



Survival Pattern and Toxin Production of Pathogens in Spiced and Non Spiced Fried Rice

**Afolake Atinuke Olanbiwoninu^{1*}, Oluwatooni Oluwaniran², Faidah Oladosu²,
Kolawole Banwo² and Sunday Odunfa²**

¹*Department of Biological Sciences, Faculty of Natural Sciences, Ajayi Crowther University, Oyo State, Nigeria.*

²*Department of Microbiology, Faculty of Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors SO, AAO and KB designed the study and supervised the research. Authors OO and FO carried out the research. Author AAO wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2020/v12i430213

Editor(s):

(1) Dr. Johnson Akinwumi Adejuyitan, Ladake Akintola University of Technology (LAUTECH), Nigeria.

Reviewers:

(1) Bora Ekinci, Mugla Sıtkı Koçman University, Turkey.

(2) Tarek Ahmed El-Desouky, National Research Centre, Egypt.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/56430>

Received 24 February 2020

Accepted 02 May 2020

Published 08 May 2020

Original Research Article

ABSTRACT

Aims: There is no data regarding the survival rate of pathogens in party foods held at different temperature and time interval in Nigeria. Hence, the need to study the survival rate of selected food borne pathogens in Fried rice (spiced and non – spiced) served at parties, monitor toxin production and the rate at which it is produced with respect to time and holding temperature as well as determine the effect of spices on the survival of food pathogens.

Place and Duration of Study: Lagelu and Ibadan North Local Government Authority Area of Ibadan, Oyo State, Nigeria, between May 2017 and September 2017.

Methodology: We obtained 10 fried rice samples (5 spiced and 5 non- spiced) from different parties inside sterile food warmers and transferred to the Food Microbiology Laboratory. Pathogens inoculated were obtained from the culture collection unit of the Food Microbiology laboratory and they were *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli*. Inoculum size of the pathogens was determined prior to inoculation. Initial temperature of the fried rice sample was taken and after inoculation, samples were taken at an interval of 2 hours to determine the survival rate of the pathogens. Brine Shrimp Lethality (BSL) assay was used to determine the level of toxin produced.

*Corresponding author: Email: flakyolanbiwoninu@yahoo.com, aa.olanbiwoninu@acu.edu.ng;

Results: The microbial load of all pathogens inoculated increased from its initial size as the holding temperature reduces and holding time increases. For *E. coli*, there was an increase from 5.3 log₁₀CFU/g to 9.3 log₁₀CFU/g and 8.3 log₁₀CFU/g in non-spiced fried rice and spiced fried rice, respectively, initial load for *S. typhi* also increase from 4.1 log₁₀CFU/g to 9.1 and 8.4, for *B. cereus* and *S. aureus* there was an increase form 6.3 log₁₀CFU/g to 9.0 and 8.4 for non-spiced and spiced rice respectively. Toxicity test shows that the lethal dose was high at 8 hours (LC₅₀:1.5) which connotes that the toxicity was high at this holding time.

Conclusion: Holding cooked foods at ambient temperature for 6 h or longer without appropriate reheating constitute a major critical control point for party food. Keeping foods at the right temperatures is an essential food safety practice. Adequate knowledge and application of this by caterers will reduce the rate of food borne illness in the country.

Keywords: Party food; survival; pathogens; time; temperature; brine shrimp; LC₅₀.

