wjpmr, 2017,3(6), 43-51

SJIF Impact Factor: 4.103

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

IN VITRO EFFICACY OF TEMPERATURE AND PRESERVATIVES ON FAST FOOD BACILLI, AND THEIR ANTIBIOTIC SUSCEPTIBILITY PROFILE

***Aruwa Christiana Eleojo and Akinyosoye Felix Akinsola**

Assistant lecturer/Research Scientist, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

***Corresponding Author: Aruwa Christiana Eleojo**

Assistant lecturer/Research Scientist, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

Article Received on 10/05/2017 **Article Revised on 31/05/2017** **Article Accepted on 20/06/2017**

ABSTRACT

Background and Objective: Species within the *Bacillus* genus are ubiquitous, and cause food infections and intoxications. *Bacillus* species are however rarely assayed for in convenience foods. Furthermore, consumer health protection as it relates to the keeping quality of convenience/fast foods (prior to sale to consumers), remain a subject of global concern. Therefore, this study focused on the i*n vitro* efficacy of temperature and preservatives on fast food bacilli. **Materials and Methods:** A study of chemical preservative and thermal effect on test bacilli isolates was done, with spectrophotometric measurement of optical density at 600nm. Several concentrations of chemical preservatives (0.1-1% for potassium metabisulphite, sodium nitrite, sodium benzoate, and sorbic acid; and 1-10% for sodium chloride) were prepared. Test *Bacillus* species were subjected to the concentrations, incubated over a 72-hrs and readings taken periodically. Statistical analysis was carried out using one way ANOVA in SPSS version 15 package for separation of means at 95% confidence interval. **Results:** Findings showed that at 60°C holding temperature growth of test bacilli were effectively inhibited. Also, 8% sodium chloride, 0.3% sorbic acid, 0.4% sodium benzoate, 0.3% sodium nitrite and 0.4% potassium metabisulphite effectively inhibited all test bacilli. Antibiotic susceptibility results showed that *B. megaterium* and *B. stearothermophilus* were resistant to vancomycin, while *B. cereus*, *B. subtilis* and *B. thuringiensis* were susceptible to vancomycin. Other test bacilli were resistant to clindamycin except *B. cereus* and *B. stearothermophilus*. **Conclusion**: This study showed the importance of heat and chemical preservatives in the inactivation of *Bacillus* species. Holding temperatures (55-60°C) and/or preservatives (at minimum inhibitory levels) could improve the shelf life and quality of ready-to-eat foods prior to purchase, and ensure consumer health protection. Antibiotic susceptibility profile of test species would be efficacious in alleviating symptoms of *Bacillus* related food borne illness.

KEYWORDS: Food quality, health, temperature, preservatives, antibiotic, *Bacillus*.

INTRODUCTION

Bacillus species spores are ubiquitous and frequently contaminate a variety of food products and supplements.[1] Food-borne illnesses associated with toxins produced by *Bacillus cereus* usually result in self-limiting diarrhoea or vomiting.[2] Their detection in foods can cause disease of varying severity, including death (especially if they were not properly preserved during exposition for sales. This creates good conditions for growth and the microorganisms reach considerable levels of contamination). Thus, food safety and preservation issues are of major importance to local, national and global health.[3] Foods that undergo mild/gentle heat treatments of between 65-95°C are known to enable the survival of spores, especially those of *B. cereus*. The presence of *Bacillus* species in foods demonstrates a potential health risk.[4,5] *B. cereus* can give rise to two distinct forms of food-borne disease, the emetic and diarrhoeal syndromes. Storage of cooked food products at room temperature for long periods encourages germination of resistant spores and their proliferation. Illness would follow the consumption of such poorly stored/preserved cooked foods containing significant numbers of *Bacillus*.[4,6]

The global incidence of food borne illnesses is difficult to estimate. Illness resulting from the consumption of contaminated food is a widespread public health problem in contemporary society. Food poisoning is caused with presence of bacteria in food due to improper food preparation or preservation. Some of its symptoms include nausea, vomiting, diarrhoea and abdominal pain. Milk, dairy products, fatty foods, bread, cakes and seafood can easily be contaminated with *Bacillus* spp.[7] Based on the report of European Food Safety Agency (2005) 1–33% of food-borne poisonings are caused by *B.*

cereus. In Nigeria, as in many developing countries, a major source of ready-to-eat foods [Street foods (SFs)] are prepared and sold at public places such as schools, markets places, along the streets. The SFs are relatively cheap and at easily accessible.[8] Traditional meals which are quite laborious and take time to prepare are also offered. Thus, with the increase work hours SFs have become increasingly important among all socio-economic groups.[9] Given the risk they pose to human health, experts recommended the introduction of the Hazard Analysis and Critical Control Point (HACCP) system, temperature control, correct washing of hands, disinfection of equipment, and rapid chilling of products for reducing the number of spores in products.[7] It is impossible to eliminate *Bacillus* species from food products. Hence, it is essential to continually educate street food vendors (especially local vendors whose foods do not go through any form of prior inspection) on the need for good food hygiene and preservation practices.[10]

