Antimicrobial Activities of Black Pepper (*Piper guineese*) Seeds and Leaf Extracts on Catfish (*Clarias gariepinus*)

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Abstract— *Antimicrobial activities of plants can also be explored using their different parts because they abound more in some of these parts than others. Extracts (hot water, cold water and ethanolic) of black pepper (Piper guineese) were used to preserve catfish (Clarias gariepinus) post-slaughter by dipping in separate bowls. The leaf extracts were more effective and ethanol the best solvent of extraction. Ethanolic extract of the leaves extended the shelf life of the fish from 20hours in the unpreserved sample to 36hours; it was followed by the ethanolic extract of the seeds (32hours). This observation makes black pepper seeds and leaves extracts useful natural preservatives for fish.*

Keywords— Black pepper seed, Preservative, Extract, Catfish.

I. INTRODUCTION

Fish is a major source of food providing a significant portion of the protein intake of a large proportion of the people, particularly in developing countries. In Nigeria, fish is the preferred source of high quality animal protein compared to pork or beef with little or no religious rejection bias against it (Ephraim, 2010). Fish has the highest protein profile of essential sulphur-containing amino acids such as cysteine, Methionine and lysine which are limiting in some legumes and most cereals-based diets (Ephraim, 2010). Fish is a very perishable, high-protein food that typically contains a high level of free amino acids. As soon fish dies, spoilage begins and this is the result of a whole series of complicated changes brought about by bacteria (Agha, 2011). Fish spoilage is manifested by a gradual change in the natural characteristics of the fish after death. Using his/her sense of smell, the consumer can detect changes in the flavour of the fish from that of the sweet (acceptable) falvour to that of the putrid (unacceptable) odour. Spoilage in fish is accompanied by physical and chemical changes in the gills, eyes, tissues/flesh and skin. Such changes are undesirable where fresh fish is required. This study therefore was undertaken to preserve catfish (*Clarias gariepinus*) using black pepper seeds and leaves extracts and to determine which of the two plant parts can extend the shelf life of fresh catfish (*Clarias gariepinus*) more.

II. MATERIALS AND METHODS

PREPARATION OF FISH SAMPLES

The fish samples (*Clarias gariepinus*) were purchased from Monday Market at New Bussa, Niger State and taken to the Fish Museum at Federal College of Freshwater Fisheries Technology, New Bussa, Niger State for shelf life studies. The fish were gutted and washed before dipping them in the extracts in separate bowls.

COLLECTION OF PLANT MATERIALS

The plant materials used were the seeds and leaves of Black pepper (*Piper guineese*) which were purchased from Monday Market at New Bussa, Niger State.

PREPARATION OF BLACK PEPPER SEEDS AND LEAVES EXTRACTS

300g each of black pepper seeds and leaves were ground into powder and macerated respectively. These were divided into three separate bowls and the extraction done using hot water, cold water and ethanol as solvents of extraction according to Epraim (2010).

Black pepper Seeds Extract Preparation

a. 300g of ground black pepper seeds was soaked in 150ml of cold distilled water for 24 hours. The pulp obtained was left in clean glass container and shaken vigorously to allow for proper extraction. Filtration was done using a sterile muslin cloth and the extract obtained air-dried and stored at ambient temperature until it was used Epraim (2010).

b. 300g of ground black pepper seeds was soaked in 150ml of hot water for 24 hours. The resultant extract was air-dried and stored as done in (a) above.

c. 300g of ground black pepper seed was soaked in 150ml of 95% ethanol for 24 hours. The resultant extract was air-dried and stored as done in (a) above.

The black pepper leaf extract was prepared using the procedure above

PRESERVATION OF FISH USING EXTRACTS

The fish were dipped in the black pepper seeds and leaf extracts in separate bowls.

SHELF LIFE STUDIES

The fish samples which had been preserved in the plant extracts were monitored for spoilage 4-hourly for 48hours and the organoleptic characteristics noted.

