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Antibacterial effects of spices on steak (Suya)

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ABSTRACT

The antibacterial effects of some local spices were studied. Bacterial loads and types associated with steak were evaluated. The popular local spices-ginger (*Zingiber officinale*), garlic (*Allium sativum*) and pepper (*Piper nigrum*) were tested. The effects of the spices were measured after direct application of the processed (powdered) spices on the meat immediately prior to roasting as practiced commercially. Total viable counts were determined following a spread plate technique. Also, the effect of combination of the spices (synergism) on bacterial load and flora on the steak was determined. These effects were tested on commercially available steak and laboratory prepared steak samples. The finding showed that there were limited effects on the garlic treated sample which had a bacterial load of 5.2% less than the control. The test for synergism showed that a combination of garlic and ginger had appreciable less bacterial load of 52.7% less than the control. The organisms isolated included *Pseudomonas Staphylococcus, E.coli, Bacillus Streptococcus* and *Proteus* species. It was concluded that spices had little or no bacteriostatic effects. The combination of garlic and ginger was recommended for use, since there was need for use of spices for taste. Observation of good hygiene and good sanitation during production of steak was also recommended.

INTRODUCTION

Spices and condiments are plant products used in flavouring foods and beverages. For thousands of years, aromatic plant materials have been used in food preservation and preparation[11,5].

The World Health Organisation (WHO) considers a spice and or condiment as a culinary term, not a botanical category. It does not refer to a specific kind of plant or plant part and has a unique aroma and flavour, which it derives from compounds known as phyto chemicals or secondary compounds because they are secondary to plants basic metabolism[7].

Throughout recorded history, food borne bacteria especially species of *Clostridium, Escherichia Listeria, Salmonella, Shigella* and *Vibrio* or their toxins have been of serious health concerns and they still are[16]. If spices were to kill such microorganisms or inhibit their growth before they could produce toxins, use of spices then might reduce food borne illness and food poisoning[1], if these antimicrobial hypotheses were true several predictions should be fulfilled: (a) spices should exhibit antibacterial and antifungal activity multiple techniques have been used in investigating inhibition and the primary data vary considerably in quality and quantity for different spices[12] (b) Use of spices should be greatest in hot climates where unrefrigerated food spoil especially quickly. Meat dishes that are prepared in advance and stored at room temperature build up massive bacterial population especially in tropical climates. (c) Quantities of spices called for in recipes should be sufficient to produce antimicrobial effects and cooking or roasting should not destroy the potency of phytochemicals[10].

Many spices exhibit greater antibacterial potency when they are mixed than when used alone such as black pepper a bioavailability enhancer meaning that it acts synergistically and absorbs phytotoxins[16]. Individual spices and blends are employed as coloring

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agents, antivirals (including suppressing HIV), brain stimulants and aphrodisiacs. Among traditional societies, many spice plants also have ethnopharmacological uses often as tropical or ingested antibacterial and vermiciders[3]. A few spices particularly garlic, ginger, cinnamon and chillies, have for centuries been used to counteract a broad spectrum of ailments including dysentery, kidney stones arthritis and high blood pressure[6].

This work investigated the antibacterial effect of three commonly used spices namely pepper(*Piper nigrum*), garlic(*Allium sativum*) and ginger (*Zingiber officinate*) on the bacterial load of steak(Suya). Investigation based on the fact that they are often used as seasoning in household and business purposes without a scientific test of their antimicrobial effects or potency[4].

MATERIALS AND METHODS

Source of materials

The three spice plants ginger, pepper and garlic were purchased in their unprocessed form from the Umuahia central market and their botanical identities were authenticated by the Chief plant officer of the Horticulture unit, National Root Crops research Institute, Umudike. All the materials used were sterilized according to Cheesbrough[2].

