The Antimicrobial Properties of Well-Fermented Cassava Grit Steep Liquor Against Some Food-Borne Pathogens

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ABSTRACT

Fermentation provides an economic means of preserving food and inhibiting the growth of pathogenic organisms. Fermented food, enjoyed across the globe, conveys health benefits through lactic acid fermentation. Cassava grit (garri) is widely known in Nigeria and other West African countries. This research work is based on the efficacy of cassava grit steep liquor obtained from roasted well-fermented cassava mash to inhibit the growth of some target microbes. The fungi isolated from the rotten food samples were *Fusarium monoliforme, Aspergillus carbonariu*s, *Aspergillus niger* and *Fusarium oxysporum.* The three target bacterial strains which were isolated from poultry soil sample were *Salmonella enterica*, *Shigella dysentriae* and *Escherihia coli*. At 100% concentration, the inhibitory property of the cassava grit soft liquor ranged from 0.45mm against *Fusarium oxysporium* to 7.1mm against *Aspergillus carbonarius* between the 1st and 3rd day after visible growth and from 6.41mm against *S. dysenteriae* to 18.10mm against *E. coli* between the 2nd and 4th day after inoculation. The growth of the microbial strains was not in any way inhibited by the presence of the distilled water (control). The pH of the cassava grit soft liquor showed a positive result for acidity (3.9) and ethyl alcohol (3.5%). By this investigation, it can be deduced that the cassava grit steep liquor had a slightly impressive antifungal capability `but an impressive bacteriostatic efficacy against the test organisms employed, under routine conditions. The physico-chemical properties of the soft liquor, like the presence of bacteriocins and some intrinsic factors, should be investigated and optimized for better results.

Keywords: Fermentation, Bacteriocin, Efficacy, Physico-chemical properties, pH

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1. INTRODUCTION

Cassava is a tropical plant occurring in the humid tropics and semi-arid areas. It provides large tuberous roots in which significant reserves of starch are stored. The starch content in tubers of fresh cassava ranges from 25% to 30%Wt (Ajao and Adegun, 2009). Cassava starch is composed of about 17-24% amylase and 76-83% amylopectin. Cassava is a commodity consumed by a large number of people, mainly in Latin America, Africa and Asia. The world annual cassava production is estimated at about 255.7million, in the form of tubers (FAO, 2013). Africa ranks first with 53.7% of the world production, followed by Asia with 33.0%. South America ranks third with 12.7%, while in Oceania its production is estimated at about 0.6% of the production each year. Cassava is a versatile crop and can be processed into a wide range of products.

These products include starch, flour tapioca, beverages and cassava chips for animal feed. Cassava is also gaining prominence as an important crop for the emerging biofuel industry and as corroborated by Ziska *et al*. (2009), it is a potential carbohydrate source for ethanol production.Cassava varieties are frequently described as sweet or bitter. Sweet cassava varieties are low in cyanogens with most of the cyanogens present in the peels. Bitter cassava varieties are high in cyanogens that tend to be evenly distributed throughout the roots (Eze and Ugwuoke, 2010). Environmental factors (soil, moisture, temperature) and other factors also influence the cyanide content of cassava. Low rainfall or drought increases cyanide level in cassava roots due to water stresses on the plants. Cassava, like other foods, also has anti-nutritional and toxic factors. Of particular concern are the cyanogenic glucosides of cassava (linamarin and lotaustralin). On hydrolysis, these release hydrocyanic acid (HCN) (Agbor and Lape, 2006). Apart from acute toxicity that may result in death, consumption of sub-lethal doses of cyanide from cassava production over a long period of time results in chronic cyanide toxicity and that increases the prevalence of goiter and cretinism in iodine deficient situations. Symptoms of cyanide poisoning from consumption of cassava with high level of cyanogens include vomiting, stomach cramp, dizziness, headache and weakness (Okafor *et al*., 1984). Cooked cassava starch has a digestibility of over 75%.