1. INTRODUCTION

The battle against foodborne diseases is facing new challenges due to the globalization of the food market, climate change and changing patterns of human consumption as fresh and publicly cooked food have been more embraced [1]. More than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with foodborne diseases in humans [2,3]. The infections range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes caused by either toxin from the “disease-causing” microbe, or by the human body’s reaction to the microbe itself [4]. Out of the many thousands different bacteria species, more than 90% of food-poisoning illnesses are caused by species of *Clostridium botulinum*, *Campylobacter*, *Staphylococcus*, *Salmonella*, *Bacillus* and Enteropathogenic *Escherichia coli* [5]. Other factors regarding the food-borne illness relate to the impact of good hygienic practices on the safety and quality of the food [6]. Statistics have shown that cross contamination during the food preparation contributes notably to the occurrence of the foodborne diseases. Cross contamination can occur due to the fact that microorganisms need certain elements to survive and grow and favourable conditions such as water, food, proper temperature, time, oxygen and proper pH or acidity. These elements determine the survival rate of the pathogens in foods [6,7,8]. Natural substances such as spices also have effective antimicrobial properties where they have been used as seasonings for centuries [9]. They have been used extensively to reduce microbial activities in food despite other qualities such as appetizing effects, prevention of food deterioration and extension of shelf life. Hence, they are considered natural preservatives and acceptable in the production and preparation of

food [10]. Incidence of food borne disease caused by consumption of party foods or outdoor sources can be traced to relatively poor handling, post cooking contamination, the holding times and temperatures which can allow the growth and survival of these pathogens. Their ability to survive in the food poses a great risk to the health of consumers. There is need for food handlers to know the holding time, temperature and keeping conditions of party foods. This knowledge would enhance distribution of microbiologically safe foods. This study is aimed at investigating the survival rate of food pathogens within the food matrix and determines the effect of spices on the survival of food pathogens at different holding time and temperatures.

2. MATERIALS AND METHODS

2.1 Sample Collection

The sample used is fried rice (spiced and non – spiced). Food pathogens used: *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhi* and *Escherichia coli* were obtained from Food Microbiology Laboratory, University of Ibadan, Oyo State, Nigeria.

The isolates were plated out using selective media for each and after ascertaining that they are pure cultures they were Gram stained and viewed under the microscope, preliminary biochemical test was carried out for confirmation of each pathogen. Tryptose Soy Agar (Difco) for *Bacillus cereus*, Mannitol Salt Agar (Oxoid) for *Staphylococcus aureus* and *Salmonella* and Shigella agar (Oxoid) for *Salmonella typhi* was used as selective media for each organism.

The inoculum size of pathogens to be inoculated into the food samples was determined using the

modified method of Omafvube et al. [11,12]. The pathogens were grown on Nutrient agar at 37°C for 24 h. Each of the culture was harvested into 10 mL sterile peptone water and was serially diluted to give an absorbance of 0.03 at 540 nm using a Jenway 6405 UV/VIS spectrophotometer.

2.2 Survival of Pathogens at Different Temperatures

The initial temperature of the food samples was determined using a sterile thermometer and this was repeated at every 2 hours interval. At the initial temperature of 80°C, inoculum size of 0.1 mL of each of the pathogens determined was transferred aseptically into 100 g of the food sample and stored in food warmers at holding time of 0, 2, 4, 6 and 8 h. Measurement of temperatures was carried out at each holding time using a sterile thermometer. At each holding time 10 g of each food sample (spiced and non – spiced fried rice) inoculated with a particular pathogen was serially diluted in 90 mL of sterile distilled water of dilutions of 10^{-1} to 10^{-6} . Desired dilutions were plated out and previously sterilized selective media of respective pathogens was poured and swirled gently to disperse the inoculum. The media was allowed to solidify; the labeled plates were inverted and incubated at 37°C for 24 h. After the incubation period, distinct colonies were counted and recorded to determine the viable population [13].

2.3 Antimicrobial Effects of Spices on the Microbial Population of the Inoculated Foods

The spices used for this study are *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), Thyme, Curry powder and Capsicorn. The spices were blended and cooked with the fried rice so as to compare the microbial load of the food matrix inoculated without spices and those inoculated with spices. The spiced fried rice was inoculated with the different pathogens and stored in different food warmers at an initial temperature of 80°C. Pour plating technique was carried out at different holding times of 0, 2, 4, 6 and 8 h. The plates were incubated at 37°C for 24 h and the microbial load was also counted and recorded.

Data were entered and analysed using SPSS version 20. P-values <0.05 were taken as statistically significant association.

