THE USE OF EXTRACTS OF GINGER (*ZINGIBER OFFICINATE*), GARLIC (*ALTIUM SATIVUM*), AND LIME (*CITRUS* AURAN*TIFOLIA*) JUICE IN MEAT PRESERVATION

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ABSTRACT
Use of extracts of *Zingiber officinale* (Ginger), *Allium sativum* (Garlic) and *Citrus aurantifolia* (Lime) juice in meat preservation were carried out in the laboratory using standard methods. The bacterial isolates were *Escherichia coli*, *Salmonella* sp, *Pseudomonas s*p, *Bacillus* sp, *Enterococcus* sp, *Proteus* sp, *Klebsiella* sp and *Staphylococcus* sp. The isolates grew on fresh meat with steady increase from 1.2 x 10^2cfu/g at 6h to 0.5 x 10^3cfu/g for 24h. Meat spoilage was inhibited for 1, 2 and 7 days by Ginger (10%w/v), Lime (10%v/v) and Garlic (10%w/v) respectively. The Minimum Inhibitory concentration (MIC) of the extracts on the meat isolates showed decreasing inhibition from 10% w/v to 5% w/v to 1%v/v of the extracts and from Garlic extract to Ginger extract to lime juice. Thus garlic (10%w/v) alone could play a role in meat preservation for up to 7 days.

Keywords: Extracts, Preservation, Meat., Spoilage, Ginger, Lime, Garlic.

INTRODUCTION

Meat, a major source of protein to man, contains an abundance of all nutrients required for the growth of bacteria, yeasts and molds and an adequate quantity of these constituents exist in fresh meat. Frazier and Westhoff (1991) reported that in the event of slaughtering an animal, a chain of events occur beginning with cessation of circulation through reduction of redox potential; slow development of rancidity; cessation of respiration with the concomitant stoppage of ATP synthesis; fall of pH from 7.4 to about 5.6 which initiates protein denaturation followed by an exchange of divalent and monovalent cations on the muscle proteins which lead to cessation of the reticuloendothelial system which permits microorganisms to grow unchecked. These events require between 24 – 36h at normal holding temperature (35 – 40^{o}C) of freshly slaughtered animal. Part of the normal flora of the meat is from the animal's own lymph nodes, the knife used for the slaughtering, the animal's hide, intestinal tract, dust, hands of the handlers, cutting knives, storage bins, utensils, tables and water. If unchecked, beef could be unfit for consumption after about 48h (Frazier and Westhoff, 1991).

In Nigeria, beef distribution network often involves hawkers, sellers on open tables in open markets and sellers on foot, bicycles or any other means of transportation to the hinterland which makes the beef vulnerable to microbial contamination. There is usually a lag time between slaughtering of the meat and when consumers receive it often resulting in the sale of spoilt beef. Fresh beef is highly perishable unless appropriate and adequate steps are taken e.g. packaging, transportation and storage at refrigerated temperatures. With the epileptic electricity supply prevalent in urban centers in Nigeria and most of the hinterlands not yet connected to the National grid, coupled with the pertinent fear of carcinogenicity and/or bioaccumulation of chemical preservatives, the search for preservation of beef using plant extracts has become imperative. Various plant extracts have been reported worldwide to be efficacious on known pathogens (Ibekwe *et al*,2001; Brown, 2002; Akujobi *et al.,* 2004; Onyeagba *et al.,* 2004; Ejechi and Akpomedaye, 2005; Akujobi *et al.,* 2006; Zaria *et al.,* 2006).

This study focuses therefore on the use

of extracts of garlic, ginger and lime juice to extend the shelf-life of fresh beef beyond 36 – 48h with a view to ensuring its safer delivery to consumers in the hinterland in an acceptable state fit for consumption.

MATERIALS AND METHODS

Collection of samples: Fresh meat (1Kg) was collected from the abattoir in Abraka market at about 9am in a sterile cellophane bag and kept in a clean plastic container with cover. The meat sample was taken immediately to the laboratory for analyses.

Determination of microbial population of fresh meat: One gram (1g) of meat was placed in sterile water for 15mins after which the water was serially diluted and inoculated in duplicates on nutrient agar and MacConkey agar using pour plating technique. Inoculated nutrient agar plates were incubated at 37^{o}C for 24h while MacConkey agar plates were incubated at 37^{o}C for 24 – 48h.