In some regions, mainly in African countries, fermentation plays an important role in the nutrition of infants and young children, as it is used for the preparation of complementary foods. Fermentation provides an economic means of preserving food and inhibiting the growth of pathogenic bacteria, under conditions where refrigeration or other means of safe storage are not available (Yasmine, 2000). It also enhances the nutritional quality of certain foods. In many parts of the world, particularly in Asia and in Africa, the technology has been traditionally used as a preservation method to ensure food safety. Fermented food, enjoyed across the globe, conveys health benefits through lactic acid fermentation. The fermentation process can transform the flavour of food from the plain and mundane to a mouth–puckering sourness by colonies of beneficial bacteria and enhanced micronutrients. For spoilage microorganisms, for instance, the acid produced by the dominant organisms inhibits the growth of all other micro organisms (Adepoju, 2010). Fermentation can produce important nutrients or eliminate anti-nutrients. Food can be preserved by fermentation, since fermentation uses up food energy and creates conditions unsuitable for most microbes.

Garri (cassava grit) is a creamy-white, granular flour with fermented flavour and a slightly sour taste made from fermented, gelatinized fresh cassava tubers (Abass *et al*., 2012). Garri is widely known in Nigeria and other West African countries. Cassava, when dried to a powdery (or pearly) extract, is called tapioca; its fermented, flaky version is named 'garri.' When properly stored, it has a shelf-life of six months or more (Olumide, 2004). Food-borne pathogens, particularly fungi associated with food rot and members of the family Enterobacteriaceae, commonly and constantly remain a major public health issue because they are often associated with the contamination of food; one of life's necessities for survival. The gradually growing resistance of microorganisms to synthetic antimicrobial agents is alarmingly becoming a serious concern, hence, the need for alternative, locally prepared and yet affordable and beneficial fermented food item with potent, naturally-occurring antimicrobial agents and effective intrinsic parameters which affords little or no toxicity.

2. MATERIALS AND METHODS

Sample Collection

Part of the investigation was carried out in the Microbiology laboratory at The Federal Polytechnic, Ilaro, Ogun State, Nigeria. Healthy cassava tubers were purchased from a local farmer in Ilaro town, Ogun State, for processing into roasted cassava mash at a local cassava processing site in Ilaro. A bread loaf and yam tuber was also purchased at the same market and left to naturally rot, for fungal isolation. A soil sample was obtained from a poultry farm for bacterial isolation.

Medium Preparation

The PDA used was prepared according to the manufacturer's instruction which is to dissolve 39g of dehydrated PDA in 1litre of distilled water and then autoclaved at 121°C for 15mins (Cheesebrough, 2000). The medium was allowed to cool to about 35^0C, an antibiotic (streptomycin) was incorporated to suppress bacterial contamination. Approximately 12ml of the molten PDA was aseptically dispensed into the sterile disposable 90mm Petri plates and then allowed to gel (Okigbo and Emeka, 1993). The bacteriological media (Salmonella-shigella and McConkey agar) were used to isolate the target bacterial cultures. Each agar medium was prepared according to the manufacturer's instructions i.e. to dissolve 28g of dehydrated nutrient agar in 1litre of distilled water, to dissolve 27g of dehydrated Salmonella-Shigella agar in 1litre of distilled water and to dissolve 49.53g of dehydrated McConkey agar in 1liter of distilled water. About 250ml of each medium was prepared by first homogenizing in Erlenmeyer flasks and then autoclaved at 121^oC for 15minutes. The medium was allowed to cool to about 35^oC. Approximately 12ml of the molten medium was aseptically dispensed into sterile disposable 90mm Petri plates and then allowed to gel before inverting them (Cheesebrough, 2004).

Isolation

The method described by Chukwu *et al.* (2010) was employed to isolate fungi from the rotten food samples. A sterile scalpel was used to cut off some infected portions of the samples. The cut infected portions were used to directly inoculate the already prepared PDA plates. The plates were incubated at 28^0C $\pm$ 2^0C for four days. A six-fold serial dilution was conducted using 1ml stock homogenate of the soil sample containing faecal matter. 10^{-2} and 10^{-4} diluents were used for the bacterial enumeration (Hassan *et al*., 2014). 1ml from each diluent for each sample was taken and then introduced into each plate containing the molten McConkey agar and Salmonella-Shigella agar respectively. The plates were incubated at 37^0C ± 2^0C for 2 days.