Foodstuffs durability may be achieved by physical procedures: drying, cooling, deep-freezing and heating.[11] Nevertheless, chemical preservation also plays a prominent role. The use of preservatives is often combined with physical methods. A preservative is a naturally occurring or synthetically produced substance that is added to products such as foods, pharmaceuticals, paints, biological samples, etc. to prevent decomposition by microbial growth or undesirable chemical changes.[12] Chemical preservatives are substances which, under certain conditions, either delay the growth of microorganisms without necessarily destroying them or prevent deterioration of quality during manufacture and distribution, thereby extending shelf life. The application of preservatives has a long history, such as the use of common salt, smoke or sulphur dioxide.[13] The extent of inactivation of bacterial endospores by chemicals is dependent upon many factors including the chemical used, form and concentration of the chemical, time and temperature of the treatment and genus, species and strain of the microorganism.[14] Preservatives inhibit the processes that culminate in food spoilage such as the process of fermentation, acidification, and decomposition.[15] They may be antimicrobial; antioxidant and antienzymatic or metal chelators.[13] Several studies have shown that bactericides and fungicides may evince their effects on a variety of microbial cellular targets, for example; the cell wall, the cytoplasmic membrane or the cytoplasm. It is often difficult to assign a precise target for a specific class of preservative. The target can, and does change with preservative concentration. As a consequence, preservatives can often interfere with several different microbial cellular mechanisms.[13] Sulphites inhibit microbial growth by reacting with cell adenosine triphosphate; inhibition of metabolic pathways, and blocking of cellular transport systems. Other antimicrobials alter microbial membrane or cell wall permeability or destroy the genetic material.[13] Sorbate compounds are salts based on sorbic acid, and like benzoates, are often chosen for their solubility in water.[16] Common functions for chemical preservatives in food include colour retention, flavour protection, mould inhibition, spoilage retardation, and general preservation.[14]

Research has also shown that almost all isolated *B. cereus* cultures from milk and meat products show low susceptibility to ampicillin and oxacillin antibiotics. Except for the resistance to streptomycin; resistance to other antimicrobial agents such as clindamycin, erythromycin, tetracycline, and neomycin vary significantly among species/strains.[17] This research was therefore aimed at assessing the *in vitro* efficacy of heat and chemical preservatives on selected *Bacillus* species isolated from street foods. It also reports the antimicrobial susceptibility profiles of the bacilli isolates. Furthermore, the use of chemical preservatives besides common salt in SFs is not common among local vendors. Results from this study would also provide wider preservation options to street food vendors.

MATERIALS AND METHODS

I. Sample collection and analysis

Bacillus species were isolated from rice, egg, meat and farinaceous fast food from various vendors; and characterized using the Analytical Profile Index (API) kit (50CHB/20E). Freshly prepared foods were purchased, and transported in cold packs under aseptic condition to the laboratory for microbiological analysis using the serial dilution and pour plate method, within one hour of collection[18].HiChrome *Bacillus* Agar (HiMedia) was used for isolation of *Bacillus* species, and Mueller Hinton (MH) Agar for assessment of antibiotic profile, and sterilized at 121°C for 15 min.[19]

II. Analytical Profile Index (API) kit(50CH/CHB/20E) identification

Test isolates were identified using the API 50CH/20E strip according to Aruwa and Olatope.[20] It consists of 50 biochemical tests for the study of the carbohydrate metabolism of microorganisms. The API 20E strip consists of 20 microtubes containing dehydrated substrates. The strip is humidified and tests are inoculated with a bacterial suspension that reconstitutes the media during incubation. Metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table, and the identification is obtained by referring to the identification software.

III. Standardization of inocula

Standardization of inocula was carried out according to Oyeleke *et al*.[21] A loop full of test organism was aseptically inoculated into nutrient broth and incubated for 24 hrs. Exactly 0.2 ml from the 24-hr culture of the organism was dispensed into 20 ml sterile nutrient broth for 3-5 hrs to standardize the culture to 0.5 McFarland standards (1.5x10^8cfu/ml) before use.

IV. Subjection of isolate(s) to heat

Separate bacilli suspensions of purified isolates were subjected to varying temperatures (range 30°C-70°C), using a shaking water bath.[22] One millilitre of the standardized inocula was suspended in 9ml of 1% peptone water.