Isolation and characterization of organisms from meat: Colonies from the incubated plates were picked and subcultured on fresh nutrient agar plates to produce pure colonies. Morphological and biochemical tests for identification were carried out in accordance with procedures reported by Harrigan and McCaine (1976) and Cowan and Steel (2004).

Inoculation of fresh meat with the isolates: A suspension of each isolate was prepared by inoculating two wire-loopfuls of the isolate in sterile nutrient broth, incubated at 37^{o}C for 18h and determined the microbial count of each isolate. Sliced fresh meat of 2 x 2 x 2cm dimensions were placed in 250ml flasks, sterilized by autoclaving and on cooling, inoculated with 1ml of suspension (of known microbial load) of the isolates . After 6, 12, 18 and 24h, slices were withdrawn using sterile forceps and the microbial population was determined as before. The meat samples were then observed for spoilage (Idise, 2007).

Preparation of plant extracts: Bulbs of garlic were peeled, sliced and 100g blended and put in a 1L flask. Hexane (500ml) was added and kept at room temperature with intermittent shaking for 24h. The mixture was filtered using Whatman No. 1 filter paper and the filtrate was concentrated at 60^{o}C in a water bath for 4 days which gave a milky brown solution which was stored at 4^{o}C till needed.

Roots of ginger were sliced and dried in an oven at 60^{o}C for 24h after which it was blended into powder. Fifty grams (50g) of the powder was soaked in 500ml sterile hot water in a 1L flask and kept at room temperature for three days and concentrated in a water bath at 80^{o}C for 7 days which gave a semi-solid dark brown solution which was stored at 4^{o}C until needed.

Unripe lime fruits were washed with detergent and rinsed thoroughly with water and sterilized by immersing in 10% hypochlorite for 15mins and rinsed thoroughly with water. Using a knife, sterilized by dipping in methylated spirit, the fruits were cut and the contents manually squeezed into a sterile beaker. The extract was filtered using a clean white muslin cloth to obtain lime. The extract was stored at 4^{o}C until needed.

Treatment of the fresh meat with the plant extracts: Twenty-four (24) pieces of beef each 1g were aseptically cut, out of which eight pieces were placed in each extract for 15mins. The plate count of one of the pieces in each extract was carried out and the procedure was repeated daily for 7days as reported by Harrigan and McCane (1976).

Observation Panel: A panel of five observers daily inspected the beef samples to determine color, odor and texture and fitness for consumption (Idise, 2007).

Determination of Minimum Inhibitory Concentration of the Extracts on Isolates:

Two-fold serial dilutions were made using nutrient broth according to the method of Akujobi *et al*. (2006) and the following concentrations were obtained: 250mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 v and 15.625 mg/ml. The initial concentration was obtained by dissolving 1.0ml of the extract in 4ml of nutrient broth. Having obtained the different dilutions and concentrations, three drops of the overnight broth cultures of the test organisms were inoculated into the dilutions and incu-

bated at 37°C for 24h.The lowest concentration of the extract that in habited the growth of the test organisms was recorded as the Minimal inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC) of the Extracts: Tubes showing no visible growth from the MIC test were sub cultured into nutrient agar and incubated at 37°C for 24h. The lowest concentration of the extracts yielding no growth was recorded as the Minimal Bactericidal Concentration (MBC).

RESULTS AND DISCUSSION

The isolates from fresh meat included *Staphylococcus* sp, *Escherichia coli*, *Salmonella* sp, *Proteus* sp, *Bacillus* sp, *Enterococcus* sp, *Pseudomonas* sp and *Klebsiella* sp (Table 1) and these are in agreement with reports by previous workers (Hess, 1973; Stiles and Lai-King, 1978; Cox and Merouri, 1987).

Table 1: Characterization and identity of the Isolates

Key: *+ = Positive. - = Negative. NA = Not applicable.*

Tests	Gram reaction	Aerobic growth	Catalase test	Motility test	Endospore test	Indole test	Voges Proskauer test	Oxidase test	Growth on SSA medium	Shape	Identity
A	+	+	+	-	-	-	+	-	NA	Cocci in clusters	*Staphylococcus* sp
B	-	+	+	+	-	+	-	-	-	Rods	*Escherichia* sp
C	-	+	+	+	-	-	-	-	+	Rods	*Salmonella* sp
D	-	+	+	+	-	+	-	-	-	Rods	*Proteus Isp*
E	+	+	+	+	+	-	+	+	NA	Rods	*Bacillus* sp
F	+	+	-	-	-	+		-	-	Cocci in chains	*Enterococcus* sp
G	-	+	+	+	-	+	-	+	-	Rods	*Pseudomonas* sp
H	-	+	+	-	-	-	+	-	-	Rods	*Klebsiella* sp

SSA = Salmonella/Shigella agar.