2.4 Toxicity Assay

2.4.1 Brine Shrimp Lethality (BSL) assay

Artemia salina cysts (Brine shrimp eggs) and sea water were obtained from the Department of Pharmacognosy, University of Ibadan and placed in a tank with dividing compartment and a lamp which serves as source of light to attract shrimps was placed closed to the tank. The eggs were added to the first compartment, covered with aluminium foil and sea water to about two-thirds of the tank. It was left for 24 - 48 h to hatch and mature as nauplii. Plain bottles were used for test and each test was done in triplicate for 1000, 500, 100, 10 and 1 µg.

50 µg of food sample extract inoculated with *Bacillus cereus* was weighed and dissolved in 5 mL of sea water. An aliquot of 0.5 mL was pipetted into the sterile plain bottles in triplicate for the 1000 µg while 0.25 mL was pipetted into plain bottles labelled 500 µg also in triplicate. For 100 µg, 0.5 mL of the extract was measured into a separate plain bottle and 0.4 mL of sea water was added and 0.5 mL was measured into bottles labelled 100 µg and this was repeated for both 10 and 1 µg.

For the positive test, Potassium dichromate (standard drug) was used. All the nauplii survived at the concentrations of 1000, 500 and 100 µg/mL but died at 100 and 1 µg/mL concentration. The ED50 was 0.5273 which made it highly toxic. The standard drug must be very toxic.

3. RESULTS AND DISCUSSION

3.1 Confirmation of Food Borne Pathogens

The *Bacillus cereus* is a gram positive rod. It tested positive to catalase, citrate, hemolysis, growth in KCN and motility test, while it is negative to gelatin hydrolysis, indole and methyl red test. *Staphylococcus aureus* is gram positive cocci, non sporing organism that tested positive to catalase, citrate utilization, coagulase, gelatin hydrolysis and tested negative to indole production. For *Salmonella typhi*, it is a gram negative rod, it tested negative to indole, growth in KCN, citrate, gelatin hydrolysis and oxidase test, while it was positive to catalase, methyl red, nitrate reduction and hydrogen sulphide production. *Escherichia coli* is a Gram negative, non sporing rod that tested negative to oxidase, VP (Vogel Proskeaur), citrate utilization, nitrate

reduction, coagulase. They are positive to catalase, methyl red, indole and are motile.

3.2 Survival Rate of Food Pathogens in Relation to Holding Temperature and Holding Time

The population of *E. coli* initially introduced to fried rice without spice at 80°C increased significantly ($P \leq 0.05$) as the holding temperature reduces and the holding time increases from 5.3 log₁₀ CFU/g to 9.3 log₁₀CFU/g after 8 hours in the holding equipment. For spiced fried rice, the rate of survival of *E. coli* was not as high as in non-spiced fried rice. The population increased from 5.3 log₁₀CFU/g to 8.3 log₁₀CFU/g after 8 h (Table 1) with a considerable reduction in temperature from 80°C to 30°C.

Non spiced fried rice inoculated initially with 4.1 log₁₀CFU/g of *Salmonella typhi* at 80°C experienced a drop in temperature to 60°C after 2 hours thereby leading to an increase in the

microbial load to 8.4 log₁₀CFU/g which then increased significantly to 9.1 log₁₀CFU/g after 8 hours of exposure. Population (Table 2) of *S. typhi* in spiced fried rice increased significantly from 4.1 log₁₀CFU/g at 80°C to 8.4 log₁₀CFU/g after 8 hours in the food warmer as the temperature reduces and holding time increases. There is a significant difference between the rate at which the pathogens survived in non - spiced fried rice and spiced fried rice.

The survival rates of *B. cereus* in non - spiced fried rice is significantly high because there was a significant increase from the initial microbial load of 6.3 log₁₀CFU/g at 80°C to 9.0 log₁₀CFU/g after 8 hours (Table 3) of holding in the food warmer at a reduced temperature of 30°C. In spiced fried rice, the population of *B. cereus* increased to 8.4 log₁₀CFU/g after holding the rice for 8 hours. There is a significant increase in the survival rate of pathogen in non – spiced fried rice compare to their rate of survival in spiced fried rice.

