

Full Length Research Paper

Microbial load in ready-to-eat rice sold in Benin City

Wogu, M. D., Omoruyi, M. I.*, Odeh, H. O. and Guobadia, J. N.

Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, P.M.B. 1100, Benin City, Edo State, Nigeria.

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The microbial load of ready-to-eat rice from both local fast food centers (local restaurants) and Standard fast food centers (high class restaurants) within Benin City was investigated. The total colony count ranged from 2.0×10^4 to 1.2×10^6 for bacteria and 8.0×10^4 to 2.0×10^5 for fungi. Four bacteria were isolated: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *B. cereus*, which is mainly associated with food poisoning because of its ability to produce toxins, was present in 37.5% of samples alongside *E. coli*. Two fungi were isolated: *Saccharomyces cerevisiae*, occurring in 50% of samples, and *Aspergillus niger*. Ready-to-eat rice from standard fast food centers was found to have more microbial load and more microorganisms compared to ready-to-eat rice from local fast food centers. The results of our study indicated that most of the ready-to-eat rice samples examined did not meet bacteriological quality standards. Hence, it is recommended that a more close supervision of ready-to-eat food should be carried out by relevant authorities.

Keywords: Microbial load, ready-to-eat rice, standard fast food centers and local fast food centers.

INTRODUCTION

Rice is a grain of a monocotyledonous plant *Oryza sativa* and is the most important staple food for a large part of the world human population, especially in Africa, Southeast Asia, the Middle East, Latin America, and the West Indies (Boyce et al., 1996).

Rice is the grain with the second highest worldwide

production, after maize (corn). Since a large portion of maize crops are grown for purposes different from human consumption, rice is probably the most important grain with regards to human nutrition and caloric intake, providing more than one fifth of the total calories consumed worldwide by humans. In Africa, rice has been used to improve nutrition quality, boost food security, foster rural development and support sustainable landcare (Chomvarin et al., 2006). It is basically grown as an annual plant, and its cultivation is well-suited to countries and regions with low labor cost and high rainfall, as its cultivation is very labor-intensive and requires plenty of water.

Research has shown that the nutritional value of rice is provided by its content in carbohydrate, sugar, fiber, energy, fat, protein, water, iron, calcium, magnesium and zinc (Kaneko et al., 1999). Rice is used for making a number of products and beverages such as amazake, horchata, rice milk and sake. Rice flour has also been recommended for people on a gluten-free diet because of the absence of gluten in the grain (Ali et al., 2008).

Almost one in every five adult Americans is reported to eat at least half a serving of white or brown rice per day (Addo et al., 2007). In Nigeria, rice is the prevalent food

*Corresponding author. E-mail: uyiwithintegrity@yahoo.com.
Tel: +234(0)8062764607.

Abbreviations: LRK1, Ready to eat rice from point of sales located at Ikpoba-Hill, sampling point 1; LRK2, ready to eat rice from point of sales located at Ikpoba-Hill, sampling point 2; SRK1, ready to eat rice from standard fast food located at Ikpoba-Hill, sampling point 1; SRK2, ready to eat rice from standard fast food located at Ikpoba-Hill, sampling point 2; LRM1, ready to eat rice from point of sales located at M.M. Way, sampling point 1; LRM2, ready to eat rice from point of sales located at M.M. Way, sampling point 2; SRM1, ready to eat rice from standard fast food located at M.M. Way, sampling point 1; SRM2, ready to eat rice from standard fast food located at M.M. Way, sampling point 2; Point of sales, local fast food centers.

in most fast food centers, because of its easy preparation and the varieties with which it can be served. The patronage of ready-to-eat food centers within Benin City have also increased over time, as parents and guardians have become busy and as such prefer to buy convenient food instead of preparing it. Four out of every five Nigerian visit a fast food center at least once a day. Due to the increased consumption of rice as a staple food in most parts of the world, there has been an increased need to determine its safety, especially when prepared and sold in fast food centers and cafeterias.

Moreover, the microbiological quality of ready-to-eat rice is said to be influenced by a number of factors such as cuisine type, rice type, cooking, serving methods and management/food handling (FEHD, 1995). In 2009, De Bess et al. (2009) reported that 32% of food handlers in ready-to-eat centers in Washington had no knowledge of food safety practices and of prevention of foodborne diseases. This may result in the transmission of foodborne pathogens to the people consuming such food. As a consequence, Nichols et al. (1999) demonstrated that the microbiology quality of ready-to-eat rice from some eateries is low compared to some others. Due to the number of outbreaks of food poisoning from ready-to-eat food centers, it has been suggested that laws should be enacted on the establishment of ready-to-eat food centers, and that the staff of such centers should be trained on proper hygienic procedures as well as the transmission of foodborne diseases, especially in developing countries.