V. Subjection of bacilli isolates to chemical preservatives

Preparation of different concentrations of chemical preservatives (sodium nitrite, sorbic acid, sodium chloride, potassium metabisulphite) was carried out according to Wood *et al.*[23] Five (5) selected and representative bacilli isolates were subjected to the different concentrations of chemical preservatives so prepared. Four different concentrations ranging from 0.1 to 1.0% w/v of each synthetic chemical preservative, and 1 to 10% w/v for sodium chloride, were weighed and poured into the bottles containing 100ml of sterile distilled water. Each concentration prepared was labelled and shaken vigorously.

The method of Ilesanmi *et al.*[24] was adopted in determining the minimum inhibitory concentration (MIC). Different concentrations of chemical preservatives already prepared ranging from 0.1-1% were used. Broth cultures of the bacteria were prepared and 9ml of sterilized peptone water was pipet into McCartney bottles with 1.0 ml of the test bacteria. One millilitre (ml) of different concentrations of chemical preservative was added too, and incubated at 37°C for 24 hours. The optical density/growth of the organism was monitored at 600 nm absorbance on the coring colorimeter at 0, 12, 24, 36, 48, 60 and 72 hours. The McCartney bottles were checked for growth. Growth was indicated by turbidity. The last concentration at which growth inhibition was noticed was taken as the minimum bactericidal concentration. Preservative concentration which showed the least observable optical density was taken as the minimum inhibitory concentration (MIC) for the preservative.

VI. Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out according to the method of Zanpantis *et al.*[25] The antibiotic sensitivity test was carried out in order to compare the sensitivity of the bacterial isolates to the different commercially available antibiotics. The commercial antibiotic discs (HiMedia Biological) which included CPD – Cefpodoxime (10 µg); C – Chloramphenicol (30 µg); VA – Vancomycin (30 µg); S – Streptomycin (10 µg); RIF – Rifampicin (5 µg); LE – Levofloxacin (5 µg); CTR – Ceftriaxone (30 µg); CD – Clindamycin (2 µg); AMC – Augmentin (30 µg); AK – Amikacin (30 µg); CFM – Cefixime (5 µg); TE – Tetracycline (30 µg); were used for the bacterial isolates. Sterile Petri dishes were seeded aseptically with 1 ml each of the standardized broth cultures of the test organisms, while about 20 ml of sterilized Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile forceps, the antibiotic discs were placed firmly on the solidified plates and incubated for 24 hrs at 37°C. After incubation, clear areas around the discs were measured, which represents the zones of inhibition. Seeded agar plates without antibiotics served as the control experiment. The zones of inhibition were measured in millimeter (mm). The experiment was carried out in triplicate.

VII. Statistical analysis of data

All experiments were carried out in triplicates, and data obtained from the study were subjected to one-way analysis of variance (ANOVA) at 95% confidence limits using the SPSS window 15 version, and Duncan's Multiple Range Test for the separation of means to determine the significance of any difference between the means.[4]

RESULTS

Five fast food borne test *Bacillus* species used in this study were identified to be *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium, Bacillus thuringiensis* and *Bacillus stearothermophilus* using the Analytical Profile Index (API) kit method (Table I). Figures Ia –VIb showed optical density results for *Bacillus* species subjected to heat and varied concentrations of chemical preservatives. *Bacillus stearothermophilus* optical density (OD) reading increased from 0.03-0.06±0.01at 10 min to 0.15±0.01 after 1 hr heating at 30-50°C.*Bacillus thuringiensis* was most affected by longer heating with OD of 0.13 to 0.001 after 1 hr of heating (Figs. Ia, b). *B. cereus* and *B. subtilis* were most affected at 0.4% potassium metabisulphite concentration ($K_2S_2O_5$) after 24 and 48 hr with OD of 0. An insignificant increase in OD was observed for *Bacillus megaterium* at 0.2% metabisulphite concentration but with subsequent decrease at higher concentrations after 24 (Fig. IIa) and 48 hrs (Fig. IIb). Sodium benzoate at 0.4% concentration was bactericidal for *B. megaterium* and *B. stearothermophilus* after 24 and 48 hrs (Figs. IIIa, b) with an OD of 0. Sodium nitrite was also bactericidal for *B. megaterium, B. stearothermophilus*, and *B. thuringiensis* as well after 24 hrs (Fig. IVa), but bactericidal for all test bacilli except *B. subtilis* at 0.3% after 48 hrs (Fig. IVb).

