Malaya Journal of Biosciences 2017, 4(2):56-62
ISSN 2348-6236 print /2348-3075 online

Malaya Journal of Biosciences

www.malayabiosciences.com

RESEARCH ARTICLE

Open Access Full Text Article

Bacteriological and Mycological Quality Assessments of some Ready-to-eat foods sold in Kaduna State University market, Kaduna, Nigeria

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Article Info: Received 15 Nov 2017; Revised: 26 Dec 2017; Accepted 28 Dec 2017.

ABSTRACT

The bacteriological and mycological quality assessment of selected ready-to-eat foods sold in Kaduna State University (KASU) were investigated. A total of one hundred and sixty ready-to-eat food samples: including rice, beans, yam and waina (rice cake) were collected from twelve food vending sites which serves as the major ready-to-eat food vending centres to the student community. Pour plate methods were used for the isolation of bacteria on different media and the fungi on potato dextrose agar (PDA). The bacteria and fungi isolates were characterized and identified using standard techniques. A total of five species of bacteria and three species of fungi were isolated and identified. These bacteria species include; *Escherichia coli*, *Staphylococcus* sp, *Salmonella* sp, *Pseudomonas* sp, *Shigella* sp and the fungi species include; *Aspergillus* sp, *Mucor* sp and *Rhizopus* sp. The mean total aerobic bacteria plate count ranged from 2.3 $x10^1$ to 6.2$x10^9$ and fungal count ranged from 5.3$\times10^1$ to 4.5$x10^9$. The level of some food contaminations were within acceptable microbiological limits in relation to the specifications by International Commission for Microbiological Specification for Foods (ICMSF), except for waina which constituted about 30% of the total microbial isolates having *E.coli* and *Aspergillus* sp as the most predominant and *Shigella* sp as the least predominant. This could be attributed to extensive handling, mixing and processing of the waina. The Hazard analysis critical control point (HACCP) systems should be enforced in foods sold on campus through stringent supervision of the ready-to-eat foods by relevant authorities to prevent possible outbreak of food borne illness.

Keywords: Biomass carbon, Plantation forests, Regression equation, Stand density, Volume equations

1. INTRODUCTION

Street-vended foods or street foods are those foods and beverages that are prepared and/or sold by vendors on the street and in other public places for immediate consumption or for consumption at a later time without further processing or preparation [1]. Street-vended foods are prone to contamination because they are sold in the open and are often not covered. Additionally, because street vendors prefer to take their products to their customers, they often operate from places such as bus terminals, industrial areas, schools, market places, streets. Such locations usually do not meet food and safety requirements [2]. Sale of food in the streets is very controversial from a

health standpoint. The main health hazard associated with street foods is microbial contamination. A number of observational studies have shown that street foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings [3] that make them prone to contamination. In addition, most of the vendors had either no formal education or few years of schooling. People are more attracted to non-homemade foods like, restaurant foods and street foods. It is not always about the fascination it is about the time to prepare food to eat, e.g. lack of time people of all age from student to job holder have to rely on non-homemade foods [4]. Usually non-homemade foods are not hygienic, to keep the attention of customer and earn more money with less effort vendors compromise with food quality. People suffer a lot due to consumption of unhygienic foods [5]. Safe food is a basic human right despite many foods are frequently contaminated with naturally occurring pathogenic microorganisms. Such pathogens cannot be detected organoleptically (seen, smelled or tasted), but can cause disease of varying severity, including death specially if the way they are conserved during exposition for sales provides conditions for those microorganisms to grow and reach considerable levels of contamination. Thus, food safety issues are of major importance to world health [1]. Illness resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society [6].