In Nigeria, there is little or no knowledge of foodborne diseases and their transmission among food handlers working in ready-to-eat food centers, and no rules are provided from the establishment of ready-to-eat food centers. Most proprietors of ready-to-eat food centers are not duly licensed, and their staff properly selected. Hence, there is need to examine the microbiological quality of ready-to-eat rice from food centers to reduce the risk of food poisoning.

Aims and objective

The aims and objectives of this study are:

1. To isolate microorganisms (bacteria and fungi) from ready-to-eat rice, obtained from different fast food centers within Benin metropolis.
2. To compare the microbial load of ready-to-eat rice collected from these fast food centers.

MATERIALS AND METHODS

Sample collection

Forty eight different samples of cooked rice (fried rice, Jollof rice and white rice) were randomly obtained from High class fast food centers and from local fast food centers within Benin metropolis,

between January and June, 2010. All samples were collected in sterile containers, and were taken under aseptic condition to the laboratory for microbiological analysis.

Microbiological analysis

Bacterial colony count

3 g of each sample was dissolved in deionized water, and serial dilution of each sample was made, to form 10^{-3} , 10^{-2} and 10^{-1} dilutions.

1 ml sample from each dilution (10^{-3} , 10^{-2} and 10^{-1}) were seeded on plate count agar using spread plate method, and the medium was then incubated at 37°C for 24 h. The colony count was reported as colony forming units per gram of food sample (cfu/g).

Isolation of bacteria

0.1 ml sample of the dilution was spread on culture plates with a sterile glass rod onto Nutrient Agar, Blood Agar and MacConkey Agar, and then incubated at 37°C for 24 h. After the incubation time, the different culture plates were examined for microbial growth. Sub-cultures were made, to get discrete colonies, and different morphological tests were performed on the colonies, which were then stored in a slant at 4°C for further biochemical investigations, in order to identify microorganisms in the isolate.

Isolation of fungi

0.1 ml of each sample was seeded on Potato Dextrose Agar and incubated at room temperature for about four days, after which the plate was examined to detect fungal growth.

Identification of isolates

The different isolates obtained from the different plates were macroscopically examined and then biochemically tested, to identify the organism to the species level, using Bergey's manual of determinative bacteriology.

The growth portion of the fungal mycelia on the Potato Dextrose Agar medium was cut and placed on grease free microscopic slide containing few drops of Lacto phenol cotton blue, and covered with a cover slip. The mycelium was then examined under the microscope at a magnification of x10.

Total colony count

The plate count agar was examined and colonies present were counted and recorded after incubation at 37°C for 24 h, to get the total colony count in cfu/g, as shown in Tables 1 and 2.

RESULTS

Table 1 shows mean values for total colony count for bacterial isolates. Four bacteria were isolated: *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *B. cereus*, which is mainly associated with food poisoning because of its ability to produce toxins, was present in 37.5% of samples, alongside *E. coli*.

Table 1. Mean values for total colony count for bacterial isolates.

Sampling point/Code	Total colony count for bacteria (cfu/g)
LRK1	$2.4 \times 10^5 \pm 34058$
LRK2	$6.0 \times 10^5 \pm 56328$
SRK1	$2.0 \times 10^4 \pm 2489$
SRK2	$2.0 \times 10^4 \pm 2446$
LRM1	$8.8 \times 10^5 \pm 98434$
LRM2	$3.8 \times 10^5 \pm 39876$
SRM1	$1.2 \times 10^6 \pm 265344$
SRM2	$1.0 \times 10^5 \pm 18932$

Results are given as mean value \pm standard deviation.

Table 2. Mean values for total colony count for fungi isolates.

Sampling point/Code	Total colony count for fungi (cfu/g)
LRK1	$6.0 \times 10^4 \pm 7457$
LRK2	$1.6 \times 10^5 \pm 20548$
SRK1	$8.0 \times 10^4 \pm 8656$
SRK2	$1.2 \times 10^5 \pm 16325$
LRM1	$2.0 \times 10^5 \pm 18785$
LRM2	$1.0 \times 10^5 \pm 14132$
SRM1	$8.0 \times 10^4 \pm 6898$
SRM2	$1.2 \times 10^5 \pm 22344$

Results are given as mean value \pm standard deviation.

Table 3. Distribution of bacteria isolates in the different sampling sites.

