Enumeration of Microorganism in Dried cassava Powder (Garri); a Comparative Study of Four Methods.

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Abstract: Evaluation and relative appraisal of various viable count techniques in individual food should be considered as a standard protocol of obtaining representative viable number of microorganisms in food samples due to differences in the chemical composition of such foods. This research was therefore carried out to compare four different viable count techniques viz ; pour plate, plate count, surface spread and miles and misra method for the enumeration of microorganism in garri.0.1ml of 0.5McFarland standard of *Esherichia coli ATCC 25922* were inoculated in 10g of pre autoclaved garri samples and then enumerated using standard plate count techniques after six hour of incubation at 37^{o}C. A variation in count between the methods were observed from 3.16% to 5.71%. The order of increasing of the viable counts for each of the methods investigated were pour plate<plate count <spread plate<miles and misra technique . The study showed that Miles and Misra count was significantly higher than other viable count techniques ($P<0.05$) and more economical in term of materials requirement. It should therefore be of interest for enumerating microorganisms in garri and for other food in developing countries including, Nigeria where resources are limited.

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1. Introduction

Garri is a roasted granule of cassava that is widely accepted in both rural and urban areas(FAO,2010).It is by far the most popular form in which cassava is consumed by both young and old in Nigeria and indeed in west Africa (Ikediobi et al.,1980).However, the practices associated with production, processing and post process handing of garri such as spreading on the floor, display in open bowl in the markets and sales points, movement from rural to urban areas may exacerbate microbial contamination(Ogiehor and Ikenebomeh,2005).Thus, there is need for increasing attention to be paid to microbiological quality control of garri processing establishments throughout the production areas and a regular appraisal of the techniques used is necessary.

The international commission on microbiological specification for foods was formed in 1962 in response to the need for internationally acceptable and authoritative decisions on microbiological limits for foods. This commensurate with public health safety and particularly for foods in international commerce. This commission reviewed current methods for the microbiological examination of foods and published interim recommendations for their use(Thatcher and Clark,1968).For the enumeration of mesophilic aerobic microorganisms in food, the ICMSF favored the pour plate or standard plate count method(Kramer and Gilbert,1978).IDF (1985) and (1990) also favoured the pour plate method for the enumeration of *Psychrotrophs* and *Enterobacteria*. But ,since the pour plate method is both time consuming and costly, laboratories committed to the examination of large numbers of samples have sought more rapid and economical procedures for estimating the numbers of bacteria in food (Kramer and Gilbert,1978).Researches had also shown that all other methods have also been used at one time or the other. The need to evaluate the most efficient method for enumerating microorganisms in different food has however become imperative since each food differs in their chemical composition which can also indirectly influence the final outcome of the

enumeration of microorganism of any given food(Ogunledun,2007).

The purpose of the present study was therefore to compare the Pour plate, Surface spread , Plate count and Miles and Misra techniques for the enumeration of microorganisms in garri in Ogun State, Nigeria.

2. Materials and Methods

2.1. Test products and Inoculation of microorganisms

Garri samples was obtained from markets in Ogun State, Nigeria. Prior to inoculation of the garri samples, they were autoclaved and 10g each of the sterilized garri samples was placed in a presterilized aluminium pan. These were then inoculated with 0.1ml of 0.5McFaland standard of overnight pure culture of *E.coli ATCC25922*.The samples were then mixed with a sterilized glass rod before incubating it at 37^{o}C for six hours.

2.2.Microbiological Analysis

2.2.1.Preparation of initial suspension

This was prepared using the method described by ISO(1999) with slight modification. 10g of each sample was added to 90ml of 0.1% (w/v) peptone-water and homogenized by rolling between the palms at medium speed. Serial decimal 10 fold were done by transfer of one millimeter of initial suspension (10^{-1}) into a tube containing 9ml of sterile 0.1% (w/v) peptone water. The mixture was then homogenized to make 10^{-2} dilution. To prepare further decimal dilutions, 1ml of the 10^{-2} dilution was transferred into a tube containing 9ml of sterile 0.1% (w/v) peptone water to make 10^{-3} dilution. These operations were repeated by using a new sterile pipette to obtain 10^{-4} through 10^{-10} dilutions.

2.2.2.Media Preparation

Plate count agar (PCA.,Oxoid) from a single production batch was used in all the methods .For the Pour plate and Plate count techniques, the agar were melted and cool to 46^{o}C in a water bath before use. Pre poured plate were required for other methods and these were dried thoroughly at 36^{o}C.