In Nigeria, as in many developing countries, a major source of ready-to-eat foods (Street foods (SFs) are prepared and or sold at public places such as schools, markets places, along the streets. The SFs offer food at relatively cheaper rate and at easily accessible places [7]. Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming. Thus, with the increase in the number of hours spent at work places by parents and schools, the importance of SFs in the human feeding is increasingly becoming very important among all socio-economic groups. A number of observational studies have shown that these foods are sometimes held at improper temperatures, excessively handled by food vendors and sold at very dirty surroundings [8] and [9]. In addition, the vendors practice poor personal hygiene and reports of food vendors being carriers and therefore could serve as a potential source of transmission of enteric fevers. Most of the vendors have either no formal education or few years of schooling and therefore, lack knowledge on proper food handling and their role in the transmission of pathogens [7]. Some pathogenic microbes associated with contamination and food poisoning include *Bacillus cereus, Listeria monocytogenes, Salmonella* sp, *Shigella* sp, *Clostridium perfringens, Campylobacter* sp, *Escherichia coli* O157 (and other Shiga toxin-producing *E. coli* (STEC) Shiga toxin-producing *E. coli* (STEC) is a group of *E. coli* that produces one or more verocytotoxins (VT), also known as Shiga-like toxins. This group of bacteria is also called Verocytotoxin-producing *E. coli* (VTEC) [10,11]. The STEC is transmitted to humans primarily through consumption of contaminated foods, such as raw or uncooked ground meat products, contaminated fruits and vegetables and direct contact with animals and their environment. Direct person-to-person transmission through the oral-faecal route can also occur. *E. coli* O157:H7 is the predominant serotype in a pathogenic subset of STEC, designated enterohaemorrhagic *E. coli* (EHEC). The designation is based on their capacity to cause attaching and effacing lesions in epithelial cells of intestine, and their ability to cause haemorrhagic colitis (bloody diarrhoea) and a life-threatening complication haemolytic uraemic syndrome (HUS) in humans. Other non-O157 serogroups, including O26, O91, O103, O104, O111, O113, O117, O118, O121, O128 and O145, have been associated with occasional outbreaks of human disease, and others may be associated with sporadic cases [12]. A combination of factors (pH, water activity) can also control the growth of *L. monocytogenes* in foods. Consuming Listeria contaminated food may lead to the development of a disease called listeriosis. Ready-to-eat foods with long shelf lives under refrigeration such as soft cheeses and ready-to-eat poultry and meat pose the greatest risk as *L. monocytogenes* may grow to significant numbers at refrigeration temperatures when given sufficient time. However, the growth of *L. monocytogenes* is not supported under freezing condition. Foodborne listeriosis is a relatively rare but serious disease with high fatality rates (20%-30%). *Listeria* predominantly affects foetuses and new borns, elderly and immunocompromised individuals such as patients with AIDS, diabetes mellitus or cancer [8].

Most student particularly frequent food vendors in kaduna state university market. There are notable features in some of these food vending site that are questionable i.e. some vendors practice poor food sanity, where foods are served in open environment and in some cases cutleries are not properly sanitized. Hence microbiological assessment is of paramount importance to tackle these in contingences. Therefore, present study aimed at bacteriological and mycological quality assessment of ready-to-eat foods

obtained from the major food vending centers in Kaduna State University (KASU) for acceptable microbiological standards and specification for foods.

2. MATERIALS AND METHODS

2.1 Collection of Samples

A total of one hundred and sixty sample samples (rice, beans, yam and waina) were collected from 12 shops over a period of five weeks from Kaduna state university market (50- rice, 50- beans, 50- yam and 10- waina (rice cake) samples). All the food samples were collected in sterile polythene bags separately from the market and were conveyed to the Kaduna State University Microbiology laboratory for analysis.

2.2 Sample Preparation

Ten grams of each food samples were homogenized using an electric blender. Before blending, the blender was sterilized for 15-20 minutes with boiled water. Samples were blended with 9ml of sterile peptone water for each respectively.

2.3 Media Preparation

Nutrient agar was prepared according to manufacturer's instruction with respect to required quantity for the analysis. Eosine methylene blue was prepared according to manufacturer's instruction with respect to required quantity for the analysis. Peptone water was prepared according to manufacturer's instruction with respect to required quantity for the analysis. Sabouraud Dextrose agar was prepared according to manufacturer's instruction with respect to required quantity for the analysis. MacConkey broth was prepared according to manufacturer's instruction with respect to required quantity for the analysis. Lactose broth was prepared according to manufacturer's instruction with respect to required quantity for the analysis.