Table 1. Survival rate of *E. coli* in fried rice

Temperature (°C)	Time (H)	Non-spiced fried rice (Log ₁₀ CFU/g)	Spiced fried rice (Log ₁₀ CFU/g)
80	0	5.3±0.10	5.3±0.10
68	2	8.4±0.06	8.0±0.10
40	4	8.7±0.06	8.1±0.00
35	6	8.8±0.06	8.2±0.05
30	8	9.3±0.30	8.3±0.04

Values represent means ± standard deviation. $p < 0.05$. (n=3)

Table 2. Survival rate of *Salmonella typhi* in fried rice

Temperature (°C)	Time (H)	Non – spiced fried rice (Log ₁₀ CFU/g)	Spiced fried rice (Log ₁₀ CFU/g)
80	0	4.1±0.6	4.1±0.6
68	2	8.4±0.6	8.0±0.2
40	4	8.6±0.6	8.1±0.6
35	6	8.7±0.1	8.2±0.0
30	8	9.1±0.0	8.4±0.2

Values represent means ± standard deviation. $p < 0.05$. (n=3)

Table 3. Survival rate of *Bacillus cereus* in fried rice

Temperature (°C)	Time (H)	Non – spiced fried rice (Log ₁₀ CFU/g)	Spiced fried rice (Log ₁₀ CFU/g)
80	0	6.3±0.10	6.3±0.06
68	2	8.8±0.20	7.8±0.06
40	4	9.0±0.00	8.1±0.10
35	6	9.1±0.06	8.2±0.06
30	8	9.0±0.10	8.4±0.00

Values represent means ± standard deviation. $p < 0.05$. (n=3)

Table 4. Survival rate of *Staphylococcus aureus* in fried rice

Temperature (°C)	Time (H)	Non – spiced fried rice (Log ₁₀ CFU/g)	Spiced fried rice (Log ₁₀ CFU/g)
80	0	6.3±0.00	6.3±0.13
68	2	8.4±0.56	8.0±0.15
40	4	8.7±0.10	8.2±0.00
35	6	8.9±0.56	8.4±0.15
30	8	9.0±0.00	8.5±0.20

Values represent means ± standard deviation. $p < 0.05$. (n=3)

The initial load of *S. aureus* introduced to non – spiced fried rice at 80°C placed in a food warmer was 6.3 log₁₀cfu/g (Table 4). As the holding temperature reduces, the rate at which the pathogen survived in the food increased significantly to 9.0 log₁₀cfu/g with a reduction in temperature to 30°C after holding the fried rice for 8 hours. Spiced fried rice inoculated with 6.3 log₁₀cfu/g of *S. aureus* at 80°C also experienced an increase in growth, but the rate of survival is reduced compared to the rate of survival in non – spiced fried rice.

3.3 Toxicity Assay of *Bacillus cereus*

The Brine Shrimp Lethality (BSL) Assay was done at both the initial (0 hour) and final hour (8 hours) for non-spiced fried rice inoculated with *B. cereus*. The result shows that the LC₅₀ (lethal concentration at 50%) tallies with Clarkson's toxicity index which says if the LC₅₀ is >1000: non-toxic, 500-1000: low toxic, 100-500: medium toxic and 0-100: highly toxic. The BSL Assay at LC₅₀ for *Bacillus cereus* in non – spiced fried rice at 0 and 8 hours was 5.9 and 1.5 respectively (Fig. 1). This result shows that the lethal dose was high at 8 hours which connotes that the toxicity was high at 8 hour.

