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Successive Isolation, Identification and Antimicrobial Susceptibility Pattern of Bacteria Involved in the Processing of Two Traditionally Fermented Foods (Okpehe And Ogiri)

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Abstract

This research focuses on the Successive isolation and Antimicrobial Susceptibility Pattern of bacteria involved in the processing of two traditionally fermented foods (Okpehe and Ogiri). Successive Isolation of the bacteria was done on the 24-hour, 48-hour, 72-hour, 96 hour and 120 hours of the Fermented samples by innoculating the samples on Nutrient Agar, MacConkey Agar and Mannitol Salt Agar. Some Biochemical tests (Gram staining, citrate, Oxidase and Catalase) was done and the organisms obtained were Staphylococcus aureus, Escherichia coli and Staphylococcus epidermis.

Introduction

A condiment is an edible substance which is added to some foods to impart/enhance its flavor or texture. They are healthy diets which boosts brain power, protects the heart and prevents cancer. Generally, they are referred to as oily paste made from oil seeds in west Africa [1].

Ogiri (fermented melon seeds) is widely used as condiments, sometimes in the daily preparation of soups, sauces and stews [1].

Okpehe (fermented mesquite seeds) is used as a food condiment in Nigeria by the Idoma and Igala people of the middle belt region and some parts of the Eastern and Southern Nigeria. It adds variety and pleasure to the otherwise monotonous traditional diet. It serves not only as a seasoning agent but also as a low-cost source of protein in the diet. Okpehe can serve as a substitute for meat for low-income earners and can reduce protein calorie malnutrition and essential fatty acid deficiencies [2].

Materials and Methods

Study Area

This study was carried out at the laboratory of the Department of Microbiology, University of Abuja.

Sample Collection

Fresh samples of melon and mesquite seeds (unfermented) were purchased from Gwagwalada market in FCT, Abuja and placed in a clean plastic container and transported to the laboratory.

Preparation of Sample Using Traditional Method

African mesquite condiment and Ogiri were produced traditionally using the method described by Ugwuarua [3]. Raw mesquite seeds (1000g) were cleaned to remove extraneous materials. The seeds were washed in clean water, drained and boiled for 12hours. The boiled seeds were de-hulled and cotyledons were boiled in enough quantity of water for one hour and drained. Dehulled melon seeds were also boiled for four hours. The boiled mesquite and melon seeds were allowed to cool, ground to form paste, divided into five portions of 1g each, wrapped with Nylon (NL) and aluminium foil (FL) and left to ferment for five days, moulded into shapes and stored in the refrigerator. The samples were labelled as follows;

Table 1: Samples Code

Source	Sample code	Sample code in hour
Fermented mesquite in foil	A	A24
		A48
		A72
		A96
		A120
Fermented Mesquite in nylon	B	B24
		B48
		B72
		B96
		B120
Fermented Melon in foil	C	C24
		C48
		C72
		C96
		C120
Fermented Melon in nylon	D	D24
		D48
		D78
		D96

Microbiological Analysis of Fermented Sample

One gram of each labelled sample was diluted serially in seven folds dilution. 10^2 Diluted sample was streaked Successively into sterile molten agar medium plate. Mannitol Salt Agar (MSA), MacConkey Agar (MA) and Nutrient Agar (NA) were used to Isolate bacteria. Then the plates were incubated at 37°C. This process was repeated for five days and the resulting colonies of microbes were subcultured into Cled Agar (CA), and Nutrient Agar (NA) for Identification and obtaining pure culture. The cultures were maintained on nutrient agar and kept refrigerated and stored for further use.

Macroscopic Examination of Culture Plate of Isolated Sample

The colonial appearance of the organism was noted such as colour, shape and size after 24 hours incubation.

Biochemical Tests

Catalase Test

Three (3) ml of hydrogen peroxide solution was dispensed in a sterile test tube and several colonies of 18 h culture of the test organism was picked and immersed in the hydrogen peroxide solution using a glass rod. It was then observed for immediate bubbling which indicated positive result [4].

Oxidase Test

A piece of filter paper was placed in a clean petri dish and three (3) drops of freshly prepared oxidase reagent added using a glass rod, a colony of the test organism was removed and smeared on the filter paper and observed for the development of a blue-purple colour within a few seconds for a positive test.

Citrate Utilization Test

This test was carried out by inoculating an agar slant and stabbing the butt of a 5 ml Simmon's citrate agar with the test organism. An uninoculated control was setup in each case. They were incubated at 37 oC for 48 hrs, growth indicated that the organism is able to use citrate as a sole carbon source which was accompanied by the medium turning from green to bright blue [5].