III. RESULTS AND DISCUSSION

The results of this work shows that *Clarias gariepinus* can be preserved with black pepper seeds and leaves extract using hot water, cold water and ethanol as solvents of extraction. Table 1 shows the organoleptic characteristics of catfish preserved with ethanolic extract of black pepper seeds. It was observed that the ethanolic extract of black pepper seeds could keep the fish post-slaughter for 32 hours before spoilage could set in. This means that this fish can be consumed 32 hours after it has been killed when it is preserved in ethanolic extract of black pepper seeds. Table 2 shows the organoleptic characteristics of catfish preserved with ethanolic extract of black pepper leaves. It was observed that the ethanolic extract of black pepper leaves could keep the fish post-slaughter for 36 hours before spoilage began. This means that this fish can remains intact, 36 hours after it has been killed and preserved with ethanolic extract of black pepper leaves. Table 3 shows the organoleptic characteristics of catfish preserved with cold water extract of black pepper seeds. It was observed that the cold water extract of black pepper seeds could keep the fish post-slaughter for 28 hours before spoilage can set in. Table 4 shows the organoleptic characteristics of catfish preserved with cold water extract of black pepper leaves. It was observed that the cold water extract of black pepper leaves could keep the fish post-slaughter for 28 hours before spoilage can set in. Table 5 shows the organoleptic characteristics of catfish preserved with hot water extract of black pepper leaves. It was observed that the hot water extract of black pepper leaves could keep the fish post-slaughter for 24 hours before spoilage can set in. Table 6 shows the organoleptic characteristics of catfish preserved with hot water extract of black pepper seeds. It was observed from the table that hot water extract of black pepper seeds could keep the fish post-slaughter for 16 hours before spoilage can set in. It was also observed from table 7 that catfish that was not preserved with any extract can stay for 20 hours post-slaughter before spoilage can set in and make it unfit for consumption.

RESULTS

TABLE 1: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH ETHANOLIC EXTRACT OF BLACK PEPPER SEEDS MONITORED 4-HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	Eyes	Gill	Skin	Flesh
0	Convex, bright	Bright red, fresh odour	Bright and shiny, grey	Firm, elastic
4	Convex, not bright	Bright red, fresh odour	Bright with reduced shine. Silvery at the ventral region	Firm, elastic
8	Convex	Pale red, fresh odour	Grey at the dorsal region, dull, silvery at the ventral region,	Firm, elastic
12	Convex	Pale red, fresh odour	Dark grey at the dorsal region, dull, silvery at the ventral region,	Firm, elastic
16	Convex, dark pupil	Pale red, fresh odour	Dark grey at the dorsal region, dull, silvery at the ventral region	Firm, slightly elastic
20	Slightly Convex, dark pupil	Pale red fresh odour	Dark grey at the dorsal region, dull, silvery at the ventral region	Firm, slightly elastic
24	Slightly Convex, dark pupil	Pale red, fresh odour	Silvery at the ventral region, dark grey at the dorsal region, dull	Less Firm, slightly elastic
28	Concave, cloudy pupil	Brownish, offensive	Silvery at the ventral region, dark grey at	Less Firm

		smell	the dorsal region	
32	Concave, cloudy pupil	Brownish, pungent smell	Silvery at the ventral region, dark grey at the dorsal region	Soft retains finger indentations
36	Flat, cloudy pupil	Brownish, pungent smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation
40	Flat, cloudy pupil	Green, very offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation
44	Flat, cloudy pupil	Green, very offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation
48	Flat, cloudy pupil	Green, very offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation

TABLE2: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH ETHANOLIC EXTRACT OF BLACK PEPPER LEAF MONITORED 4-HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	**Eyes**	**Gill**	**Skin**	**Flesh**
0	Convex, bright pupil	Bright red, odour	Bright, pale red at the ventral region, dark at the dorsal region	Firm, elastic
4	Convex, dark pupil	Bright red, fresh odour	Bright, pale red at the ventral region, dark grey at the dorsal region	Firm, elastic
8	Convex, dark pupil	Bright red, fresh odour	Bright, pale red at the ventral region, dark grey at the dorsal region	Firm, elastic
12	Convex, dark pupil	Bright red, fresh odour	Pale red at the ventral region, dark grey at the dorsal region, dull	Firm, elastic
16	Convex, dark pupil	Bright red, fresh odour	Pale red at the ventral region, dark grey at the dorsal region, dull	Firm, elastic
20	Convex, dark pupil	Bright red, fresh odour	Pale red at the ventral region, dark grey at the dorsal region, dull	Firm, elastic
24	Concave, cloudy pupil	Red, fresh odour	Silvery at the ventral region, dark grey at the dorsal region, dull	Firm, slightly elastic
28	Concave, cloudy pupil	Red, fresh odour	Silvery at the ventral region, dark grey at the dorsal region	Less Firm, slightly elastic
32	Concave, flat	Brownish, offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Soft with slight retention of finger indentation
36	Concave, sunken	Brownish, pungent smell	Silvery at the ventral region, dark grey at the dorsal region	Very Soft
40	Concave, sunken	Green, offensive	Silvery at the ventral region, dark grey at the dorsal region	Very soft, leaves finger indentation
44	Concave, sunken	Green, very offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, leaves finger indentation
48	Concave, sunken	Green, very offensive smell	Silvery at the ventral region, dark grey at the dorsal region	Very soft, leaves finger indentation