Distinct colonies were sub-cultured for both the fungal and bacterial cultures to obtain axenic cultures by repeatedly streaking. The platted axenic cultures were then incubated appropriately. The axenic cultures isolated were preserved by streaking in McCartney bottles until the appearance of appreciable growth before they were then stored in a refrigerator. The pure cultures were kept refrigerated as stock cultures. The experiment was conducted in duplicate. Labeling of the sample containers was done accordingly.

Cultural Identification

The fungal isolates were identified on the basis of their morphological and cellular characteristics, as described by Barnet and Hunter (1999). The physical characteristics were compared to a Fungi Atlas for identification.
The bacteria isolated were identified based on their Gram's status, cultural and colonial characteristics, sugar fermentation and various biochemical tests according to Cheesebrough (2000). Bergey's manual of systematic bacteriology was used as a reference for the identification of the bacterial isolates (Cowan, 1974).

3. PRODUCTION OF CASSAVA GRIT (GARRI)

The cassava grit was prepared at the processing site according to Olumide (2004):
Sorting of cassava tubers – Peeling – Washing – Grating - Fermentation/Steeping – Pressing – Sifting – Roasting – Cooling – Packing – Storing

Some cassava grit was steeped in water, enough to leave some water afloat, to obtain the "soft liquor" infused with extract from the steeped grit. The liquor was aseptically incorporated into sterile PDA and Nutrient agar media respectively by pour plate technique.

Agar Diffusion Assay

The inocula for this test were prepared using 4-day old cultures of the pure fungal isolates and 24-hr old cultures of the pure bacterial isolates respectively. Fresh Potato Dextrose and Nutrient agar plates were prepared for the assay, the plates were inoculated with the axenic cultures respectively and a sterile 5mm cork borer was used to make a well at the centre of each plate. 1ml of the product at 100% concentration was introduced into each well. The plates were then heavily inoculated with each axenic culture and then incubated at 28^0C $\pm$ 2^0C for 4 days for the fungi and 37^0C $\pm$ 2^0C for 24hrs for the bacteria. The zones of inhibition were marked by using a pair of venier calipers and then placed on a transparent ruler to take the exact measurement or reading. The zones of inhibition readings were taken daily for three consecutive days from the fourth day of incubation for the fungi and from the second day of incubation for the bacteria. A control without the soft liquor but instead distilled water was setup. The potential ability of the soft liquor to inhibit the growth of the isolates was indicated by the appearance of clear zones within the medium for sensitivity, otherwise resistance (Oduwobi and Afuye, 2017).

4. RESULTS AND DISCUSSION

Four different fungal strains belonging to two genera were repeatedly isolated from the food samples; rotten Bread and Yam. The fungi isolated were *Fusarium monoliforme, Aspergillus carbonariu*s, *Aspergillus niger* and *Fusarium oxysporum.* The three target bacterial strains belonging to three different genera which were repeatedly isolated are *Salmonella enterica*, *Shigella dysentriae* and *Escherihia coli*. Cassava grit soft liquor is denoted as CGSL. At 100% concentration, the inhibitory property of the cassava grit soft liquor ranged from 0.45mm against *Fusarium oxysporium* to 7.1mm against *Aspergillus carbonarius* between the 1st and 3rd day after visible growth, as shown in table 1.

Table 1: Susceptibility or Resistance of the Fungi to the CGSL (mm)

SN	ISOLATES	DAY 1	DAY 2	DAY3
1	*Fusarium oxysporium*	0.5mm	4.1mm	6.5mm
2	*Aspergillus niger*	0.7mm	4.6mm	5.9mm
3	*Aspergillus carbonarius*	0.78mm	4mm	7.1mm
4	*Fusarium monoliforme*	0.45mm	2.7mm	4.8mm

At 100% concentration, the inhibitory property of the cassava grit soft liquor ranged from 6.41mm against *S. dysenteriae* to 18.10mm against *E. coli* between the 2nd and 4th day after inoculation, as shown in table 2.

