shelf life of stored fish.

Key words: Microbial; Grass-eater; Evaluation; leaf-extract; Smoke-cured; *Carica-papaya*; Shelf life.

INTRODUCTION

Estimates show that about a million people worldwide rely on fish as their primary source of animal protein (Mello *et al.,* 2008). Fresh fish is perishable. Various methods have been devised to prolong its' shelf life. Smoke curing is the predominant means of fish preservation in the third world. Unfavourable environmental conditions shorten the shelf life of smoke cured fish (Nisar -Ahmad, *et al.,* 2011). Vital nutrients, chemical spoilage, microbial spoilage and consequent economic losses are associated with this problem (Robert Kral, 1997).This results in scarcity of protein and fish mongers and Artisanal fishermen do not reap the full benefits of their effort (HAGS, 2002). Deleterious results have been obtained from use of synthetic materials to check microbial and chemical spoilage of fish (Harper Douglas, 2012). Due to this problem, attention has been turned to spices and other plant materials that are user friendly and medicinal in the processing and

preservation of fish and meat products (Doughari, 2007 and Doughari 2006) UNICAF, 2014). Several of these including Carica papaya, make the long list that serve as food, medicine, etc. and grow well in the West African sub region. *C. papaya*, a member of the family Caricacaea are palatable feedstuff whose leaves, fruits and byproducts are used to feed domestic animals (Dawkins, 2003). Extracts of different parts of the plant have antibiotic properties (Baskaran et al., 2002; Fakeye et al., 2007; Ayoola and Adeyeye, 2010; Ahmed et al., 2010). The enzyme, Papain from *C. papaya* is still in use as a meat tenderizer of which the leaves contain 2% of the enzyme. Latex from the plant is known to heal wounds while the leaves are used in dressing wounds. The plant does not contain cyanogenic glycosides and is thus safe for inclusion into edible products (Imaga et al., 2009). Extracts of dried leaves of the plant have demonstrated antioxidant properties (Onah, 2002; Clucas,1996). *Distichodus rostratus* (Grass eater) is commonly landed by artisanal fishermen and it is a common fish commodity on the table of fish mongers in fish markets (Idodo, 2003) and therefore deserves to be well preserved since they are caught in large quantities. Literature search does no show that *C. papaya* leaves have been used in fish preservation. The aim of this work is to evaluate the inhibitory potentials of aqueous leaf extracts of *C. papaya* on the microbial load of smoke cured pre-treated fish in an attempt to extend its shelf life.

MATERIALS AND METHODS

Sample collection

12 Specimens of *D.rostratus* of mean weight 1120 ± 0.45 g were purchased from Yenagoa, Bayelsa State and transported to the fish processing laboratory of Imo State Polytechnic, Umuagwo, Ohaji, South eastern Nigeria, in a 20 L ice chest filled with crushed ice but without direct contact to avoid microbial cross contamination.

Sample preparation

Fish were sorted into four groups (T_1, T_2, T_3 and T_4), weighed, degutted, washed and weighed again to obtain the dress weight. The extracts were made by soaking 250 g, 500 g and 800 g of squashed *C. papaya* leaves in 10 L of water to which 300 g of salt had been added for 4 hours. These were soaked for one hour as follows: T_1 + 3% brine, T_2 + 3% brine + 2.5% *C. papaya* extract,T_3 + 3% brine + 5% *C. papaya* extract and T_4 + 3% brine + 8% *C. papaya* extract.

Fish processing

The extract pre-treated samples were then drained on muslin and cured with wood smoke for 4 hours with turning at intervals to prevent charring and promote even curing, according to the methods of Agbabiaka, (2002). Cured fish was left under ambience for one day. Fish was weighed to obtain dry weight before being wrapped in sterile polythene bags and storage in refrigerator until analysis.