Processing and preparation of spices

The spice plants were first examined for the presence of extraneous materials like insects or their larva, pest, dried and diseased portions. Some extraneous materials were removed before the processing of the good ones. The garlic bulbs, were washed, the scally leaves were removed and the succulent portions were cut into thin slices, spread in a laboratory tray and put in the oven for drying at 65^0C for 24 hours, similarly the ginger rhizomes were washed and the scaly leaves and some roots were removed before it was cut into thin slices and spread in a laboratory tray for drying in the oven at 65^0C. The pepper fruits were dried directly after spreading in a tray. The samples were dried in the oven until they were brittle. They were then ground in a Thomas-Willey laboratory mill in which the ground samples were sieved through 1mm test sieve to obtain a powdered processed sample.

Preparation of steak (suya) sample

The steak (suya) type commonly called or referred to as "Belengu" by the vendors was prepared. The assistance of a professional "suya" man was enlisted. Five different steak (suya) treatments were prepared in addition to the market type and control purchased directly from the vendors. The samples were designated as follows:

i. Market sample
ii. Control sample produced without any additions
iii. Suya sample treated with 5g ginger powder
iv. Suya sample treated with 5g garlic powder
v. Suya sample treated with 5g pepper powder

All the samples were produced using the normal roasting method. They were then subjected to analysis.

A second set of steak (suya) samples were produced in which the test spices were combined and tested for possible synergistic effect. The samples were designated as follows: (a) Ginger and garlic (b) Garlic and pepper (c) Pepper and ginger (d) Control (e) Market types.

In the preparation of the samples only fresh meat from freshly slaughtered cow were purchased to reduce contamination. The preparation was done immediately after purchase.

Preparation of media

Media used for the work includes nutrient agar, Christenson's urea agar, tryptone broth and peptone iron agar medium. These were obtained in commercial powdered form and prepared according to the manufacturers specifications and instructions.

Determination of bacterial load

Bacterial load determination was done by the plate count method following a spread plate technique as described by Iroegbu et al.[15]. The method is in line with the specification of International Commission on Microbiological Specifications for Foods[14]. Counts were taken in gallenkamp colony counter from triplicate culture plates of serially diluted diluents of each test sample.

Procedure

One gram of the steak (suya) sample was weighed in a sterile bijou bottle using a sartorious digital weighing balance. The sample was crushed with sterile glass rod, blended in 2mls of the sterile normal saline and serially diluted in 10-fold. Triplicate plates of nutrient

TABLE 1: Bacterial load of spice treated steak

Sample identity	1	2	3	Mean
Garlic	138	132	134	1.346×10^8
Ginger	144	152	151	1.490×10^8
Pepper	161	159	165	1.616×10^8
Market type	164	165	168	1.656×10^8
Control	139	142	142	1.423×10^8

TABLE 3: Bacterial load of combined spice treatment on steak

Sample	1	2	3	Mean
Garlic+pepper	122	128	125	1.25×10^8
Ginger+ginger	61	66	63	6.30×10^8
Pepper+pepper	296	298	298	2.97×10^8
Market type	289	290	286	2.88×10^8
Control	133	132	135	1.33×10^8

TABLE 2: Bacteria isolates of spice treated steak

Sample	*Pseudomonas sp.*	*Streptococcus sp.*	*Bacillu sp.*	*Escherichia sp.*	*Staphylococcus sp.*	*Proteus sp.*	*Salmonella sp.*
Garlic	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Ginger	+ve	-ve	+ve	+ve	+ve	-ve	-ve
Pepper	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Market	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Control	+ve	-ve	+ve	-ve	+ve	-ve	-ve

TABLE 4: Bacterial isolates of combined spice treated steak

Sample	*Pseudomonas sp.*	*Streptococcus sp.*	*Bacillus sp.*	*Escherichia sp.*	*Staphylococcus sp.*	*Proteus sp.*	*Salmonella sp.*
Garlic+pepper	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Garlic+ginger	+ve	-ve	+ve	+ve	+ve	-ve	+ve
Garlic+Pepper	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Market	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Control	+ve	-ve	+ve	-ve	+ve	+ve	-ve