OD for sorbic acid concentrations levelled off at 0.05±0.01 at 0.3% for all test bacteria at 24 hrs (Fig. Va). OD readings decreased after 48 hrs at the same concentration and varied between 0.02-0.05±0.01 ($p = .05$). A significant decrease was observed with *B. stearothermophilus* at 0.1-0.2% sorbic acid concentrations, with OD from 0.55 to 0.16±0.0 after 48 hrs exposure (Figs Va, b). In exposures to sodium chloride, OD for *B. megaterium* fluctuated between 0.22-0.59±0.01 ($p = .05$). A significant decrease at 8% to 0.10±0.00 OD followed. Other test *Bacillus* showed a steady increase on OD/growth below 8% salt concentration after 24 (Fig. VIa) and 48 hrs (Fig. VIb)

exposure. Observed experimental minimum inhibitory concentrations for each test microbial isolate was depicted in table II. The antimicrobial susceptibility and resistance profile of each test *Bacillus* to commercial antibiotics is shown in table III. All species were resistant to augmentin, except *B. subtilis*. There were significant differences within and across groups of antibiotics. *B. stearothermophilus* (16±0.1 mm) showed susceptibility to clindamycin, but mostly to levofloxacin and tetracycline. *B. megaterium* was most susceptible to chloramphenicol (32±0.1 mm). All test bacilli showed susceptibility to Amikacin (16-24±0.1 mm); levofloxacin (19-27±0.1 mm), streptomycin (16-27±0.1 mm), and tetracycline (22-29±0.1 mm). *B. cereus* was most susceptible to tetracycline (29±0.1 mm) and clindamycin (21±0.1mm). *B. subtilis* was most susceptible to the 5µg levofloxacin concentration with 34±0.1 mm inhibition zone compared to other test *Bacillus*.

Table 1: Identity of selected test bacilli used for further study.

Isolate code	API Identification
A	*Bacillus cereus*
B	*Bacillus subtilis*
C	*Bacillus megaterium*
D	*Bacillus thuringiensis*
E	*Bacillus stearothermophilus*

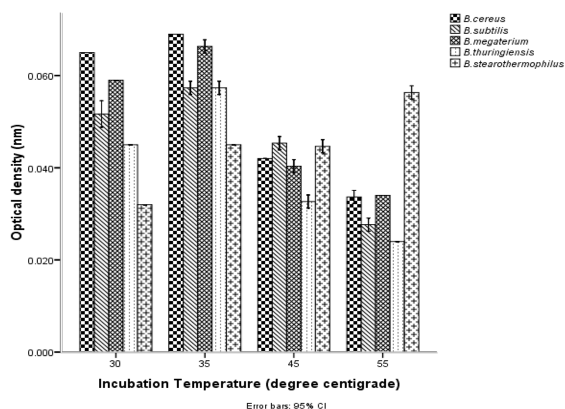

Fig. 1a: Effect of different heat temperatures on bacilli isolates after 10 min exposure.
Key: Bars OD mean range=0.024-0.071; Each bar value represents a mean ± SD (n=3) at *p*=0.05; Standard bars: ±2 SD.

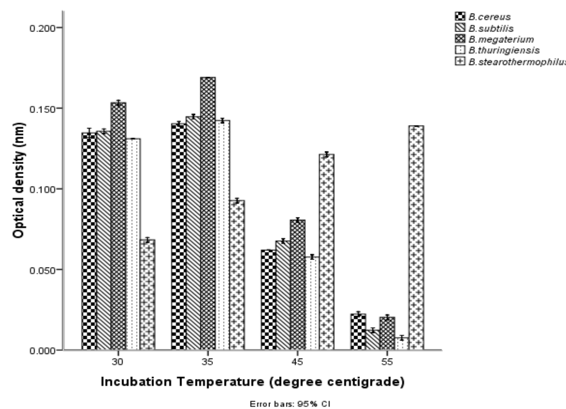

Fig. 1b: Effect of different heat temperatures on bacilli isolates after 1 hr exposure.
Key: Bars OD mean range = 0.010 - 0.164; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

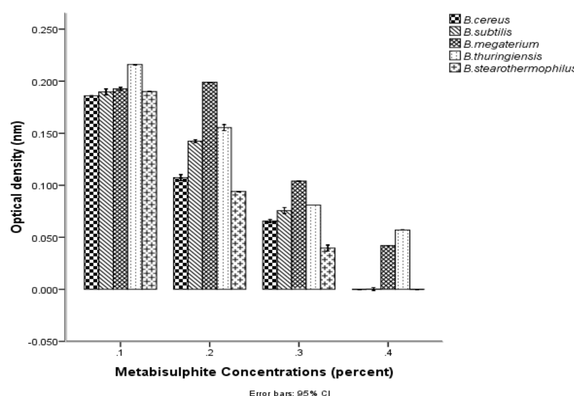

Fig. 2a: Effect of different concentrations of potassium metabisulphite on bacilli isolates after 24 hrs.
Key: Bars OD mean range = 0 - 0.22 Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