Gram Reaction

The bacteria isolates were gram stained according to the method outlined [5]. A thin smear of the organism was made on a grease free microscopic slide. It was air-dried then heat-fixed by passing briefly over flame. Two drops of crystal violet were added to the smear for 60secs and then rinsed with water. Grams iodine (Mordant) was added for 60secs and rinsed with water. It was then decolorized with acetone for 30secs and rinsed with water and counter stained by flooding with safranin solution for 60secs. The slide was then rinsed with slow flowing water and air dried before examining under x 100 oil immersion objective lens of the microscope.

Motility Test

This test was done using the Agar method. A straight needle was touched to a colony of a young (18- to 24-hour) culture growing on agar medium. It was then stabbed once to a depth of only 1/3 to ½ inch in the middle of the tube, incubated at 35°-37°C and examined daily for up to 7 days and Observed for a diffused zone of growth flaring out from the line of inoculation.

Preparation of Extract

35g each of the Samples of fermented and unfermented samples was weighed for aqueous and ethanolic extraction. The samples

were ground using mortar and pestle after which the macerate was transferred into two beakers containing 100ml of sterile water and 100ml of ethanol, filtered and the liquid was allowed to evaporate.

Antimicrobial Susceptibility Testing

Bacteria used for the susceptibility study was obtained from pure culture of the isolated bacteria. Using the agar disc-diffusion method, the antibiotic susceptibility / resistance patterns of the ethanol and aqueous extract of melon and mesquite seeds was carried out. Whatmann no 1 filter paper was used to prepare susceptibility discs of 4mm in diameter which was sterilized in hot air oven. The extract was mixed with ethanol and water in varying concentration. Each concentration was diffused into a sterile sensitivity disc; similar preparations was done with ethanol and sterile distilled water. Sensitivity testing agar media was innoculated with the selected organism. Discs impregnated with appropriate concentrations of ethanol and water extracts was carefully placed on the innoculated agar media. The preparation was incubated overnight at 37 degree Celsius and the zone of inhibition observed.

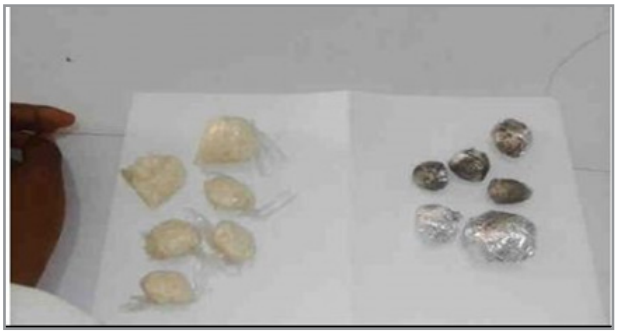
Results

Dehulled Mesquite seeds

Table 2: Morphological characteristics of bacteria from different samples.

Sample code in hour	No of Colony	Size	Shape	Optical property	Elevation	Margin	Colour	Media
A24	19	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	71	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
	21	Tiny	Circular	Opaque	Raised	Entire	Pink	MaC
A48	21	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	63	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
	18	Tiny	Circular	Opaque	Raised	Entire	Pink	Mac
A72	69	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
A96	62	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
A120	57	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	37	Tiny	Circular	Opaque	Raised	Entire	Pink	Mac
B24	19	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	73	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
	17	Tiny	Circular	Opaque	Raised	Entire	Pink	MaC
B48	23	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	64	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
	14	Tiny	Circular	Opaque	Raised	Entire	Pink	Mac
B72	73	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
B96	67	Medium	Circular	Opaque	Convex	Entire	Pink	MSA
B120	52	Small	Circular	Opaque	Convex	Entire	Cream	MSA
	32	Tiny	Circular	Opaque	Raised	Entire	Pink	Mac
C24	14	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
C48	19	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
C72	16	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
C96	18	Large	Irregular	Translucent	Raised	Entire	Pink	Mac

C120	20	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
D24	19	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
D48	21	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
D72	17	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
D96	14	Large	Irregular	Translucent	Raised	Entire	Pink	Mac
D120	18	Large	Irregular	Translucent	Raised	Entire	Pink	Mac