2.4 Isolation of Bacteria from Food Samples

For the isolation of the total bacteria counts, serial dilution of the samples were prepared using a 5 fold dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and10^{-5}) respectively. Using a sterile syringe, 1ml of the sample were transferred aseptically into the first tube labelled as 10^{-1}. 1ml was then withdrawn from the tube after shaking to ensure thorough mixing and transferred to the second tube making a 10^{-2} dilution. And the same process was repeated serially to obtain 10^{-3}, 10^{-4} and 10^{-5} dilution [13,15].

2.5 Test for Coliform

Presumptive test: One gram of each food samples were transferred to sterile McCartney bottles containing MacConkey broth and inverted Durham tubes separately. Incubation was for 24-48 hours at 37ºC. Tubes showing gas production and/or colour change of dye were streaked on EMB plates. Incubation of plates for confirmatory test was at 37ºC and 44ºC for 24 hours; colonies from EMB plates were picked and inoculated into tubes containing lactose broth for complete test and onto nutrient agar slants for further characterization. Inoculated tubes and slants were incubated for 24 hours at 37ºC [14].

2.6 Characterization and Identification of Bacteria Isolates

The standard procedures as reported in [13] were employed for the bacteria colony, cell morphology identification and biochemical tests. The biochemical tests include: Gram's staining, Catalase test, Coagulase test (Slide test), Indole test, Methyl-Red Voges Proskauer test (MR-VP) for bacteria characterization and identification.

2.7 Isolation of Fungi from Food Samples

Potato dextrose agar was used for fungal count. Pour plate technique was employed using10^{-3}, 10^{-4} and 10^{-5} dilutions. Exactly 1ml aliquots was aseptically withdrawn and introduced into plates separately and were allowed to solidified and were incubated at ambient temperature for 3 to 5 days. The bacteria and fungi isolates were counted using a colony counter. The colony forming unit (CFU/g) was determined using the formula:

$$\text{CFU/g} = \frac{\text{No of colonies x reciprocal of dilution factor}}{\text{Volume of inoculums used}}$$

3. RESULTS AND DISCUSSION

Table 1, showed the total bacteria, total fungal and coliform counts of rice analyzed. The highest fungal count in rice occurred in shop 6 with $6.8x10^5$cfu/g and the lowest count was recorded as $2.5x10^2$ cfu/g in shop 1. The highest fungal count in rice was recorded in shop 4 with 3.0×10^5 cfu/g and least in shop 5 with 3.1×10^3 cfu/g. The total bacteria count, total fungal count and coliform count of beans is shown in Table 2. The highest bacteria count in beans occurred in shop 10 with $2.2x\ 10^5$ cfu/g and least was $2.7x10^1$ cfu/g in shop 2. The highest fungal count in the beans analyzed was $3.4x10^3$ cfu/g and lowest was $7.3x10^1$ cfu/g in shop 10 and shop 3 respectively. The total bacteria count, total fungal count and coliform