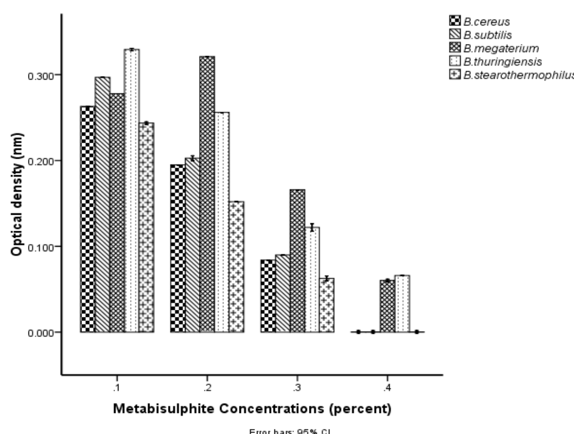

Fig.2b: Effect of different concentrations of potassium metabisulphite on bacilli isolates after 48-hr exposure.
Key: Bars OD mean range = 0 - 0.33; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

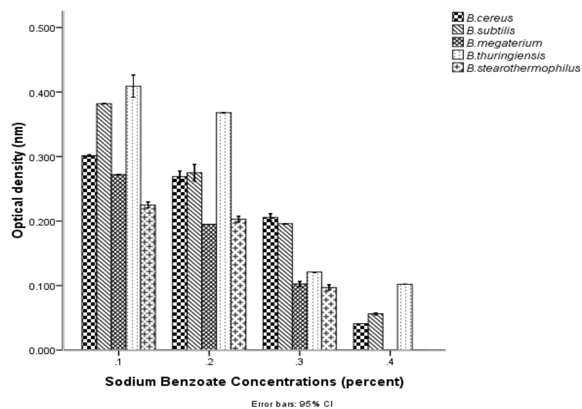

Fig. 3a: Effect of different concentrations of sodium benzoate on bacilli isolates after 24-hr exposure.
Key: Bars OD mean range = 0 - 0.42; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

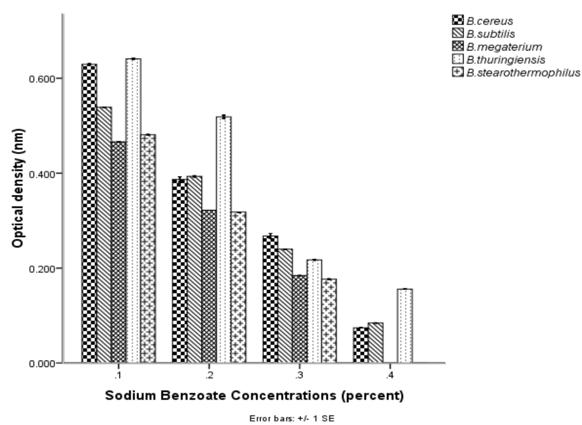

Fig. 3b: Effect of different concentrations of sodium benzoate on bacilli isolates after 48-hr exposure.
Key: Bars OD mean range = 0 - 0.63; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

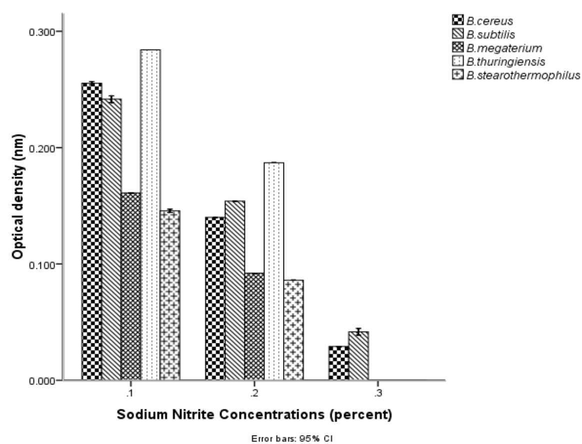

Fig. 4a: Effect of different concentrations of sodium nitrite on bacilli isolates after 24-hr exposure.
Key: Bars OD Mean range = 0 - 0.291; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

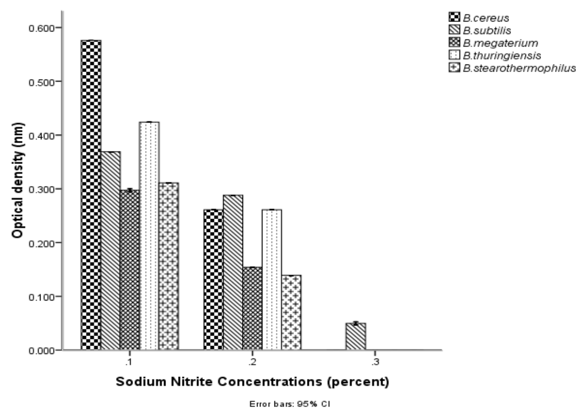

Fig. 4b: Effect of different concentrations of sodium nitrite on bacilli isolates after 48-hr exposure.
Key: Bars OD Mean range = 0 - 0.588; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