TABLE 5: Isolation and identification of microorganisms isolated during the determination of bacteria load of steak

	1st isolate	2nd isolate	3rd isolate	4th isolate	5th isolate	6th isolate
Colonial morphy	Smooth, circular colonies, shiny and mucoid	Smooth, circular opaque, creamy and butyrous	Smooth, colonies with edges entire, creamy, dull faced and translucent	Large irregular colonies, translucent on colonies, sooth nutrient agar	Small, tiny colonies, smooth circular and translucent nutrient agar	Large expanding, smooth, circular and translucent on nutrient agar
Microscopy						
Motility test	+ve	-ve	+ve	+ve	-ve	+ve
Grain stain	-ve	+ve	-ve	+ve	-ve	-ve
Spore	-ve	-ve	-ve	+ve	-ve	-ve
Flagella	+ve	-ve	+ve	+ve	-ve	+ve
Capsule	-ve	-ve	-ve	-ve	-ve	-ve
Cell arrangements	Small rods, straight curved	Single oval cells arranged in clusters	Short rods in singles	Single short rods	Organisms short rods in chains	Slender, short, single rods
Biochemical tests						
Catalase	+ve	+ve	+ve	+ve	-ve	+ve
Oxidase	+ve	-ve	-ve	-ve	-ve	-ve
Coagulase	+ve	+ve	-ve	+ve	+ve	-ve
Indole	+ve	-ve	+ve	-ve	-ve	+ve
Urease	-ve	-ve	-ve	-ve	-ve	-ve
Methyl red	+ve	+ve	+ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve	+ve	+ve
H_2S	+ve	-ve	-ve	+ve	-ve	+ve
Citrate	+ve	-ve	-ve	+ve	-ve	+ve
Carbohydrate utilization						
Glucose	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	-ve	+ve	-ve	+ve	-ve	+ve
Maltose	+ve	+ve	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	-ve	+ve	+ve	+ve	+ve	+ve
Xylose	-ve	+ve	+ve	+ve	-ve	+ve
Probable identity	*Pseudomonas spp*	*Staphylococcus spp.*	*E.coli*	*Bacillus spp.*	*Streptococcus spp*	*Proteus spp*

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TABLE 6: Isolation and identification of micro organisms isolated from the combined treatment of spices on steak

	1st isolate	2nd isolate	3rd isolate	4th isolate	5th isolate	6th isolate
Colonial morphology	Smooth, circular colonies, shiny in consistency	Smooth, circular opaque, creamy on nutrient agar	Smooth, small circular butyrous creamy colonies	Large irregular colonies, dull faced	Tiny, smooth colonies translucent	Large expanding, colonies nutrient agar
Microscopic						
Grain stain	-ve	+ve	-ve	+ve	+ve	+ve
Spore	+ve	-ve	-ve	+ve	-ve	-ve
Flagella	+ve	-ve	-ve	+ve	-ve	+ve
Capsule	-ve	-ve	-ve	-ve	-ve	-ve
	small rods, straight curved	single short rods	oval shaped cells in clusters	single short rods	short single cell in chains	slender, single rods
Biochemical tests						
Catalase	+ve	+ve	+ve	+ve	-ve	+ve
Oxidase	+ve	-ve	-ve	-ve	-ve	-ve
Coagulase	+ve	-ve	-ve	-ve	+ve	-ve
Indole	-ve	+ve	+ve	-ve	-ve	+ve
Voges proskauer	+ve	-ve	+ve	-ve	-ve	+ve
Methyl red	+ve	+ve	+ve	+ve	+ve	+ve
H_2S	+ve	-ve	-ve	-ve	-ve	+ve
Citrate	+ve	-ve	-ve	+ve	+ve	+ve
Nitrate	+ve	+ve	+ve	+ve	+ve	+ve
Urease	-ve	-ve	-ve	-ve	-ve	-ve
Carbohydrate utilization						
Glucose	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	-ve	-ve	+ve	+ve	-ve	+ve
Maltose	+ve	+ve	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	-ve	+ve	+ve	+ve	+ve	+ve
Xylose	-ve	+ve	-ve	+ve	-ve	+ve
Probable identity	*Pseudomonas spp*	*Staphylococcus spp.*	*E.coli*	*Bacillus spp.*	*Streptococcus spp*	*Proteus spp*