4. DISCUSSION

Party foods which are usually prepared in the early hours of the day and kept in warmers to be served later can support the growth of pathogens in different ways. A major gap in our knowledge is the lack of information about the holding time and temperature. The temperature at which a food is kept before serving can support rapid bacterial multiplication, inactivation of the organisms or kill pathogenic organisms. For most foods, the temperature and time at which food is held are the most significant factors. The commonest temperature and time food processing errors are: putting too much food in a

large container when wanting to cool it, holding food at room temperature (between 20°C and 50°C) to cool, storage of cold or hot food in the danger zone (4°C and 60°C), cooking temperature too low to kill pathogens and preparing foods more than 12 hours before they are to be served [14]. The control of the holding temperature of foods after processing and before consumption is crucial for safe food production. General recommended holding temperatures are < 8°C for cold foods and > 63.8°C for hot food [15]. The result of this study shows that the microbial load of all the food pathogens increased at longer holding time and as the holding temperatures reduces. *Bacillus cereus*, has traditionally been associated with foods linked to food borne illness usually caused by inadequate hot holding. The prevalence of this organism in raw and processed foods has been established and is given the range of 1 – 3 CFU/g in fried rice, indicating that *B. cereus* represent a persistent risk for food borne illness from foods that are held hot. Inadequate hot holding was attributed to 7% and 24% of the investigated outbreaks of food poisoning in New York and Washington states, respectively. The numbers of associated cases (suspected or confirmed) indicate that inadequate hot holding was the 7th and 2nd leading contributing factor for food borne illness in these states. Inadequate hot holding was associated with approximately 50% of cases attributed to both *C. perfringens* and *B. cereus*. Although spore-forming pathogens contributed significantly to cases of food borne illness when hot holding was inadequate, *Salmonella* and *S. aureus* were also shown to be causative agents. This suggests that post-cooking contamination and subsequent survival by vegetative infectious and toxigenic agents can occur. However the heat stability of *Staphylococcus* enterotoxin makes it impossible to determine if staphylococcal contamination occurs more commonly pre- or post-cooking. During hot holding, food that is held at a constant temperature loses heat to the surrounding

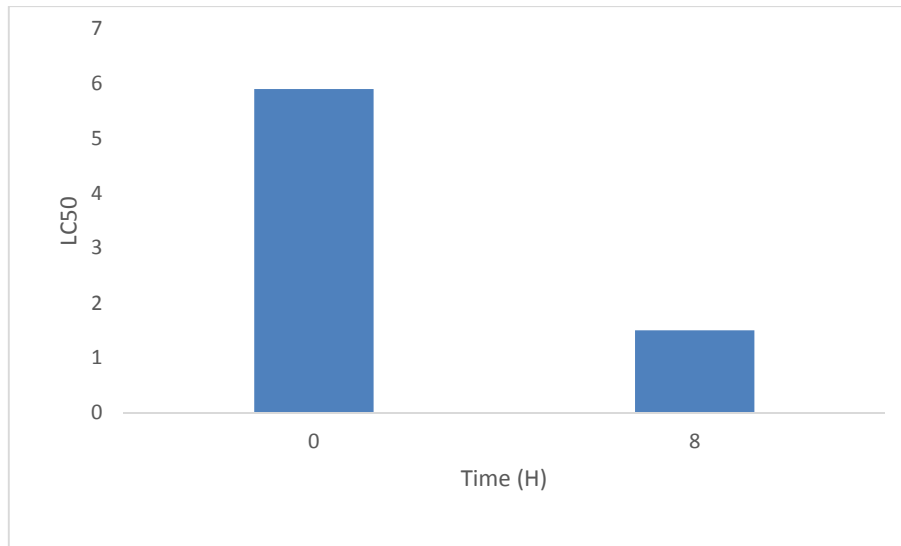


Fig. 1. BSL Assay at LC₅₀ for *Bacillus cereus* in non-spiced fried rice at 0 and 8 hours

