Growth and Survival of Gastroenteritis Pathogens in Dried Cassava Powder (Garri)

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Abstract: Gastroenteritis rank with respiratory tract infection as the most common infectious disease syndrome of humans. However, the survival of the commonly implicated gastroenteritis pathogens on the most popular staple food in Africa has yet to be investigated despite food borne gastroenteritis been the etiology of over 15-30 percent of all death in developing countries. This research therefore aimed at investigating the growth and survival of selected gastroenteritis pathogens in dried cassava powder (garri) at room temperature and at different time. Prior to inoculation of the garri samples with each of the gastroenteritis pathogens, the garri samples were autoclaved before being inoculated with 0.1 ml of 0.5 McFarland standard and incubated at room temperature. The enumeration of the gastroenteritis pathogens were carried out according to standard microbiological method at six hour intervals. Counts of *Escherichia coli* 0157:H7 decreased by approximately 5log units from 5 to 1.3 log in white garri and 5 to 1.31 log units in yellow garri. The cells decreased rapidly at a death rate of 0.0371 and 0.0374 per hours in white and yellow garri respectively.This mean that at a specific time, the number of cells in white and yellow garri were decreasing by 3.71 and 3.74% per total number of cells at that points. The type of garri samples have no significant effect ($t=-4.00$, $p>0.05$) on the specific death rate of *Escherichia coli* 0157:H7 per hour but does had an apparent effect on the survival of this organism ($t=11.00$, $p<0.05$). However, *Salmonella typhimurium*, *Salmonella gallinarum*, and *Staphylococcus aureus* were all very sensitive to the garri environment as they were not detectable after 24 hours of inoculation in both yellow and white garri. The counts of these organisms decreased rapidly from 5log unit to 1.2,1.3, and 1.21 respectively in white and yellow garri. No obvious difference occur in the specific death rate of these organisms for both yellow and white garri samples ($t=0.00$, $p>0.05$). Also, the type of garri samples have no significant influence on the survival of these organisms ($t=0.00$, $p>0.05$). This study showed that all the tested organisms cannot grow but all survived in the two garri samples to varying degrees of time**[**B.T.Thomas, H.I.Effedua, O.D.Popoola , A.Oluwadun. **Growth and Survival of Gastroenteritis Pathogens in Dried Cassava Powder (Garri).** *New York Science Journal* 2012;5(2):9-14]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 2

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1. Introduction

Over the last few years, food poisoning and food safety have become very topical subjects eliciting a great deal of public concern to many people all over the world (Mamajoro,2009).This is as a result of emerging food borne pathogens that continue to cause outbreaks of gastroenteritis in different countries. A wide variety of diseases can be caused by eating food contaminated with preformed bacterial toxin or multiplication of live microorganisms in food (Ojo, 2009), thereby resulting in gastroenteritis which is of public health concern (Venugopal *et al*., 2001;Flint *et al*., 2005).

Gastroenteritis rank with respiratory tract infection as the most common infectious disease syndrome of humans. Approximately five billion episodes of diarrhea occur worldwide annually accounting for 15-30 percent of all death in some countries (Flint *et al.*, 2005).

Garri is the most popular of the cassava products in Africa (Oluwole *et al.*, 2004) that is widely consumed in both rural and urban areas in Africa and can be consumed without any additives or can be consumed with variety of additives such as groundnut, fish, meat and stew (FAO, 2010). Tauxe (2002) reported that when a broad spectrum of microbial pathogens contaminate human food and water supplies, they cause illness especially when the pathogens or its toxins are consumed. The implication of this is that garri can pose a health risk especially if it is poorly handled and get contaminated. The aim of this study was to investigate the growth and survival of gastroenteritis pathogens in garri at room temperature and at different time.

2.0. Materials and Methods

2.1. Cultures

Bacterial strains were collected from Nigeria institute of Medical Research Yaba, Lagos and Department of Veterinary Microbiology and Parasitology, University of Agriculture, Abeokuta. The strains comprises of *Staphylococcus aureus, E.coli 0157:H7,Salmonella typhimurium, Salmonella gallinarum*. All were grown on selective media. Each strain was maintained on nutrient agar (Biolab, Germany) slants at 4^{o}C.