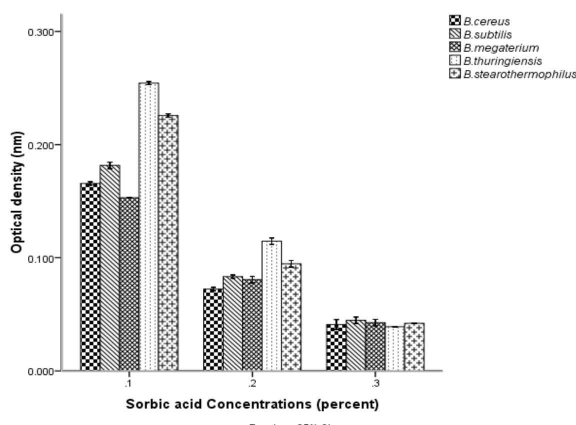

Fig. 5a: Effect of different concentrations of sorbic acid on bacilli isolates after 24-hr exposure.
Key: Bars OD Mean range = 0.051 - 0.202; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

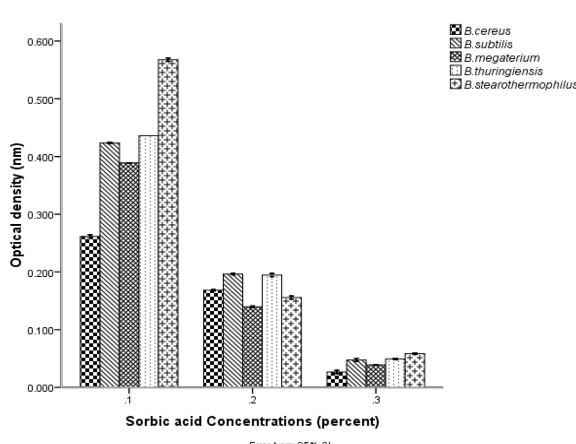

Fig. 5b: Effect of different concentrations of sorbic acid on bacilli isolates after 48-hr exposure.
Key: Bars OD Mean range = 0.02 - 0.591; Each bar value represents a mean ± SD (n=3) at *p* =0.05; Standard bars: ±2 SD.

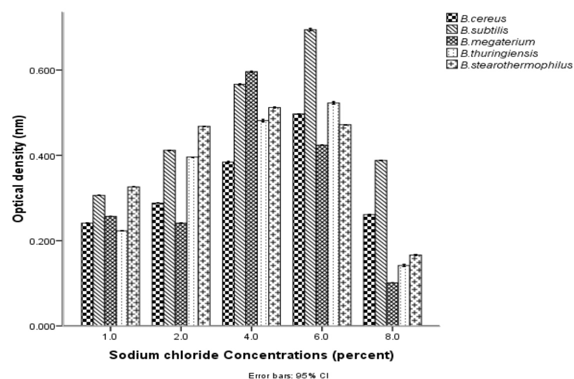

Fig. 6a: Effect of different concentrations of sodium chloride on bacilli isolates after 24-hr exposure.
Key: Bars OD Mean range = 0.11 - 0.72; Each bar value represents a mean ± SD (n=3) at p =0.05; Standard bars: ±2 SD.

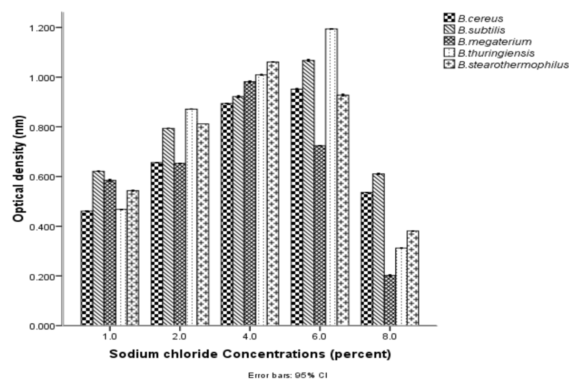

Fig. 6b: Effect of different concentrations of sodium chloride on bacilli isolates after 48-hr exposure.
Key: Bars OD Mean range = 0.23 - 1.18; Each bar value represents a mean ± SD (n=3) at p =0.05; Standard bars: ±2 SD.

Table II: Observed minimum inhibitory concentration (MIC) for all test bacilli.

Test *Bacillus* sp.	Potassium metabisulphite ($K_2S_2O_5$)	Sodium benzoate	Sodium nitrite ($NaNO_3$)	Sorbic acid	Sodium chloride (NaCl)
	MIC (%)				
Bacillus cereus	0.3	0.4	0.3	0.3	10
Bacillus subtilis	0.3	0.4	0.3	0.3	10
Bacillus megaterium	0.4	0.3	0.2	0.3	8
Bacillus thuringiensis	0.4	0.4	0.2	0.3	8
Bacillus stearothermophilus	0.3	0.3	0.2	0.3	8

Table III: Antibiotic susceptibility profile of test *Bacillus* species.