TABLE 3: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH COLD WATER EXTRACT OF BLACK PEPPER SEED MONITORED 4-HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	Eyes	Gill	Skin	Flesh
0	Convex, bright, iridescent	Bright red, fresh odour	Bright, shiny,	Firm
4	Convex, bright, dark pupil	Bright red, fresh odour	Silvery at the ventral region, grey at the dorsal region	Firm
8	Convex, bright, dark pupil	Bright red, fresh odour	Silvery at the ventral region, grey at the dorsal region	Firm
12	Concave, dark, pupil	Pale red, fresh odour	Silvery at the ventral region, grey at the dorsal region, dull	Firm
16	Concave, dark pupil	Pale red, fresh odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm
20	Concave, dark pupil	Pale red, fresh odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm
24	Slightly concave	Pale red, natural odour	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Less Firm, slightly elastic
28	Slightly concave	Pale red, offensive odour	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Less Firm
32	Slightly concave	Brownish, offensive odour	Silvery at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, non elastic
36	Slightly concave	Brownish, offensive odour	Red at the ventral region, dark grey at the dorsal region	Soft, non elastic
40	Concave, flat	Brownish, offensive odour	Red at the ventral region, dark grey at the dorsal region	Very soft, non elastic
44	Concave, sunken	Brownish, offensive odour	Red at the ventral, dark grey at the dorsal region	Less firm, non elastic
48	Concave, sunken	Brownish, offensive odour	Red at the ventral region, dark grey at the dorsal region	Less firm, non elastic

TABLE 4: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH COLD WATER EXTRACT OF BLACK PEPPER LEAF MONITORED 4-HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	Eyes	Gill	Skin	Flesh
0	Convex, bright	Reddish, fresh odour	Shiny, bright, firm belly	Firm
4	Convex, not bright, dark pupil	Reddish, fresh odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm, elastic
8	Convex, not bright, dark pupil	Reddish, fresh odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm, elastic
12	Concave, dark pupil	Reddish, fresh odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm, elastic
16	Concave, cloudy	Brownish, natural odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm, elastic

20	Concave	Brownish, natural odour	Reddish at the ventral region, grey at the dorsal region, dull	Firm, slightly elastic
24	Slightly concave	Pale red, fresh odour	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Firm, slightly elastic
28	Slightly concave	Pale red, natural odour	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Firm, slightly elastic
32	Concave	Brownish, pungent smell	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retains finger indentation
36	Concave	Brown, pungent/sharp smell	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retains finger indentation
40	Concave	Brown, pungent/sharp smell	Reddish at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retains finger indentation
44	Concave	Brown, pungent smell	Reddish at the ventral, dark grey at the dorsal region	Soft, retains finger indentation
48	Concave	Brown, pungent smell	Reddish at the ventral region, dark grey at the dorsal region	Soft, retains finger indentation

TABLE 5: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH HOT WATER EXTRACT OF BLACK PEPPER LEAF MONITORED 4 HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	**Eyes**	**Gill**	**Skin**	**Flesh**
0	Convex, bright	Reddish, fresh odour	Shiny, bright, firm belly	Firm and tender
4	Convex, not bright, dark pupil	Reddish, fresh odour	Silvery at the ventral region, grey at the dorsal region, dull	Firm, elastic
8	Convex, not bright, dark pupil	Reddish, fresh odour	Silvery at the ventral region, grey at the dorsal region, dull	Firm, elastic
12	Concave, dark pupil	Reddish, fresh odour	Silvery at the ventral region, grey at the dorsal region, dull	Firm, elastic
16	Concave, cloudy	Brownish, natural odour	Silvery at the ventral region, grey at the dorsal region, dull	Firm, elastic
20	Concave	Brownish, natural odour	Silvery at the ventral region, grey at the dorsal region, dull	Less Firm
24	Slightly concave	Brownish, pungent smell	Silvery at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retaining finger indentation
28	Slightly concave	Brownish, pungent smell	Red at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retaining finger indentation
32	Slightly concave	Brownish, pungent smell	Red at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retaining finger indentation
36	Slightly concave	Brownish, pungent smell	Red at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retaining finger indentation
40	Slightly concave	Brownish, pungent smell	Red at the ventral region, dark grey at the dorsal region, red along the vertebral column	Soft, retaining finger indentation