2.2.3.Plating and Enumeration of Microorganism

The plating methods used were Pour plate(Thatcher and Clark,1968),Surface spread(Ogiehor and Ikenebomeh,2006),Miles and misra(Miles and misra,1938) and Plate count(Ogunledun,2007).The plate count technique were carried out as follows ;10ml amount of PCA in tubes were melted and cooled to 45^{o}C in a water bath. Two Petri dishes per dilution to be tested were set out and labelled with appropriate dishes, using a fresh pipette for each dilution. The serially diluted garri samples were aseptically put at the centre of each of the Petri dishes after which the PCA contents of each tube were subsequently poured gently on the samples aseptically. Each dish was moved gently six times in a clockwise circle of diameter about 150mm.The movement was repeated counter-clockwise. Then the dish was moved back and forth six times with an excursion of about 150mm.The medium was allowed to set. All the plates were inverted and incubated at 37^{o}C for 24-48hour.Viable counting of bacterial colonies were done using a colony counter with a digital read out(Gallenkamp).The colony count was calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution and reported as colony forming unit per gramme of the garri samples.

3.Statistical Analysis

All data were analyzed using statistical package for social science version 15. Comparison of mean counts of the different viable count techniques were determined by ANOVA(Analysis of variance).

4.Results

Table1 depicts the viable count of Pure culture of *E.coli* ATCC 25922 using different techniques namely; Pour plate, Surface spread,Plate count and Miles and Misra techniques. The Miles and Misra technique was significantly higher than every techniques in terms of bacterial density while Pour plate had the lowest bacterial count. A variation in count between the methods was observed from 3.16% to 5.71%,that is Miles and Misra was 5.71% higher than the surface spread, surface spread was 3.16% higher than the plate count and plate count techniques was 4.74% higher than the pour plate techniques, all in terms of bacterial density. Table 2 represents the materials requirement by each of the four viable count techniques. The table shows that, if twenty plates of Plate count agar is needed for each of the pour plate, surface spread and plate count techniques, then five plates count agar is enough for Miles and Misra method. The figures shown for peptone water only represent the percentage of diluent of the garri samples but do not include materials such as 9ml volumes of diluent used for the preparation of 1/10 homogenate. The pour plate, spread plate and plate count techniques also required large numbers of Petridishes and agar. In addition, the materials required for each of pour plate, spread plate and plate count were found to occupy 4-5times the operating and storage space needed for the Miles and Misra technique. The time taken to test one sample by the pour plate method, Plate Count method, and surface spread method, two or more samples would have been processed by miles and misra techniques. All the methods examined relied on support labour both for the preparation of culture media, diluent and sterile equipment and for the disposal of used materials.

However, the amount of support labour for each method varied from substantial support for pour plate, spread plate and plate count to little support for Miles and Misra techniques (Table 2).

Table 1 Comparison of the Viable Count Techniques.

Viable Count Techniques	n	Bacterial Density Log CFU/g Mean±SEM	Highest bacterial Density
Pour plate	2	9.83±0.01	Miles and miles techniques
Surface Spread	2	10.73±0.01	
Plate Count	2	10.37±0.06	
Mile &Misra	2	11.38±0.01	

P <0.05, F value =757.72,n=number of time the experiment is replicated

Table 2. Materials requirements for the examination of garri samples by the four methods.

Media/Equipment	**Pour Plate**	**Surface Spread**	**Plate Count**	**Miles & Misra**
Plate Count Agar	20.0	20.0	20.0	5.0
Peptone water	0.1%(w/v)	0.1%(w/v)	0.1%(w/v)	0.1%(w/v)
Petridishes	20.0	20.0	20.0	5.0
Dilution vessels (n/d)	20.0	20.0	20.0	20.0
Sterile Pasteur pipette (d)	Nil	Nil	Nil	20.0
Additional Items	Nil	Glass Spread	Nil	Nil
Sterile pipette	20	20	20	Nil

5.Discussion

Previous comparison between methods of enumeration in various commodities have been carried out under strict conditions of pure culture of bacteria, spore suspensions or relatively small number of food(Kramer and Gilbert,1978.,Birgit et al.,2005.,Alatossava et al.,2007).The standard plate count (SPC) has been reported to be by far the most widely used method for determining the numbers of viable cells or colony forming units (CFU) in a food product(AOAC,1984)..Among the four different methods of SPC employed in the present study for the viable count of 18 hour pure culture of *Escherichia coli* ATCC25922, Miles and Misra technique gave the highest mean count and this was also significant (P<0.05) (Table 4.1).This finding is contrary to the finding of Ogunledun(2007) who reported the pour plate method as the most efficient method of recovering bacterial from cocoa based beverages. This difference could be due to the difference in the nature of the two food substrates. In terms of cost of material needed, support labour and time used, the method of Miles and Misra was observed to be economical, faster and effective (Alatossava et al.,2007).As early as 1916, the difficulty that food supply can be an issue, that colonies close to each other on the plate may merge and that neighbour colonies may inhibit or conversely stimulate the growth was pointed(Breed and Dotterer,1916).Of all methods aim to detect the absolute number of bacteria, no one is perfect and approaches rely on differences in growth rate between different species (Alatossava et al.,2007). It seemed that Miles and Misra has never been used for garri analyses, it should therefore be of interest for enumerating microorganisms in garri especially where the viable bacteria is needed without delay.

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