Biological evaluation

The weight loss was computed as follows:
Dressed weight = Carcass weight- weight of offals
Total weight loss= Carcass weight- weight after smoking
%weight loss= (Total weight loss/ Carcass of fish) ×100.

Microbiology

The procedures described in Agbabiaka et al., (2016) were adopted.

Media preparation

Nutrient Agar (NA), Eosin Methylene Blue (EMB) Agar and Potato Dextrose Agar (PDA) were used for the media preparation according to Cheesebrough, (2000). 28 g of Nutrient Agar (NA), 31.2 g of Potato Dextrose Agar (PDA), and 17.28 g of Eosin Methylene Blue (EMB) Agar were measured out according to the manufacturer's direction. Thereafter, the three measured media were dispersed into 3 conical flasks and 1litre of distilled water was added respectively and shook vigorously for proper mixing. The media were autoclaved for 15 minutes at 120°C and cooled at room temperature respectively. After that, 20 ml of each of the media were poured into 12 plates, i.e., quadruplet per agar sample. Other instruments such as wire loop, petri dishes, pipette, and beakers were sterilized. In the preparation of Potato Dextrose Agar (PDA), broad spectrum antibiotic (Chloramphenicol) was added to prevent the growth of bacteria.

Serial dilution

Serial dilutions of each homogenate were prepared as described by Cheesbrough, (2000).Ten (10) fold serial dilution was made for each fish sample, 5 test-tubes were filed with 9 ml of peptone water, 1 g of the sample was dissolved and later transferred with syringe into assigned test tube (making it 10 ml) and thoroughly mixed; further sequential dilution were made by taking 1 ml from each of the 10 ml mixture into other test tubes respectively.

Culturing, incubating, colony count and identification

These methods were carried out according to

Cheesbrough (2000). After the serial dilution, 1 milliliter of each sample taken from 2nd and 3rd (10^{-2} and 10^{-3}) test tubes were transferred to petri- dishes that have been appropriately labelled. The spread plate method was used for culture. 2-3 drops of the diluents sample were dropped in each of the media and a bent glass rod dipped into ethanol and sterilized in an open flame was used to distribute the dropped samples in the media evenly and was repeated for other samples. The plates for bacterial count were kept on laboratory bench and allowed for 24 hours, while that of fungi and coliform were kept for 48 hours at room temperature. Thereafter, the bacterial count was done and the colonies that appeared as clusters in each plate was counted and recorded. Similar counts were done on fungi and coliform. The numbers were counted and recorded; identification was carried out using standard methods with biochemical tests such as Gram Staining Techniques, Catalase Test, Motility Test, Indole Test, Citrate Test, Methyl-Red and Vogues Proskaur according to Cheesebrough, (2004).

Microscopic examination of microbes

The traditional method in the microscopic examination of bacteria in the laboratory is the grams staining method. The description of the staining method was extracted from Cheesebrough (2000), while the method of the microscopic study of fungi was conducted according to Harrigan and Mclance, (1990).

Procedure of the gram staining

The Gram stain is basically four step involving water rinses after each step. The smear was air dried and gently heat fixed. Flood the slide with crystal violet (30 seconds) and wash with tap water. Flood with Grams iodine (brown) for 30 seconds and wash with tap water. Carefully decolorize with 70% ethanol for 10-15 seconds until the thinnest parts of the smear are colourless. Wash with tap water. Flood with Safranin (red) for 30 seconds and wash with tap water. Thereafter, place it at the draining rack for drying before viewing under microscope. Representative colonies of the microorganisms were gram-stained, purified and stored in nutrient agar slants at 4°C.

Statistical analysis

Analysis of variance (ANOVA) test and DMRT for mean separation were used (Duncan, 1955) were used.