44	Slightly concave	Brownish, pungent smell	Red at the ventral, dark grey at the dorsal region	Soft, retaining finger indentation
48	Slightly concave	Brownish, pungent smell	Red at the ventral region, dark grey at the dorsal region	Soft, retaining finger indentation

TABLE 6: ORGANOLEPTIC CHARACTERISTICS OF CATFISH (Clarias gariepinus) PRESERVED WITH HOT WATER EXTRACT OF BLACK PEPPER SEED MONITORED 4 HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	**Eyes**	**Gill**	**Skin**	**Flesh**
0	Convex, bright	Red, fresh odour	Bright, shiny, firm belly	Firm
4	Convex, dark pupil	Brownish, fresh odour	Reddish at the ventral region, grey at the dorsal region, bright	Firm
8	Convex, dark pupil	Brownish, fresh odour	Reddish at the ventral region, grey at the dorsal region, bright	Firm
12	Concave	Brownish, natural odour	Reddish at the ventral region, grey at the dorsal region, dull	Less Firm
16	Concave, cloudy	Brownish, natural odour	Reddish at the ventral region, grey at the dorsal region, dull	Soft, less firm
20	Concave, cloudy	Pale green, offensive smell	Reddish at the ventral region, grey at the dorsal region, dull not bright, open belly	Soft retaining finger indentations
24	Slightly concave	Green, offensive smell	Reddish at the ventral region, dark grey at the dorsal region	Soft retaining finger indentations
28	Slightly concave	Green, offensive smell	Reddish at the ventral, dark grey at the dorsal, red along the vertebral column, dull, soft belly	Soft retaining finger indentations
32	Slightly concave	Green, offensive smell	Reddish at the ventral, dark grey at the dorsal, red along the vertebral column, dull, soft belly	Soft retaining finger indentations
36	Slightly concave	Green, offensive smell	Red along the vertebral column, reddish at the ventral, dark grey at the dorsal, dull	Soft retaining finger indentations
40	Concave	Green, offensive smell	Red at the ventral region, dark grey at the dorsal region, soft belly	Very soft, retains finger indentation
44	Concave	Dark green, offensive smell	Red at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation
48	Concave	Dark green, offensive smell	Red at the ventral region, dark grey at the dorsal region	Very soft, retains finger indentation

TABLE 7: ORGANOLEPTIC CHARACTERISTICS OF UNPRESERVED CATFISH (Clarias gariepinus) MONITORED 4 HOURLY AT AMBIENT TEMPERATURE

Time (Hr)	**Eyes**	**Gill**	**Skin**	**Flesh**
0	Convex, bright	Bright red, fresh odour	Shiny, bright grey at the dorsal, silvery at the ventral region	Firm, elastic
4	Convex, bright	Bright red, fresh odour	Shiny, pale red at ventral region	Firm, elastic
8	Convex, cloudy	Red, fresh odour	Pale red at the ventral region, grey at the dorsal region, dull	Firm, elastic
12	Convex, cloudy	Red, fresh odour	Pale red at the ventral region, grey at the dorsal region, dull	Less firm, elastic

16	Convex, flat	Red, fresh odour	Pale red at the ventral region, grey at the dorsal region, dull	Less firm
20	Convex, dark pupil	Pale red, sour odour	Red at the ventral region, dark grey at the dorsal region, dull	Soft retaining finger indentation
24	Slightly concave	Brownish, sour odour	Red at the ventral region, dark grey at the dorsal region	Soft retaining finger indentation
28	Slightly concave	Brownish, very sour odour	Red at the ventral, dark grey at the dorsal, dull	Soft retaining finger indentation
32	Slightly concave	Brown, irritating smell	Red at the ventral region, dark grey at the dorsal, dull	Very soft retaining finger indentation
36	Concave, sunken	Pale green, irritating smell	Red at the ventral region, dark grey at the dorsal region, dull	Very soft retaining finger indentation
40	Concave, sunken	Pale green, irritating smell	Red at the ventral region, dark grey at the dorsal region, soft belly	Very soft retaining finger indentation
44	Concave, sunken	Green, irritating smell	Red at the ventral region, dark grey at the dorsal region, dull	Very soft retaining finger indentation
48	Very concave	Green, irritating smell	Red at the ventral region, dark grey at the dorsal region	Soft, retains finger indentation