2.2. Microbiological Analysis

The growth and survival of gastroenteritis pathogens in the two garri samples (yellow and white) at room temperature and at different time were determined using the method described by Maria *et al*.(2001) with slight modification. These organisms were identified by cultural, morphological, and biochemical tests using standard procedures (Cheesborough, 2005). Each of the gastroenteritis pathogens used was inoculated onto appropriate selective media. The plates were incubated at 37^{o}C for 24h in order to reactivate the organisms. A loopful of each of the resulting growth were then resuspended in 9ml of Tryptic Soy broth. The resulting turbid culture were standardized to the turbidity of 0.5Mcfarland (A_{625nm}=0.85-0.9) using 0.85% Nacl solution as diluents to give bacterial density of 10^5cells/ml. The number of cells per ml were assessed by viable counts using the plate count technique (Collins and Lyne, 1976). Prior to inoculation, all samples of garri were autoclaved at 121^{o}C for 15minutes and 10g of sterilized sample were placed in presterilized flasks. Then 0.1ml of the 0.5McFarland standard of each of the gastroenteritis pathogens were inoculated into the flask containing the garri samples and preparation were mixed by shaking and then incubated at room temperature. Sampling were carried out at 6hrs intervals for seven days. Two grammes portion of the incubated garri sample culture were thoroughly mixed with 18ml of sterile 0.1% (v/v) peptone water and homogenized by swirling at medium speed. A serial ten fold dilutions were prepared in peptone water and 1ml from each dilution were plated in appropriate selective media using plate count technique. The plates were incubated at 37^{o}C for 18-24h and then the number of CFU per gramme of sample calculated.

3. Statistical Analysis

The results were statistically evaluated using SPSS Version 19. Independent student t test was used for comparing the mean specific death rate and survival period in white and yellow garri while $P<0.05$ was set as the significant level. The death equation was also used to calculate the length of survival of all the gastroenteritis pathogens using the equation below:

$X_t = X_0.e^{-kd.t}$

Where;

X_t = Final concentration of viable cells

X_0 = Initial concentration of viable cells

Kd = Specific death rate

t = time

Therefore; $t = \frac{-(\ln X_t - \ln X_0)}{Kd}$

4. Results

Table 1 depict the Media used for the enumeration of the presumptive gastroenteritis pathogens and also their description on the corresponding selective media. The strain of *Escherichia coli* 0157:H7 used in this study was the most adaptable to the garri environment compared to all other pathogens tested as it survived for the longest period in both yellow and white garri. Counts of *Escherichia coli* 0157:H7 decreased by approximately 5log units from 5 to 1.3 log in white garri and 5 to 1.31log units in yellow garri. The cells decreased rapidly at a death rate of 0.0371 and 0.0374 per hour in white and yellow garri respectively. This mean that at a specific time, the number of cells in white and yellow garri were decreasing by 3.71 and 3.74% per total number of cells at that points. The type of garri samples have no significant effect ($t=-4.00$, $p>0.05$) (table4) on the specific death rate of *Escherichia coli* 0157:H7 per hour but does have an apparent effect on the survival of this organism ($t=11.00$, $p<0.05$) (table 7). However, *Salmonella typhimurium*, *Salmonella gallinarum*, and *Staphylococcus aureus* were all very sensitive to the garri environment as they were not detectable after 24hours of inoculation in both yellow and white garri. The counts of these organisms decreased rapidly from 5log unit to 1.2, 1.3, and 1.21log units respectively in white and yellow garri. No obvious difference occur in the specific death rate of these organisms in both yellow and white garri samples ($t=0.00$, $p>0.05$). Also,the type of garri samples have no significant influence on the survival of these organisms ($t=0.00$, $p>0.05$).

Table1. Media used for the enumeration of the presumptive gastroenteritis pathogens and the description of colonies of each pathogens.

Bacterials	Selective broth	Selective agar	Description of colonies
E.coli 0157:H7	Tryptic Soy broth (Merk,Germany).	Eosin Methylene Blue agar	Blue-Black with a metallic sheen.
Salmonella spp.	Tryptic Soy broth	Salmonella-Shigella* agar	Transparent black colonies
S.aureus	Tryptic Soy broth	Mannitol salt agar*	Yellow Halo growth

Table 2.The Specific Death Rates of the Presumptive Gastroenteritis Pathogens in White garri

Pathogens	Period(Days)	Specific death rate(d^{-1})	Specific death rate(h^{-1})
E.coli 0157:H7	0d-7d	0.89	0.0371
Salmonella typhimurium	0d-1d	6.00	0.250
Salmonella gallinarum	0d-1d	6.24	0.260
S.aureus	0d-1d	5.76	0.240

Table 3.The Specific Death Rates of the Presumptive Gastroenteritis Pathogens in Yellow garri