RESULTS AND DISCUSSION

The results of the weight characteristics of Carica papaya leaf extract pre–treated smoke cured *Distichodus rostratus* is presented in (Table 1). The range of the weight loss is 60.2% - 74.8%. The mean weight loss is 67.0% and this range is in agreement with the 65.00% recommended by Benne, (1997). The results of total bacterial count are summarized on (Table 2). The highest microbial count recorded in T^1 was 2.6×10^7 cfu/ml while the lowest were 2.2×10^5 cfu/ml, 1.5×10^5 cfu/ml and 1.24×10^4 cfu/ml recorded in samples T2, T3 and T4 respectively. It is interesting to note that the high microbial count in control (T_1) is in excess of 10^6 cfu/ml, which if exceeded in microbiology, product may be declared unfit for human consumption (Cheesebrough, 2000). Results in leaf extract pre-treated smoke cured fish at increasing concentrations (T2-T4) proved to be progressively potent in their bactericidal activity. The counts recorded were of the order of $\leq 10^5$ which is the normal limit of plate count in microbiology for food fit for consumption (ICSMF, 1986 and PHLS, 1992). The higher microbial count in sample T1 (control) may be as a result of the salt pre–treatment on it without any added treatment.

Bhattacharyya, (1982) noted that most organisms are salt tolerant. There is evidence to prove that smoke curing may not be as efficient as gas and electric oven in moisture removal hence permitting some degree of increased water activity that may aid microbial growth (Eyo, 2001). Hygroscopy is a renown attribute of salt. While these factors may aid intrinsic and extrinsic facilitators of microbial establishment and survival in untreated fish (T_1), inhibition may be promoted with addition and increasing concentrations of leaf extract. In corroboration of this supposition, lowest microbial count in sample T_2 and T_3, especially T_4 can be attributed to the presence of anti – microbial substances in the leaf extract of *C. papaya*. Aravind et al. (2013) reported that *C. papaya* contains proteolytic enzymes that have anti-viral, anti-bacterial and anti–fungal properties. This report supports the findings in this experiment.The probable organisms' colonial morphology of bacterial pure culture is shown on (Table 3). The identified organisms were *Staphylococcus* Sp (T_1), *Proteus* Sp (T_2) and (T_3) and *klebsiella* sp (T_4). This result is in agreement with the findings of Akah *et al.,* (1997) and Orhue *et al*., (2013), who proved that the water extract of *C. papaya* leaf terminated such bacteria as *Staphylococcus aureus*, *Escherica coli*, *Bacillus* Sp. The mentioned organisms were not found in any of the treated samples. Table 4 is the summary of the result of the cell morphology and biochemical characteristics of the bacteria isolates. The organism found in T_1 was Staphylococcus sp. The organisms found in T_2 and T_3 was Proteus sp. The organisms found in T_4 were Klebsiella sp. The result of biochemical and cell morphology characteristics of bacteria isolate confirms that T_1 was 'cocci,' T_2 and T_3 are 'rod,' and T_4 is also 'rod.' The identified organisms are therefore *Staphylococcus* Sp (T_1), *Proteus* sp. (T_2) and

Table 1. Weight characteristics of smoked cured fish, *Distichodus rostratus* pre-treated with leaf extracts.

Samples	Live weight	Dressed weight	Weight after smoking	Total weight loss	% weight loss
T_1	800	600	317	483	60.3
T_2	1500	1200	453	1047	69.8
T_3	1400	1200	352	1048	74.8
T_4	1800	1500	661	1139	63.2

Mean weight loss = 67.0%.

KEY:
T_1 = Fish sample treated with 3% brine
T_2 = Fish sample treated with 2.5% *Carica papaya* leaf extract and 3% brine.
T_3 = Fish sample treated with 5% *Carica papaya* leaf extract and 3% brine
T_4 = Fish samples treated with 8% *Carica papaya* leaf extract mix with 3% brine.

Table 2. Total bacterial hetorotrophic fungi plate count on *Carica papaya* leaf extract pre–treated smoke cured fish.