	Antibiotics											
Test *Bacillus* species	**CPD**	**C**	**VA**	**S**	**RIF**	**LE**	**CTR**	**CD**	**AMC**	**AK**	**CFM**	**TE**
B. cereus	0 ± 0.0^{a}	17 ± 0.1^{c}	16 ± 0.1^{c}	27 ± 0.1^{g}	23 ± 0.1^{f}	19 ± 0.1^{d}	0 ± 0.0^{a}	21 ± 0.0^{e}	0 ± 0.0^{a}	24 ± 0.0^{f}	13 ± 0.1^{b}	29 ± 0.1^{h}
B. subtilis	0±0.0a	15 ± 0.1^{b}	18 ± 0.1^{c}	27 ± 0.1^{d}	22 ± 0.1^{c}	34 ± 0.0^{e}	0 ± 0.0^{a}	0 ± 0.0^{a}	20 ± 0.0^{c}	16 ± 0.0^{b}	17 ± 0.0^{b}	22 ± 0.1^{c}
B. megaterium	11 ± 0.1^{b}	32 ± 0.1^{e}	13 ± 0.0^{b}	16 ± 0.0^{c}	23 ± 0.0^{d}	24 ± 0.1^{d}	18 ± 0.0^{c}	0 ± 0.1^{a}	0 ± 0.0^{a}	18 ± 0.1^{c}	15 ± 0.1^{c}	24 ± 0.0^{d}
B. thuringiensis	23 ± 0.0^{d}	11 ± 0.1^{b}	21 ± 0.0^{d}	26 ± 0.1^{e}	21 ± 0.0^{c}	22 ± 0.1^{d}	17 ± 0.0^{c}	0 ± 0.0^{a}	0 ± 0.0^{a}	19 ± 0.0^{c}	13 ± 0.0^{b}	26 ± 0.1^{e}
B. stearothermophilus	0 ± 0.0^{a}	12 ± 0.0^{b}	14 ± 0.1^{b}	22 ± 0.1^{d}	20 ± 0.1^{c}	27 ± 0.1^{e}	$0\pm0.\ 0^{a}$	16 ± 0.1^{c}	$0\pm0.0_{a}$	20 ± 0.0^{c}	$0\pm0.\ 0^{a}$	28 ± 0.2^{e}

Key: Values shown are Mean ± Standard deviation;$^{a-h}$; Means across columns sharing different letter are significantly different (*P = .05*); *B. = Bacillus*; CPD = Cefpodoxime (10 µg), C = Chloramphenicol (30 µg), VA = Vancomycin (30 µg), S = Streptomycin (10 µg), RIF = Rifampicin (5 µg), LE = Levofloxacin (5 µg), CTR = Ceftriaxone (30 µg), CD = Clindamycin (2 µg), AMC = Augmentin (30 µg), AK = Amikacin (30 µg), CFM = Cefixime (5 µg), TE = Tetracycline (30 µg).

DISCUSSIONS

Optical density of the test bacilli decreased with increase in temperature and incubation time (Figs. Ia and Ib). The study showed that within the temperature range of 45-60°C for 1 hr heating all the test bacilli are favourably inhibited, and some inactivated, with the exception of *B. stearothermophilus*. The resistance of *B. stearothermophilus* was expected since the microorganism is a known thermophile. The inhibition of *Bacillus* species beyond the upper limit of growth of 45°C agrees with the submission of Grande *et al.*[26] Cooked foods not meant for either immediate consumption or rapid cooling and refrigeration could be kept at temperatures above 60°C (140°F). Microbial germination and growth generally occurs between 10-50°C.[22,27] Lovdal[28] stated that thermal treatment can improve growth and increase the risk of spore germination in food, leading to quality deterioration. With regard to growth and survival in foods, the optimum growth temperature range for *B. cereus* is around 30-35°C with an upper limit of up to 55°C. However, data on growth temperature ranges for other *Bacillus* species associated with food poisoning is limited. Although there have been occasional reports of some strains of *B. subtilis* and *B. pumilus* growing at 5°C, these microorganisms are not generally considered psychrotrophic. The vegetative cells of *Bacillus* species are not notably resistant to commonly used preservatives and sanitizers, but the spores are much more difficult to destroy. The vegetative cells of *B. cereus* are fairly heat sensitive (as was the case in this study above 35°C), being readily destroyed by typical pasteurization processes.[29]