count of yam is presented in table 3. The highest bacteria count in yam occurred in shop 8 with $3.4x10^4$ cfu/g and least was $4.2x10^1$ cfu/g in shop 6. The highest fungal count in yam was recorded as $4.6x10^4$ cfu/g in shop 8 and least was $5.2x10^1$ cfu/g in shop 7 (Table 3). Table 4 showed the total bacteria count, total fungal count and coliform count of waina (rice cake). The highest bacteria count in waina occurred in shop 6 with $4.5x10^4$ cfu/g and least was $3.5x10^1$ cfu/g in shop 7. Fungal count in waina was recorded as $7.5x10^5$ cfu/g which is the highest and $3.4x10^1$ cfu/g as the least in shop 2 and 7 respectively. Table 5 showed the result for biochemical test of bacteria isolates which confirms five different bacteria species namely, *Staphylococcus aureus*, *E. coli*, *Salmonella* sp, *Shigella* sp, and *Pseudomonas* sp. The cultural and morphological characteristics of fungal isolates were confirmed to be *Aspergillus* sp, *Rhizopus* sp and Mucor sp with distinct morphological and cultural characteristics. The isolation, characterization and identification of bacteria species such as *Staphylococus aureus*, *E. coli*, *Salmonella* sp, *Shigella* sp, *Pseudomonas* sp and fungi species such as Aspergillus sp *Rhizopus* sp and *Mucor* sp in this study could have various adverse effects ranging from nausea to vomiting and diarrhea. This corroborate with the findings of (14) and (15) who isolated and identified *Staphylococcus aureus*, *Streptococcus* sp, *Flavobacterium* sp, *Bacillus cereus*, *Klebsiella pnuemoniae*, *Shigella dysenteriae*, *Micrococcus* sp, *Pseudomonas* sp, *Enterobacter* sp, *Escherichia coli*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp, *Fusarium* sp, *Rhizopus stolonifer and Mucor* sp from street vended foods sold in High way, Onitsha, South East and Kwara Polytechnic in Nigeria. The presence of *Staphylococcus aureus* in the foods analyzed is indicative of human contamination after production. This could be from direct human contact such as fingers or indirectly through additives or utensils. The organism is associated with endotoxin, violent nausea, vomiting and diarrhea. *Aspergillus* sp is another isolate that is associated with the production of toxin; diarrheal and emetic in food which causes food poisoning. The presence of *E. coli*, *Shigella* suggested fecal contamination. Although some *E. coli* are harmless. The bacteria count obtained in this study are indicative of post contamination in the light of the amount of heating that goes into food production. Similar post treatment contamination has been reported by [16]. This contaminations can occur during cooling and exposure to the air which has been identified as the main source of microbial contamination of most street foods. The preparation of foods in advance of consumption, exposure, holding of food at ambient temperature conducive for microbial multiplication are some of the various possible reasons why there is notable contamination by microorganisms. *E. coli* and *Aspergillus* sp were predominant in waina (rice cake) which could possibly be as a result of pre-preparation and post-preparation practices like improper cleaning of the waina pot and use of bare hands to serve customers as reported similar in the study [14]. The rich medium of waina (rice cake) could equally be a factor in the increased microbial loads of the samples. *Shigella* was the least occurred organism in some of the food samples analyzed. The high level of contamination of waina (rice cake) could be as a result of extensive handling and mixing during processing microbes can be introduced contaminants via food handlers, utensils and from the environment.

4. CONCLUSION

The result of this study showed that some of the microbial counts were within the limits set by International Commission on Microbiological Specification for Food (ICMSF) with a few exceptions of waina (rice cake) especially in shop 11 which showed high level of microbial contamination. The foods provided to the students by the vending sites on campus are of acceptable microbiological quality except however, for waina (rice cake) from shop 11 and 12 with tolerable to unacceptable. The International Commission for Microbiological Specification for Foods (ICMSF) states that ready-to-eat foods with plate counts between $0 - 10^3$ is acceptable, between $10^4 \leq 10^5$ is tolerable and 10^6 and above is unacceptable. Eight (8) microorganisms namely, *Staphylococus aureus*, *E. coli*, *Salmonella* sp, *Shigella* sp, *Pseudomonas* sp, *Aspergillus* sp *Rhizopus* sp and *Mucor* sp were isolated and identified. The results revealed that there are a few lapses in the microbial quality of vended foods which could be very much responsible for the number of recorded cases of food borne illness in the school sick bay. It has been established that quality of any food is determined by the level of infestation by microorganisms. Food handling personnel play important role in ensuring food safety throughout the chain of food production, processing, preparation and storage. Mishandling and disregard to hygienic measures on the part of the food vendors have been reported by several authors. Recommendations include; (i) Local processors of foods should be enlightened on hygienic methods of processing, preservation and storage of the foods. (ii) Relevant quality control units must be reactivated to assess the quality of the foods sold in the market. i.e. the use of hazard analysis and critical control point (HACCP) systems.