Samples	Dilution	TVC on NA	TVC on MAC	Isolated conc. on NA	Isolated conc. on MAC	TFC on PDA
T_1	1×10^2	TNTC	52	–	5.2×10^5	4.8×10^3
	1×10^4	260	32	2.6×10^7	3.2×10^4	-
T_2	1×10^2	TNTC	20	-	2.0×10^4	4.0×10^3
	1×10^4	220	-	2.2×10^5	-	-
T_3	1×10^2	TNTC	12	-	1.2×10^4	3.0×10^3
	1×10^4	150	-	1.5×10^5	-	-
T_4	1×10^2	TNTC	1	-	1.0×10^4	1.0×10^3
	1×10^4	124	-	1.24×10^4	-	-

KEY
TVC = Total Viable Count
TFC = Total Fungal Count
NA = Nutrient Agar
MAC = MacConkey Agar
TNTC = Too Numerous To Count
– = No Growth

Table 3. Colonial morphology of bacterial pure culture from C. papaya leaf extract pre-treated smoke cured fish.

Samples	Media	Dilution	Shape	Elevation	Size	Chromogenese	Probable organism
T_1	NA	10^{-2}	Round	Flat	Small	Pink	*Staphyloccus* SP
T_1	NA	10^{-4}	Round	Flat	Small	Pink	
T_1	MAC	10^{-2}	Circular	Flat	Punctiform	Yellow	
T_1	MAC	10^{-4}	Irregular	Flat	Punctiform	Yellow	
T_2	NA	10^{-2}	Round	Viscid	Moderate	Cream	*Proteus* Sp
T_2	NA	10^{-4}	Round	Viscid	Moderate	Cream	
T_2	MAC	10^{-2}	Irregular	Flat	Moderate	Yellow	
T_2	MAC	10^{-4}	Irregular	Flat	Moderate	Yellow	

Table 3. Cont

T_3	NA	10^{-2}	Irregular	Convex	Large	Pink	*Proteus* sp
T_3	NA	10^{-4}	Round	Convex	Large	Cream	
T_3	MAC	10^{-2}	Circular	Flat	Moderate	Cream	
T_3	MAC	10^{-4}	Round	Flat	Moderate	Cream	
T_4	NA	10^{-2}	Circular	Viscid	Small	Cream	*Klebsiella* SP
T_4	NA	10^{-4}	Circular	Viscid	Small	Cream	
T_4	MAC	10^{-2}	Round	Flat	Punctiform	Cream	
T_4	MAC	10^{-4}	Round	Flat	Punctiform	Cream	

Table 4. Characteristics of bacteria isolate from leaf extract pre–treated smoke cured fish. (*Disticodus rostratus*).

Samples	Cell morphology	Gram reaction	Coagulase test	Catalase test	Motility test	Citrate test	Indole test	Identified organisms
T_1	COCCI		+	+				*Staphylococcus*
T_2	ROD	+	-	+	+	-	+	*Proteus* Sp
T_3	ROD	+	-	+	+	-	+	*Proteus* Sp
T_4	ROD	+	-	+	-	+	-	*Klebsiella*Sp

KEY
+ = Positive
– = Negative

Table 5. Macroscopic / microscopic morphology of fungi isolates.

Samples	Macroscopy	Microscopy	Probable fungal
T1	Powdery, dark brown, flatly spread on the surface of the medium with brown reverse	Septate and branched typhae and conidia in chains	*Aspergillus* Sp
T2	Grey colonies that were large white border. Reverse was white	Long conidiophores consisting of broom like conidia in chains	*Penicillium* Sp
T3	Grey colonies that were large with border. Reverse was white	Long conidiophores consisting of broom like conidia in chains	*Penicillium* Sp
T4	Grey to black and thick abundant cottony, mycelium and white reverse.	Now separate with sporangium containing black sporangiophores. Columella separated by septum and without rhizoids	*Mucor* Sp

(T_3), *Kleibsiella* sp. (T_4). *Staphylococcus* sp. is a gram positive bacteria while *Proteus* and *Klebsiella* species are gram negative bacteria present almost everywhere and are opportunistic pathogens, often nosocomial (Ryan and Ray, 2004). It may therefore be transmitted through equipment and handling by man in the laboratory onto the experimental samples.