DISCUSSION

Fish preservation is an important stage of fish production as it helps to reduce post harvest losses and keeping fish for time of scarcity. Fish preservation is also an important method of extending the shelf life of fish in order to improve the quality of product for a longer period of time. In the global food industry today, "natural" is a powerful force as there is increasing resistance at regulatory and consumer level against chemical food preservatives (Agha, 2011). Black pepper, *Piper guineese* has attracted attention to itself due to its possible antimicrobial activity as it shown from this study that it can be used as a natural preservative for catfish, *Clarias gariepinus* to extend its shelf life and reduce post harvest losses.

The result of this work showed that Catfish (*Clarias gariepinus*) can be preserved using natural preservatives (plants) which will extend the shelf life and reduce post harvest losses. This work showed a variation between cat fish preserved with cold water, hot water and ethanolic extract of black pepper seed and leaves and the unpreserved sample. The shelf life of catfish was extended by the extract of black pepper (*Piper guineese*) seed and leaves from 20hrs as seen in the unpreserved sample (Table 7) to 32 and 36hrs in the ethanolic extract preserved (Table 1and 2). It was also observed from the study that ethanol was the best method of extraction for the preservation of catfish since it was found to extend the shelf life of catfish to 36hrs compared to hot and cold water extracts. Research in the past has also shown clearly that ethanolic extract was more effective than hot water and cold water extract. It was also in line with the findings of Ephraim (2010) who used the cold water, hot water and ethanolic extract of black pepper seeds, grape peel and pawpaw seeds and found their ethanolic extracts more suitable for combating organisms that cause spoilage in fish. This credit to the ethanoilic extracts was also attributed to it by Ephraim (2010) and the reason was that ethanol is an organic solvent which would dissolve organic compounds better, hence liberate the active components required for antimicrobial activity. Ethanol as a solvent of extraction has its relatively high potency attributed to the high dissolving power of alcohols over water. Tables 3 and 4 show that the cold water extract of black pepper seed and leaves can preserve fish for 28hrs before it is no longer fit for consumption. It can thus be deduced from this study that cold water cannot liberate the antimicrobial compound in black pepper seed and leaves.

Tables 5and 6 show that the hot water extracts of black pepper seed and leaves can keep fish fit for consumption between 16 – 24hrs. The inability of hot water to perform well is probably because some of the active ingredients are heat labile (Azu and Onyeagba, 2007; Ephraim, 2010). The reasons for this may not be farfetched from the ones stated by Ephraim (2010) as that the antimicrobial compounds in the black pepper seeds and leaves are heat-labile and are not easily liberated in cold water. Hence, the heat applied to the water destroyed these antimicrobial compounds and raised the temperature of the extract which deactivated them. The results of the extract

show it is less effective than the ethanolic extract. The unpreserved catfish remains fit for consumption for 20hrs before spoilage could set in and this fall in line with the findings of Ajibola (2010) and Olateju (2010) while it slightly varies with that of Ephraim (2010) and Kazeem (2007). The slight variation was because the latter pair monitored catfish post slaughter at ambient temperature and found it fit for consumption till 24hours post slaughter. This variation may be due to the environmental temperature because the experiments were performed in different environments; Kazeem (2007) and Ephraim (2010) in Abeokuta, Ogun State while Ajibola (2010) and Olateju (2010) as well as the present study was done in New Bussa, Niger State.

CONCLUSION

It can thus be concluded from this study that black pepper seeds and leaves extracts are yet-to-be-exploited natural preservative for fish through which post harvest can be reduced. It can also be concluded that ethanolic extract of black pepper seeds and leaves preserved the fish best. The cold water extract of both part (leaves and seeds) could also be managed in a situation where ethanol cannot be easily got or if it would increase the farmer's cost of production.

RECOMMENDATION

The following are the recommendations

1. Education of farmers and fish consumers on the use and importance of plant extracts for fish preservation.
2. Establishment of industries that can produce various plants extracts on a large scale for fish mongers.
3. Black pepper leaves could also be used as a preservative for fish aside from its consumption as vegetables.

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