Table 1: Total Bacteria, Fungal and Coliform Counts of Cooked Rice from KASU Market

Shop code	Total bacteria count (Cfu/g)	Total fungal count (Cfu/g)	Coliform count
1	$4.0x10^3$	$3.0x10^4$	1:0:0
2	$5.3x10^5$	$4.5x10^2$	0:1:0
3	$2.3x10^2$	$3.4x10^3$	0:0:0
4	$5.0x10^2$	$2.2x10^5$	0:1:0
5	$4.5x10^2$	$3.2x10^3$	0:0:0
6	$5.1x10^4$	$4.5x10^3$	0:1:0
7	$3.3x10^3$	-	0:1:0
8	$6.0x10^3$	$4.3x10^3$	0:0:0
9	$4.5x10^5$	$4.4x10^3$	0:1:0
10	$3.8x10^5$	$2.5x10^5$	1:1:0

Table 2: Total Bacteria, Fungal and Coliform Counts of Cooked Beans from KASU Market

Shop code	Total bacteria count (Cfu/g)	Total fungal count (Cfu/g)	Coliform count
1	$2.7x10^5$	$3.4x10^4$	0:0:1
2	$3.0x10^3$	$3.0x10^5$	0:0:0
3	$4.6x10^5$	$4.5x10^3$	0:0:0
4	$2.2x10^3$	$7.3x10^4$	0:1:1
5	$4.5x10^4$	$3.6x10^3$	0:0:1
6	$5.6x10^2$	$3.2x10^5$	1:0:0
7	$4.0x10^3$	$6.8x10^3$	1:1:0
8	$6.5x10^5$	$3.4x10^2$	0:0:1
9	$5.3x10^3$	$3.0x10^4$	0:0:0
10	$3.4x10^4$	$6.4x10^3$	1:0:0

Table 3: Total Bacteria, Fungal and Coliform Counts of Cooked Yam from KASU Market

Shop code	Total bacteria count (Cfu/g)	Total fungal count (Cfu/g)	Coliform count
1	$3.4x10^2$	$2.5x10^5$	0:0:0
2	$5.3x10^4$	$8.0x10^3$	0:0:0
3	$3.5x10^3$	$3.4x10^5$	0:1:0
4	$4.2x10^4$	$6.3x10^2$	0:1:1
5	$6.0x10^2$	$5.6x10^4$	1:0:0
6	$7.3x10^5$	$3.0x10^3$	0:0:0
7	$6.3x10^3$	$7.2x10^5$	0:0:1
8	$2.4x10^4$	$5.3x10^3$	0:1:1
9	$4.5x10^2$	$3.2x10^2$	0:1:0
10	$5.0x10^3$	$5.6x10^5$	0:0:0

CFU: Colony forming unit per gram.

Table 4: Total Bacteria, Fungal and Coliform Counts of Cooked Waina (rice cake) from KASU Market

Shop code	Total bacteria count Cfu/g	Total fungal count Cfu/g	Coliform count
11	$2.9x10^8$	$5.8x10^5$	1:0:1
12	$2.1x10^6$	$7.3x10^5$	1:1:0

CFU: Colony forming unit per gram.

Table 5: Morphological and Biochemical Characteristics of Bacteria Isolates from Food Samples

Gram reaction	**Indole**	**Catalase**	**Oxidase**	**Coagulase**	**Citrate**	**Probable Microorganisms**
-	+	+	-	-	-	*Escherichia coli* 1-10
-	-	+	+	-	+	*Pseudomonas* sp 1-10
-	+	+	-	-	-	*Shigella* sp 1,4,6,7,8,9,10
+	-	+	-	+	-	*Staphylococcus* sp 1-10
-	+	+	-	-	+	*Salmonella* sp 3,4,5,7,8,9

Key: + = positive ,- = negative, Shop code:1-10.

Table 6: Cultural and Morphological Characteristics of Fungal Isolates from Food Samples

Cultural/ morphological appearance	Probable Fungi
The colonies appeared white with cotton masses of mycelium. The hyhae was non septate with smooth walled sporangiospore which stands erect.	*Aspegillus* sp 1-10
The colonies appeared white-to-gray cotton candy, darkening with time with non-septate hypae having long branched sporangiophore	*Muco*r sp 1-10
Colonies appeared white initially and turn` grey-to-yellow brown in time, non septate with root-like hypae	*Rhizopus* sp 1-10

Shop code:1-10.

Conflicts of Interest

There are no conflicts of interest.

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