The macroscopic and microscopic result of fungal isolates is represented on (Table 5). The identified species of fungi on *C. papaya* leaf extract pre – treated smoke cured fish are *Aspergillus* Sp (T_1), *Penicillium* Sp (T_2) and (T_3), *Mucor* Sp (T_4). The presence *of Aspergillus* sp is as a result of their aerobic nature and is found in almost all oxygen – rich environment where they commonly grow as moulds on the surface of a substrate, as a result of the high oxygen tension (Kirk et al., 2008).The presence of *Penicillium* Sp on sample T_2 and T_3 could be as a result of their spores in the environment, they are found in plants and most surfaces and are important in the production of drugs (Pitt, 1979). Mucor Sp. grows in every moist surface and survives high

temperature (International microbiology association, 2011).

Conclusion

The results obtained in the microbial evaluation of *C. papaya* prove that a host of bacteria present in numerous numbers on fish smoked cured by the various available methods (Abidemi-Iromini et al., 2011) are absent in *C.papaya* extract pre- treated smoke cured *D. rostratus.* This presents a new future in fresh fish handling and processing which leaves man with the option of taking full advantage of the bactericidal potency of *C. papaya* in prolonging the shelf life and also putting the anti-oxidative properties of this plant to full use in preventing oxidative rancidity of smoke cured fish.

REFERENCES

Abidemi-Iromini, OA, Olawusi-Peters OO, Fadeyi A, Bello-Olusoji OA (2011). Smoking Impact on the Microbial load of *Clarias gariepinus*. Ethiopian Journal of Environmental Studies and Management (4):3.

Agbabiaka LA, Amadi SA, Ekeocha CA (2002). Storage and organoleptic qualities of claries gariepinus smoked with woods from oil bean and oil palm trees. Animal prod- res –adv; 6 (3): 231-233.

Agbabiaka LA, Kuforiji OA, Ndumnigwe OE (2016) *Storage and Microbial Evaluation of Black Pepper Pre-Treated Oven- Dried Moon Fish (Citharinus citharus Geoffery Saint-Hilaire 1809).* J Aquac Res Development 7: 399.

Ahmed WA, Goon E, Gardner T (2010). Implication of feacal indicator bacteria for microbiological assesement of roof harvested rain water quality in south east queensland, Australia. Can. J. Microbiology 56:471-479.

Akah PA, Oli AN, Enwerem NM, Gamaniel K (1997). Preliminary studies on purgative effect of Carrica papaya root extract. Fitoterapia, 68 (4): 327 – 331.

Asian Pacific Journal of Tropical Disease. S658-S662

Ayoola PB, Adeyeye A (2010). Phytochemical and nutrient evaluation of Carica papaya (pawpaw) leave, IJRRAS 3:5.

Baskaran C, Ratha bai V, Velu S, Kubendiran Kumaran (2012).The efficacy of Carica papaya leaf extract on some bacterial and a fungal strain by well diffusion method

Bennet RN, Kiddle G, Wallsgrove RM (1997). Biosynthesis of benzylglucosinolate, cyanogenicglucosides and phenylpropanoids in Carica papaya. Phytochemistry, 45 (1): 59 – 66.

Bhattacharyya RK, Rao VNM (1982). Phenolics content of Co.2 papaya (Carica papaya L.) as affected by growth regulants. Journal of Research, Assam Agricultural University, 3 (2): 214 – 215.

Cheesebrough MD (2000).Medical laboratory manual in tropical countries, butter worth heinemen limited Oxford, Pp. 370-380.