Pathogens	Period(Days)	Specific death rate(d^{-1})	Specific death rate(h^{-1})
E.coli 0157:H7	0d-7d	0.90	0.0375
Salmonella typhimurium	0d-1d	6.00	0.250
Salmonella gallinarum	0d-1d	6.24	0.260
S.aureus	0d-1d	5.76	0.240

Table 4.The Relative Specific Death Rates of the Presumptive Gastroenteritis Pathogens in White and Yellow garri

Pathogens	N	Specific death rate(d^{-1}) in white garri	Specific death rate(h^{-1}) in yellow garri	t value	pvalue
E.coli 0157:H7	2	0.89	0.0371	-4.00	>0.05
Salmonella typhimurium	2	6.00	0.250	0.00	>0.05
Salmonella gallinarum	2	6.24	0.260	0.00	>0.05
S.aureus	2	5.76	0.240	0.00	>0.05

N* =Number of replicates.

Table 5.Survival of Gastroenteritis Pathogens in White Garri at room temperature

Pathogens	Survival Period(days)
Escherichia coli 0157:H7	9.57
Salmonella typhimurium	1.54
Salmonella gallinarum	1.36
Staphylococcus aureus	1.52

Table 6.Survival of Gastroenteritis Pathogens in Yellow Garri at room temperature

Pathogens	Survival Period(days)
Escherichia coli 0157:H7	9.46
Salmonella typhimurium	1.54
Salmonella gallinarum	1.36
Staphylococcus aureus	1.52

Table 7.Survival of Gastroenteritis Pathogens in White and Yellow garri-a Comparative analysis.

Pathogens	N	Survival Period in white garri	Survival Period in Yellow garri	tvalue	Pvalue
Escherichia coli 0157:H7	2	9.57	9.46	11.00	<0.05
Salmonella typhimurium	2	1.54	1.54	0.00	>0.05
Salmonella gallinarum	2	1.36	1.36	0.00	>0.05
Staphylococcus aureus	2	1.52	1.52	0.00	>0.05

6. Discussion

Our research was aimed at determining the potential of the investigated gastroenteritis pathogens to survive in dried cassava powder (garri). 0.1ml of the 0.5McFarland Standard of the investigated gastroenteritis pathogens were inoculated into dried cassava powder (garri) and incubated at room temperature to mimic post harvest contamination and the storage temperature. Our choice of this staple food was based on the fact that it is the most popular form in which cassava is consumed in Nigeria and indeed in West Africa (Ikediobi *et al.,* 1980). The results from this work confirmed that garri sample does not enhance the growth of the gastroenteritis pathogens tested at room temperature but only support their survival for a short period of time. The survival of these organisms for only a short period may be due to decrease in the pH of the food and

increase in the titrable acidity (Oshoma *et al.*,2009) of the garri samples. This pH reduction might have resulted from the fermentative conversion of carbohydrate in the food to organic acid (lactic and acetic) by the fermentative bacteria. Other mechanisms that might also contribute to the decline in the population of the gastroenteritis pathogens are the production of bacteriocin, hydrogen peroxide and ethanol by the fermentative microorganisms (Ogwaro *et al.*,2002) which are known to exhibit antagonistic activities against a wide range of pathogenic and spoilage microorganisms (Ouwehand, 1998). Failure to detect all the gastroenteritis pathogens after 24hrs apart from *Esherichia coli* 0157:H7 may not be unconnected to the presence of an inhibitory native microflora (Venugopal *et al.*,2001). Some microflora such as lactic acid bacteria present in fermented food like garri have the potential to inhibit the growth of pathogenic and spoilage microorganisms, thereby improving the hygienic quality and extending the shelf life of such food (Caplice and Fitzgerald,1999). The survival of *E.coli* 0157:H7 for a longer period of time than other pathogens may be explained by the ability of this organism to tolerate and withstand the low pH and high titrable acidity condition of the food (Oshoma *et al.*, 2009). According to Leyer *et al.* (1995), *E.coli* 0157:H7 has an acid adaptive response and the expression of this system enhances their survival in the presence of lactic acid and acidified food products. The apparent effect of the type of garri on the survival of *Escherichia coli* 0157:H7 was noticed as the organism survived longer in white garri than in Yellow garri. This may be due to the addition of palmoil to yellow garri which prevent penetration of oxygen and therefore provide an unconducive environment for the growth of *Escherichia coli* and other aerobic bacteria (Yeboah *et al.*, 2008). The study showed that gastroenteritis pathogens investigated cannot grow in this food but all can survive at room temperature to varying length of time. It is therefore imperative to employ a strict microbiological safety measure during production period and avoid post process contamination in order to ensure a safer product to the consumers.

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