environment at the surface interface. This phenomenon is most critical when food is particulate and the establishment does not stir the food frequently. In this case the food at the interface surface may be held at a temperature significantly lower than the interior. Frequent stirring or placing covers on the food would create more homogenous temperatures throughout the food. Covering the food also raises the humidity in the atmosphere immediately above the food. Another factor to consider is that the food is intended to be sold or served, and therefore the surface will be disrupted each time a portion is dispensed. However, the foodservice industry's practices on stirring or covering hot held food has not been evaluated. The result of this study shows that the fried rice supported the growth of these pathogens. Wogu et al. [16] and Patrick and Azanza [17] reported that, 'fried rice contains more microorganisms and can cause infections if proper precautions are not put in place'. This is probably due to the method of preparation and the ingredients used for cooking. The range of mean microbial count of *Escherichia coli* in spiced fried rice (*Zingiber officinale*, *Allium sativum* and *Aframomum danielli*) between 2- 8 h was 7.6 to 8.4 log₁₀cfu/g. The addition of spices evidently reduced the growth of the pathogen, this agrees to with the study of Dablood and Asaad [13]. The spices added which are sometimes referred to as medicinal plants contain some antimicrobial compounds such as ajoene and allicin in garlic, gingerol and geraniol, α -zingiberene, in ginger [18]. The use of these spices have been widely explored and shown to

inhibit growth of food spoilage microorganisms. The efficacy of the antimicrobial activity of these spices can also be increased by using a combination of two or more for cooking [19]. The lethality of a test sample in a simple zoological organism such as the shrimp (*Artemia salina*) has been utilized by Meyer et al. [20] in the Brine Shrimp Cytotoxicity Test (BSCT). It has been demonstrated that BSCT correlates reasonably well with cytotoxic and other biological properties [21]. The brine shrimp bioassay has been established as a safe, practical and economic method for determination of bioactivities of synthetic compound [22] as well as plant products [20]. In toxicity evaluation of plant extracts by Brine shrimp lethality bioassay LC₅₀ values lower than 1000 μ g/ml are considered bioactive [20]. The result indicate that *Bacillus cereus* produces toxins in non - spiced fried rice at 8 hours having ED50 which is in line with the findings of Meyer et al. [20], in toxicity evaluation of plant extracts by Brine shrimp lethality bioassay LC50 values lower than 1000 μ g/ml are considered bioactive.