Cheesebrough MD (2004). Collection, transport and examination of pulse from wounds abscesses, burns and sinuses. Medical laboratory manual for topical countries-volume 11 microbiology publishers. Butter worth p-124-129.

Clucas IJ, Ward AR (1996)**.** Post-Harvest Fisheries Development: A guide to Handling, Preservation, Processing and Quality. Chathman Maritime, Kent ME44TB, United Kingdom.

Dawkins G, Hewitt H, Wint Y, Obiefuna PC, Wint B (2003). Antibacterial effect of Carica papaya fruit on common wound and organism, West Indian Medical Journal. 52 (4): 290.

Doughari JH (2006). Antimicrobial activity of Tamarindus indica Linn, Tropical Journal of Pharmacology Research. 5(2): 592 – 603.

Doughari JH, Elmahamood AM, Manzara S (2007). Studies on the antibacterial activity of root extract of Carica papaya L. African Journal of Microbiology Research. 37:41.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics 11: 1-42.

Eyo AA (2001). Fish processing Technology in the tropics University of Illorin Press Pp: 112-129.

Fakeye TO, Adegoke AO, Omoyeni OA, Famakinde AA (2007). Effect of water extract of Hibiscus sabdarifa Linn (Malvaceae)-roselle on excretion of dicloffenaeformulation. Phytolher. Res. 21(1):96-98.food examiners, PHLS Microbiology Digest. 9:98-99.

Harper Douglas (2012). “pawpaw” online Etymology Dictionary. Retrieved 2012; 10 – 28.

Harrigan WF, Mclance ME (1990). Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.

Hellenic Army General Staff (HAGS) (2002). Hellenic Army General Staff (HAGS)/Medical Directorate (MD), Office of Veterinary Medicine (OVM). Enumeration of total microbial flora at 30°C. Standard Method M11. Laboratory Examination Techniques for Foods, Beverages and Water, second ed. Hellenic Army, Athens.

ICSMF, (1986). Microorganisms in foods 2, Sampling for Microbiological Analysis.Principles and Specific Applications, second ed. Blackwell Science, oxford.

Idodo UG (2003).Studies on the fresh water fisheries of Nigeria (taxonomy, Ecological notes, Diet and utilization, Phd thesis, university of Benin, Benin city, p. 412.

Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO,Oigbochie V, Kehinde MO, Bamiro SB (2009). Antisickling Property of Carica papaya leaf extract. Afr. J. Biochem. Res. 3(4): 102-106.

Kirk PM, Cannon PF, Miriter DJA (2008). Dictionary of the fungi (10th edition), walling ford U.K: (ABi.p.599 ISBN 978-0-851999-8268

Mello VJ, Gomes MT, Lemos FO, Delfino JL, Andrade SP, Lopes MT (2008). The gastric ulcer protective and healing role of cysteine proteinases from Caricacandamarcensis, Phytomedicine. 15: 237 – 244.

Nisar-Ahmad, HinaFazal, Muhammad Ayaz, BialalHaiderAbbasi, Ijaz Mohammad, LubnaFazal (2011). Dengue fever treatment with Carica papaya leaves extracts, Asian Pacific Journal of Tropical Biomedicine. 330-333.

Onah JO, Akubue PI, Okhide GB (2002). The Kinetics of Reversal of Pre-sickled Erythrocytes by the Aqueous Extract of Cajanus cajan seeds. Phytother Res., 16:1-3.

Public Health Laboratory Services (PHLS) (1992). Provisional microbiological guideline for some ready to eat foods sampled at point of sales. Notes for PHLS

Robert Kral (1997). Annonaceae in flora of North America editorial committee. Magnoliophytamagnolidate and Hammelidae.Flora of North America 3. New York NY: Oxford University press. ISBN; 978 – 0 – 511246 – 7.

Ryan KJ, Ray CG (2004). Editors, Sherris Medical Microbiology (4th Edition) Mc Graw Hill. p.370.