agar were inoculated with 0.1ml of each dilution, and the inoculum spread over the agar surface with a sterile glass hockey. After 24 hours of incubation at 37^0C, the plates were examined and colonies counted in an electronic colony counter. A mean of each triplicate count was taken and multiplied with the appropriate dilution factor.

Identification of bacterial flora

Subcultures were made, using the methods described variously by Fawole and Oso[8] and Cheesbrough[2]. Identification of each bacterial isolate was made based on cultural (colony) examination, microscopic examination and biochemical characters. Identification of the bacteria to the generic level followed the schemes of Holt et al.[13].

RESULTS AND DISCUSSION

From the results of bacteria load, it is evident that all the test samples and control contains appreciably high bacterial load ranging from 1.346×10^8 to 1.65×10^8 cfu/g representing a lower bacteria load of 5.62% (TABLES 1and 5). On the other hand the ginger and pepper treated steaks had ginger load of 1.490×10^8 and 1.616×10^8cfu/g being percentages of 8.86% and 11.67% bacterial loads respectively. The market steak had a load of 1.656×10^8cfu/g being 16.37%. The relatively lower bacterial load of the control must have come from the high sanitation that was observed during preparation, while the relatively higher load in the market steak, may be as combined effect of unhygienic environment and the groundnut (Kulikuli) additive, which probably provided more substrate for the organisms.

The bacteria flora of the test samples and the controls were normal for meat samples and the controls were normal for meat samples[9].

Five different species of bacterial made up the bacteria flora of the samples. Species of *Pseudomonas, Bacillus* and *Staphylococcus* were present in all the samples and control, while other species which include *Streptococcus, Bacillus,* Proteins, *Escherichia* and *Salmonella* were seen in the different samples.

Results as shown in TABLE 2 shows that five dif-

ferent species of bacteria make up the bacteria flora of the samples. Spices of *Pseudomonas, Bacillus* and *Staphylococcus* were present all the samples and control. The presence of *Salmonella and Escherichia spp* are indicative of poor hygiene and possible contamination during handling.

TABLES 3 and 6 show the effect of combined spices (Synergism test) on the bacteria load of steak. Combined pair of ginger and garlic had the least number of bacteria 6.30×10^{7}cfu/g while ginger+pepper had 2.97×10^{8} and the market steak had 2.88×10^{8}cfu/g.The synergism test showed that the spices tend to be more effective when combined. Garlic and ginger combination had a load of 6.3×10^{7}cfu/g(52.74%), also, garlic and pepper combination had a load of 1.25×10^{8}cfu/g.

Both the market steak and the ginger and pepper treated samples had relatively more bacterial than the control. Bacteria isolates from the combined spiced treated steak is shown in TABLE 4. *Staphylococcus spp, Pseudomonas spp and Bacillus spp* were present in all the samples and the control, while *Salmonella* and *Escherichia* species were present in the market sample only, *Proteus* was present in the control sample only.. The combination of spices decreased the bacteria load considerably.

Conclusion, therefore, it is bound that good sanitation plays better role than use of spices in bacteria control during steak production. However, the use of garlic spice had limited bacteriostatic effect as seen from the results, this effects becomes enhanced when combined with ginger.

It is therefore, generally concluded that the use of spices have little or no bacteriostatic effect in steak production. Notwithstanding since the spice are used more for taste than bacteria control, it is hereby recommended that a combination of garlic and ginger spices should be used. Also it is recommended that adequate personal hygiene and sanitation are required during production of ready to eat meat products like steak.

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