Bacteria found	Sampling site							
	LRK1	LRK2	SRK1	SRK2	LRM1	LRM2	SRM1	SRM2
<i>Bacillus cereus</i>	+	-	-	-	+	-	+	-
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	+	-	-
<i>Staphylococcus aureus</i>	-	-	+	+	-	-	+	-
<i>E. coli</i>	-	+	-	-	-	-	-	-
Type of rice	W	W	J	J	W	W	F	J

W = White rice, J = Jollof rice, F = Fried rice.

Sample SRK1 and SRK2 had the lowest bacterial colony number while SRM1 had the highest bacterial colony number of 1.2×10^6 .

The fungi isolated from samples of rice were *Saccharomyces cerevisiae*, occurring in 50% of samples, and *Aspergillus niger*. Table 2 shows mean values for total colony count for fungi isolates.

The distribution of the four bacteria species present in all samples is shown in Table 3. *B. cereus* and *S. aureus* were present in 3 samples while *E. coli* and *K. pneumoniae* were present in only one of the samples.

The distribution of fungi isolates in the different

sampling sites is shown in Table 4.

DISCUSSION

Our results revealed that the bacterial isolates from all samples collected were *B. cereus*, *S. aureus*, *E. coli* and *K. pneumoniae* (table 3). This finding is similar to previous reports by Desmarchelier et al. (1994), Nichols et al. (1999) and Mensah et al. (2002). *B. cereus* and *E. coli* were the prevalent bacteria isolates. Both microorganisms were present in 37.5% of the samples.

Table 4. Distribution of fungi isolates in the different sampling sites.

Fungi Found	Sampling Site							
	LRK1	LRK2	SRK1	SRK2	LRM1	LRM2	SRM1	SRM2
<i>Saccharomyces cerevisiae</i>	+	-	+	-	-	+	-	+
<i>Aspergillus niger</i>	-	-	-	+	-	-	-	-

Note to all tables.

B. cereus was present in rice sample collected from local fast food centers in Ikpoba Hill and in M.M. Way, as well as in one standard fast food center in M.M. Way.

K. pneumonia and *E. coli* were both present in 12.5% of samples, all from local fast food centers. Samples obtained from Standard fast food centers in M.M. Way (SRM1) produced more bacteria isolates with a higher mean colony count of 1.2×10^6 , compared with the other samples. This result however differs from that reported by Bukar et al. (2010), who found that ready-to-eat rice sold on the streets of Kano contained more microorganisms if compared to that sold in standard fast food centers.

Although few data are available on the occurrence of fungi in ready-to-eat rice, our findings showed the presence of *S. cerevisiae* and *A. niger* as fungi isolates from ready-to-eat rice: *S. cerevisiae* and *A. niger* occurred in 50 and 12.5% of samples, respectively.

Our results also showed that fried rice contains more microorganisms (*B. cereus* and *S. aureus*) in comparison with other rice preparations (Table 3), and the higher colony count (1.2×10^6). Again, this result is similar to that reported by van-Kampen et al. (1998), Gilbert et al. (1996), Patricia and Azanza (2004). However, Jollof rice did not contain a significant number of microorganisms.

Both sample collected in Ikpoba Hill from Standard fast food (SRK1 and SRK2) had the lowest microbial load for bacteria (2.0×10^4 for both), while only the former (SRK1) had the lowest microbial load for fungi (8.0×10^4 vs 1.2×10^5), respectively.

Standard fast food from M.M. Way (SFM1) had the highest colony number, 1.2×10^6 , a value exceeding the upper limit recommended by the Food Safety Institute of Ireland, that in 2001 set a recommended reference value for the bacteria colony count of ready-to-eat rice inferior to 10^5 cfu/g. However, this result is in accordance with that obtained by Patricia and Azanza (2004), who reported bacteria colony count value $> 10^5$ cfu/g.

In conclusion, our results indicated that most of the ready-to-eat rice samples examined did not meet bacteriological quality standards. Standard fast food centers within Benin metropolis are beginning to enjoy high patronage, especially as parents are becoming busier, but the microbial quality of the ready-to-eat rice they offer is rather questionable and needs to be improved. Ready-to-eat rice from standard fast food centers was found to have more microbial load and more microorganisms compared to ready-to-eat rice from local fast food centers.

In the course of our investigation, we observed that most standard fast food centers cooked food overnight and then store it in the refrigerator for a subsequent use. This practice may be responsible for the microbial proliferation which occurs when the bulk rice is stored overnight, waiting for its final cooking many hours later.

Hence, we suggest improving the hygienic measures and the conservation practices in order to minimize the microbial contamination of food so largely consumed. Moreover, it is recommended that a more close supervision of ready-to-eat food should be carried out by relevant authorities.

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