Table 2: Susceptibility or Resistance of the Bacteria to the CGSL (mm)

	Zone of inhibition (mm)		
Day	***E. coli***	***S. enterica***	***S. dysenteriae***
1	0.00	0.00	0.00
2	9.10 (± 0.30)	7.90 (± 0.10)	6.41 (± 0.20)
3	13.30 (± 0.20)	12.01(± 0.30)	10.92 (± 0.20)
4	18.10 (± 0.50)	13.70 (± 0.20)	15.60 (± 0.30)

The result for the control experiment showed no value. In other words, the growth of the microbial strains was not in any way inhibited by the presence of the distilled water. The pH of the cassava grit soft liquor showed a positive result for acidity (3.9) as expected because of the presence of lactic acid which must have been produced by the bacterial cultures during the fermentation of the cassava mash. The alcohol by volume of the cassava grit soft liquor tested to determine the presence of ethyl alcohol showed a positive result with a value of 3.5%.

The result of this study revealed that CGSL is a very promising antidote in the therapeutic and possibly prophylactic remedy of diseases associated with enteric microbes. Their inhibited growth on the CGSL incorporated medium suggests their inability to grow and survive in the presence of CGSL. The zones of inhibition increased which may probably be due to an increase in the infusion of the CGSL, containing lactic acid and other antimicrobial metabolites, into the growth media. During the cassava fermentation, the pH was expected to have declined and peaked at the 7th day. The result was more pronounced against the bacteria than the fungi, perhaps because the CGSL obtained was acidic, besides other intrinsic antagonistic factors. This is not the usual pH for growth of most bacteria and hence would mitigate the survival and growth of them. Fungi can still survive in acidic environments.

Caplice and Fitzerald (1999) attributed the anti-microbial effect of lactic acid bacteria (LAB) to lactic acid and organic acids production causing the pH of a growth environment to decrease. In addition, Lindgren and Dobrogosz (1990) stated that low pH induces organic acids to become lipid soluble, which then diffuses through cell membranes and into the cytoplasms of bacteria. LAB have long been known to produce bacteriocins (Maruggi, 1991), to a range of organisms including Gram-negative and Gram-positive bacteria. Studies of several bacteriocins have indicated that they are non-toxic and non-immunogenic. Lactobacilli have been reported by Lindgren and Dobrogosz (1990) to produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins during lactic acid fermentation. Kotz *et al.* (1990) explained that the antimicrobial effect of LAB is due to the combined effect of acids and the production of other antimicrobial metabolites by the LAB which have significant effect.

5. CONCLUSION

By this investigation, it can be deduced that the cassava grit steep liquor had a slightly impressive antifungal capability but an impressive bacteriostatic efficacy against the test bacteria employed, under routine conditions. This indicates that the soft liquor has a potential to be exploited extensively and commercially in the treatment of common fungal and bacterial gastro-intestinal infections from food poisoning, while simultaneously being enjoyed as an affordable, delicious, highly palatable and tasty beverage, if the cassava mash is left to adequately ferment.

6. RECOMMENDATIONS

Garri is, undoubtedly, one of the most consumed and enjoyed staple foods in West Africa, particularly in Nigeria. Its prospects as a natural anti-microbial should be extensively explored, hence:

- The physico-chemical properties of the soft liquor, like the presence of bacteriocins and some intrinsic factors, should be investigated and optimized for better results.
- Some potent plant extracts could be incorporated into the mash to fortify and increase the antifungal property of the soft liquor, though bearing in mind the palatability and sensory properties of the fortified product.
- Clinical trials should be conducted on experimental mice inoculated with food-borne fungal and bacterial pathogens to rate the in vivo potency of the soft liquor.
- The populace should be better educated on the health benefits of the consumption of well fermented garri, due to its potential prophylactic and therapeutic efficacy, through the various mass media platforms, besides, prolonged fermentation makes it much safer for consumption from toxic Hydrogen cyanide.

- ❖ Farmers should be educated and encouraged to propagate more of sweet cassava cultivars by Agricultural extension agents.
- ❖ The fortified steep liquor could be preserved by lyophilization for commercial purposes.

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