The isolates utilized the meat for growth with a steady increase from 1.2 x 10^2cfu/g at 6h to 9.5 x 10^3cfu/g at 24h (Table 3). It could therefore be concluded that the isolates were spoilage organisms which could either be part of the meat normal flora or gained access through either the handlers, handling implements/equipment used for the slaughtering of meat or from dust as reported by Jay (1978), Frazier and Westhoff (1991) and Madden (1992).

Table 2: Total Aerobic Counts of meat preserved with the plant extracts for seven days.

Time (days)	Ginger (10%w/v) Total Aerobic counts (cfu/g)	Lime (10%v/v) Total Aerobic counts (cfu/g)	Garlic (10%w/v) Total Aerobic counts (cfu/g)
0	1.3 x 10^5	1.3 x 10^5	1.3 x 10^5
1	8.4 x 10^4	6 x 10^4	1.5 x 10^5
2	1.22 x 10^5	1.18 x 10^5	1.64 x 10^5
3	1.53 x 10^5	1.44 x 10^5	1.76 x 10^5
4	2.5 x 10^5	1.51 x 10^5	1.9 x 10^5
5	2.8 x 10^5	2.1 x 10^5	2.13 x 10^5
6	3.2 x 10^5	2.5 x 10^5	2.35 x 10^5
7	3.5 x 10^5	3.3 x 10^5	2.5 x 10^5

Key: *0 = Fresh meat samples.*

Table 3: Growth of the isolates on sterile meat for 24h

Time (h)	Microbial counts (cfu/g)
6	1.2 x 10^2
12	8.5 x 10^2
18	6.3 x 10^3
24	9.5 x 10^3

The effects of the plant extracts on the TAC of meat with period of storage are presented in Table 2 while the physical examination of meat is shown in Table 4. Whereas there was an initial reduction in the TAC for ginger (10%w/v) and Lime (10%v/v) for Day 1, they could only suppress microbial activities for 48h after which the meat changed color, odor and developed slimy texture (Table 4), qualities detested by the consumers. Garlic (10%w/v) however had a steady but lower increase in TAC for the period of storage (7 days) and the meat did not change color, odor or change texture. It is noteworthy however, that even with the TAC of 2.6 x 10^5cfu/g for meat stored in garlic at Day 7 (which is 100% initial microbial load), spoilage had not set in.

Table 4: Physical Examination of extract–treated Beef with Storage

Time (days)	Ginger (10%w/v)			Lime (10%v/v)			Garlic (10%w/v)		
	Color	Odor	Texture	Color	Odor	Texture	Color	Odor	Texture
1	-	-	Ok	-	-	Ok	-	-	Ok
2	-	+	Ok	-	-	Ok	-	-	
3	+	+	Slimy	+	Slight	Ok	-	-	Ok
4	++	++	Slimy	+	++	Ok	-	-	Ok
5	+++	+++	Decay	++	++	Slimy	-	-	Ok
6	+++	+++	Decay	+++	+++	Decay	-	-	Ok
7	+++	+++	Decay	+++	+++	Decay	-	-	Ok

Key: *+ = Present. ++ = Slightly prominent. +++ = Very prominent. - = Absent. Ok = Okay.*

This shows that garlic possessed some chemical properties, which ensured this desirable situation, and this could be employed alongside other hurdle techniques in extending the shelf life of the meat beyond seven days. This same trend is observed in the MIC of the extracts on the isolates presented in Table 5 as growth was suppressed with 5%(w/v) and 10% (w/v) garlic but with only 10% (w/v) ginger and 10%(10%v/v) Lime.

Table 5: MIC of Plant Extracts on Beef Isolates.