5. CONCLUSION

Longer holding time of cooked fried rice have great effect on its holding temperature which contribute greatly to the rate of multiplication of organisms that contaminate the food and also on their ability to produce toxin thus leading invariably to food borne diseases. Therefore, food vendors and caterers need to be adequately enlightened about the importance of temperature and time control of foods.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Schelin J, Wallin-Carlquist N, Cohn MT, Lindqvist R, Barker GC, Rådström P. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence*. 2011;2:580-592. PMID: 22030860
PMCID: PMC3260550
Available: <https://doi.org/10.4161/viru.2.6.18122>
- Loir Y, Baron F, Gautier M. *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*. 2003;2:63–76. PMID: 12917803
- Schmidt RH, Goodrich RM, Archer DL, Schneider KR. General overview of the causative agents of foodborne illness. Food Science and Human Nutrition Department, Florida Cooperative Extension Service, IFAS, University of Florida. Publication: FSHN033. 2009;1-5.
- Teplitski SM, Wrigh AC, Lorca G. Biological approaches for controlling shellfish-associated pathogens. *Current Opinion Biotechnology*. 2009;9:185-190. PMID:19342220
Available: <https://doi.org/10.1016/j.copbio.2009.03.001>
- Nyenje ME, Tanih NF, Green E, Ndip RN. Current status on antibiogram of *Listeria ivanovii* and *Enterobacter cloacae* isolated from ready-to-eat foods in Alice, South Africa: A cause for concern. *International Journal of Environment and Resident Public Health*. 2012;9:3101-3114. PMID: 23202673
Available: <https://doi.org/10.3390/ijerph9093101>
- Lukinmaa K, Aarnisalo M, Suihk L, Siitonen A. Diversity of *Listeria monocytogenes* isolates of human and food origin studied by serotyping, automated ribotyping and pulsed-field gel electrophoresis. *Clinical Microbiology Infection*. 2004;10:562–568. PMID: 15191386
Available: <https://doi.org/10.1111/j.1469-0691.2004.00876.x>
- Bergdoll MS. *Staphylococcus aureus*. In: Doyle MP, (Ed) *Food Borne Bacterial Pathogens*, Marcel Dekper, Inc: New York. 2000;463-523.
- World Health Organization. *Global strategy for food safety: Safer food for better health*. World Health Organization, Geneva; 2002.
- Shan B, Cai Y, Brooks J, Corke H. The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Microbiology*. 2007;117:112-119. PMID: 17449125
Available: <https://doi.org/10.1016/j.ijfoodmicro.2007.03.003>
- Korukluoglu M, Sahan Y, Yigit A, Ozer E, Gucer S. Antibacterial activity and chemical constitutions of *Olea europaea* L. leaf extracts. *Journal of Food Process Preservation*. 2010;34:383-396. Available: <https://doi.org/10.1111/j.1745-4549.2008.00318.x>
- Omafuvbe BO, Shonukan OO, Abiose SH. Microbiological and biochemical changes in the fermentation of soybean for soy-daddawa – Nigerian food condiment. *Food Microbiology*. 2002;17:469-474. Available: <https://doi.org/10.1006/fmic.1999.0332>
- Omafuvbe BO. Effect of salt on the fermentation of soybean (*Glycine max*) into dadawa using *Bacillus subtilis* as starter culture. *African Journal of Bacteriology*. 2006;5:1001-1005.
- Dabool AS, Mihdhir AA. The effect of method of cooking and holding conditions on enterotoxin production by *Staphylococcus aureus* in two types of Saudi rice. *European Academic Research*. 2014;2:2286-482.
- Food and Drug Administration. *Bad bug book: Food borne pathogenic micro-organisms and natural toxins handbook*, 2nd Ed. US Food and Drug Administration, Silver Spring. 2012;87–92.
- Forsythe SJ. *The microbiology of safe food*. 2nd Ed. Blackwell Science; 2007.
- Wogu MD, Omoruyi MI, Odeh HO, Guobadia JN. Microbial load in ready-to-eat rice sold in Benin City. *Journal of Microbiology and Antimicrobials*. 2012;3: 29-33.
- Patricia MA, Azanza V. Aerobic plate counts of Philippine ready to- eat foods from take away premises. *Journal of Food Safety*. 2005;25:80-97. Available: <https://doi.org/10.1111/j.1745-4565.2005.00554.x>

18. Food and Drug Administration. *Staphylococcus aureus*. In Bad Bug Book. Food Borne Pathogenic Microorganisms and Natural Toxins Handbook, 30/11/2000; Chapter 3. Centre for Food Safety and Applied Nutrition; 2001.
Available: <http://vm.cfsan.fda.gov/~mow/chap3.html>
19. Gutierrez J, Barry-Ryan C, Bourke P. The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology*. 2008;124:91-97.
PMid: 183780323
Available: <https://doi.org/10.1016/j.ijfoodmicro.2008.02.028>
20. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*. 1982;45:31-34.
Available: <https://doi.org/10.1055/s-2007-971236>
21. McLaughlin JL. Crown-gall tumours in potato discs and brine shrimp lethality: Two simple bioassays for higher plant screening and fractionation. In: Hostett-Mann K, Ed. *Methods in Plant Biochemistry*. London: Academic Press. 1991;6:1-31.
22. Balaban N, Rasooly A. Staphylococcal enterotoxins. *International Journal of Food Microbiology*. 2000;61:1-10. PII: S0168605(00)00377-9
Available: [https://doi.org/10.1016/S0168-1605\(00\)00377-9](https://doi.org/10.1016/S0168-1605(00)00377-9)

© 2020 Olanbiwoninu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/56430>