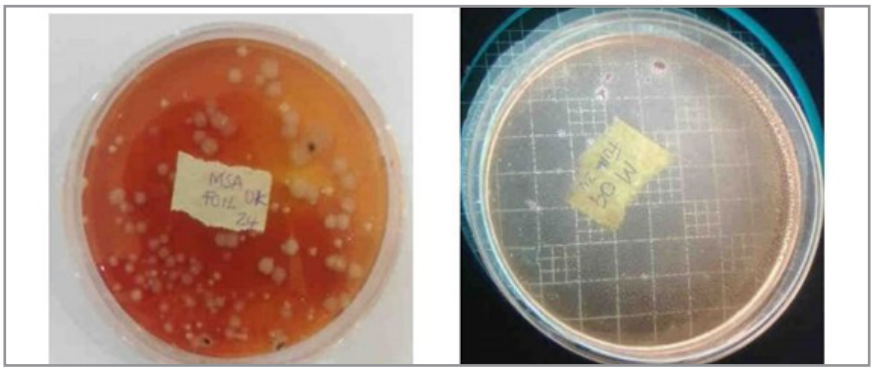


Melon seeds wrapped in Foil and Nylon

Mesquite seeds wrapped in foil and Nylon

Table 3: Biochemical test Results of the isolates

Gram Rxn	Citrate	Catalase	Oxidase	Motility	Probable Organism
+	+	+	-	-	Staphylococcus aureus
+	-	+	-	-	Staphylococcus epidermis
-	-	+	-	+	Escherichia coli



Some of the Cultured Plates

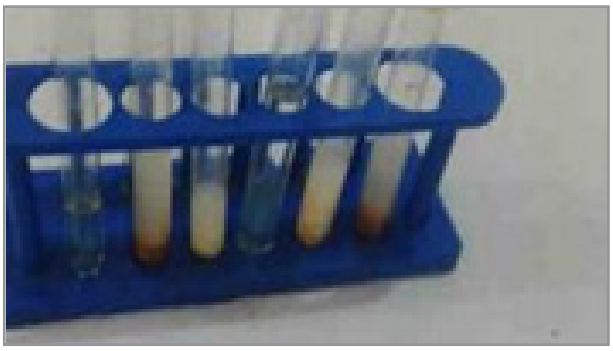
Table 4: Names of Organisms in the Samples.

Sample code in hour	No of Colony	Probable Organism
A24	19	Staphylococcus aureus
	71	Staphylococcus epidermis
	21	Escherichia coli
A48	21	Staphylococcus aureus
	63	Staphylococcus epidermis
	18	Escherichia coli
A72	69	Staphylococcus aureus

A96	62	Staphylococcus aureus
A120	57	Staphylococcus aureus
	37	Escherichia coli
B24	19	Staphylococcus aureus
	73	Staphylococcus epidermis
	17	Escherichia coli
B48	23	Staphylococcus aureus
	64	Staphylococcus epidermis
	14	Escherichia coli
B72	73	Staphylococcus epidermis
B96	67	Staphylococcus epidermis
B120	52	Staphylococcus aureus
	32	Escherichia coli
C24	14	Escherichia coli
C48	19	Escherichia coli
C72	16	Escherichia coli
C96	18	Escherichia coli
C120	20	Escherichia coli
D24	19	Escherichia coli
D48	21	Escherichia coli
D72	17	Escherichia coli
D96	14	Escherichia coli
D120	18	Escherichia coli

Table 5: Antibacterial susceptibility of the samples against Isolated Organisms.

	Extract	Staph aureus	Staph epidermis	E. coli
Unfermented Mesquite	Ethanolic	-	+	-
	Aqueous	-	-	+
Fermented Mesquite in foil	Ethanolic	+	+	-
	Aqueous	-	+	+
Fermented Mesquite in nylon	Ethanolic	-	+	+
	Aqueous	-	+	-
Unfermented Melon	Ethanolic	+	+	-
	Aqueous	-	-	+
Fermented Melon in foil	Ethanolic	-	+	-
	Aqueous	-	+	-
Fermented Melon in nylon	Ethanolic	+	+	-
		-	+	-



Serial Dilution and Sterile Discs

Table 6: Correlation Between the Number of Colonies Produce Over Time.

	Hours	Number of colonies
Hours	1	
Number of colonies	0.151547	1

The degree of correlation between the numbers of bacteria colonies produce over time in the two fermented foods, is moderate because the value lies between 0.30 and 0.49.

Discussion and Conclusion

The number of Organisms isolated is limited to only Organisms that can grow in Mannitol Salt and MacConkey agar.Organisms in both Mesquite in foil and in Nylon were the same.The Organisms Reduced from Three Organisms to one and then two Organisms.Melon in nylon had the same Organisms throughout the fermentation period while melon in foil had one Organism after 24hrs and then two Organisms after 48 hrs and then one Organism in the remaining days.The isolates were identified were Staphylococcus aureus, Escherichia coli, and Staphylococcus epidermis.

The findings from the present study show fermented Mesquite and Melon extracts to have a wide spectrum of activity. This Results agree with Findings from thefermented R. communis extracts which where are partly different from that of the unfermented extracts of the plant where water extracts showed intermediate activity at higher concentrations compared to the present increased activity of thewater extracts over alcohol at the same concentrations [6].

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