	Ginger (mg/ml)			Lime (mg/ml)			Garlic (mg/ml)		
	62.5	125	250	62.5	125	250	62.5	125	250
Escherichia coli	+	+	-	+++	++	-	+	-	-
Salmonella sp	+	+	-	+++	++	-	+	-	-
Pseudomonas sp	+	+	-	+++	++	-	+	-	-
Bacillus sp	+	+	-	+++	++	-	+	-	-
Enterococcus sp	+	+	-	+++	++	-	+	-	-
Proteus sp	+	+	-	+++	++	-	+	-	-
Klebsiella sp	+	+	-	+++	++	-	+	-	-
Staphylococcus sp	+	+	-	+++	++	-	+	-	-

Key: *+ = Slight growth. ++ = Moderate growth. +++ = Luxuriant growth. - = No growth*

The initial increase in TAC for garlic could be attributable to the conversion of Alliin to Allicin by Allinase. Allicin is a sulphur -containing compound reportedly possessing effective antimicrobial effects at concentration of 1:125,000 with a comparable effectiveness to 1% action of Penicillin. The inhibitory effects of garlic extract has previously been reported (Onyeagba *et al.,* 2004).

Thus, garlic (10%) possessed antibacterial properties that suppressed the microbial activities in meat for seven days with the meat retaining the desirable consumers' sensory qualities and could therefore be employed in meat preservation either alone or in combination with other hurdle techniques.

REFERENCES

Akujobi, C. O., Anyanuwu, B. N., Onyeze, G. O. C. and Ibekwe, V. I. (2004). Antibacterial Activities and Preliminary Phytochemical Screening of Four Medicinal Plants. *Journal of Applied Science* **7(3):** 4328 – 4338.

Akujobi, C. O., Ogbuli, J. N. and Uchegbu, U. N. (2006). Antibacterial Activities and Preliminary Phytochemical Screening of *Veronica amygdalina* and *Citrus aurantifolia*. *Nigerian Journal of Microbiology* **20(1):** 649 – 654.

Brown, D. (2002). *The Royal Horticultural Society New Encyclopeadia of Herbs and their Uses.* Dorling Kindersley, London.

Cheesbrough, M. (2002). *Distinct Laboratory Practice in Tropical Countries Part II* Cambridge University Press, London.

Cowan, S. T. and Steel, K. J. (2004). *Manual for the Identification of Medical Bacteria* (3rd ed.). Cambridge University Press. London.

Cox, N. A. and Merouri, A. J. (1987). Comparison of Two Minikits for Identification of Enterobacteriaceae Isolated from Poultry and Meat Products. *Journal of Food Protection* **41(1):** 107 – 110.

Ejechi, B. O. and Akpomedaye, D.E.(2005). Activity of essential oil and phenolic extracts of pepperfruit (*Dannetia tripetala* G,. Barker *anonceceae*) against some food borne microorganisms. *African Journal Biotech*nology **4(2):** 258 – 261.

Frazier, W. C. and Westhoff, D. C. (1991). *Food Microbiology* (3rd ed.). Tata McGraw-Hill Pub. Co., New Delhi.

Harrigan, W. F. and McCaine, M. (1976). *Laboratory Methods in Food and Dairy Microbiology*. Academic Press Inc., London.

Hess, E. (1973). *Hygiene during Meat Protection.* Academic Press, New York.

Ibekwe, V. I., Nnanyere, N. F. and Akujobi, C. O. (2001). Studies on Antibacterial Activity and Phytochemical Qualities of Extracts of Orange Peels. *International Journal of Environmental Health and Human Development* **2(1):** 41 – 46.

Idise, O. E. (2007). Microbial deterioration of Agidi during storage. *Journal of Pure and Applied Microbiol*ogy **1(1):** 29 – 32.

Jay, J. M. (1978). *Modern Food Microbiology*. 2nd ed. D. Van Nostrand Co., New York.

Madden, J. M. (1992). Microbial Pathogens in Fresh Products. *Journal of Food Products* **55:** 821 – 823.

Onyeagba, R. A., Ugbogu, O. C., Okeke, C. U. and Iroakasi, O. (2004). Studies on the Antimicrobial Effects of Garlic (*Allium sativum*), Ginger (*Zingiber officinale*) and Lime (*Citrus aurantifolia*). *African Journal of Biotech*nology **3(4):** 551 – 554.

Stiles, M. E. and Lai-King, N. G. (1978). Biochemical Characteristics and Identification of Enterobacteriaceae isolated from Meat. *Applied Environmental Microbiology* **1:** 639 – 645.