The lower concentrations of preservatives were less effective in inhibiting the growth of all test *Bacillus*. This corroborates the finding of Gopal.[30] A decrease in optical density/growth reading was observed with increase in preservative concentration. Chemical inhibition increased with increase in the concentration of chemical preservative to which the selected bacilli were subjected. This may be attributed to the fact that the preservatives bound up the water molecules, thereby making it unavailable for microbial growth. The higher concentrations of chemical preservatives had much higher inhibitory effect on the growth of the test bacilli.[31,32] Steady increase in optical density/growth at lower concentrations and prolonged incubation maybe attributed to more spore cells entering the vegetative state with continued incubation. The physiology of these microbes makes them more recalcitrant to chemical treatments. As endospores they survive and adapt to a wide range of environments. Only when conditions become favourable do the spores germinate and enter the vegetative state.[33]

The antimicrobial and inhibitory properties of sorbic acid, sodium benzoate, sodium nitrite, potassium metabisulphite and sodium chloride may have created unfavourable microenvironment, leading to physiological and metabolic distortion. In a bid to overcome the resultant hostility further stress is created resulting in metabolic exhaustion. This stress condition may have led to the gradual death and subsequent decrease in the growth of these test bacteria. The decrease in optical density toward the end of the incubation period (72hrs) reflects gradual nutrient depletion, and resultant negative effect on microbial metabolism.[34] The sensitivity of the test *Bacillus* species may be due to the molecular size of the chemical preservatives which aid the solubility in the diluents. It could also enhance their penetration through the cell wall/membrane into the bacterial cytoplasm where they act.[35] Minimum inhibitory concentration for test bacilli was observed at 0.4%, while 0.6% potassium metabisulphite concentration proved effectively bactericidal for all test bacilli. The effectiveness of potassium metabisulphite preservative increased with increase in concentration and incubation time.[30] Sodium nitrite preservative at 0.3% concentration proved bactericidal for *B. thuringiensis* and *B. stearothermophilus*. Chemical inhibition for *B. cereus* was observed after 24hrs at 0.3% and bactericidal after 36-48hrs at 0.3%. Also, 0.4% concentration was bactericidal for *B. megaterium* and *B. stearothermophilus*. 0.6% sodium benzoate concentration was bactericidal for all test bacilli. 0.4% sorbic acid preservative concentration had a bactericidal effect on all test bacilli. Results were in agreement with Jenson and Moir[36].They reported that antimicrobials which inhibit the growth of *B. cereus* group include benzoate, sorbates. The maximum salt concentration tolerated by *B. cereus* for growth was reported to be 7.5%.[37] In this study maximum salt concentration toleration was between 8-10% for all test bacilli. This falls within the range reported by Rajkowski and Bennett.[37] A higher concentration of 10% sodium chloride proved bactericidal for most of the test bacilli. Sodium nitrite and sorbic acid were however most effective in their inhibitory and bactericidal activity, and each at the lowest concentration of 0.3%. While many authors have proposed different modes of action for the different chemical food preservatives available, all agree that the effect of any chemical preservative depends on a wide range of factors. Some of these factors include chemical/preservative concentration; kind, number, age, and previous history of the microorganism; temperature, time, and the chemical and physical characteristics of the substrate in which the microorganism is found (moisture content, pH, types and amounts of solutes, surface tension, and colloids and other protective substances). A chemical agent may be bactericidal at a certain concentration, only inhibitory at a lower level, and ineffective at still greater dilutions.[30]

Test bacilli used in this study showed resistance to clindamycin. Schlegelova *et al*.[17] reported similar finding. *B. megaterium* and *B. stearothermophilus* were resistant to vancomycin, while *B. cereus, B. subtilis and B. thuringiensis* were susceptible to vancomycin. *B. thuringiensis* showed susceptibility to cefpodoxime. Considerable interspecific difference in susceptibility to vancomycin was also observed. This is in line with Cheesbrough[38]. *B. cereus* and *B. thuringiensis* have long been noted as producers of a potent broad spectrum β-lactamase that affects penicillins and cephalosporins. However, these species are often susceptible to several other drug classes including vancomycin, aminoglycosides, macrolides and quinolones that might be used to treat infections.[39]

CONCLUSION

This study showed the importance of heat and chemical preservatives in inhibiting food borne *Bacillus* species. It submits that a holding temperature of 55-60°C for an hour would be effective in inhibiting *Bacillus* species. 8% sodium chloride, 0.3% sorbic acid, 0.4% sodium benzoate, 0.3% sodium nitrite and 0.4% potassium metabisulphite readily inhibited *Bacillus* species *in vitro*. These preservatives could be effective against important food poisoning *Bacillus* species. Thirdly, antimicrobial susceptibility profile of test *Bacillus* remained in constant flux. Hence, antimicrobial susceptibility assessment of *Bacillus* species remains essential prior to use of antimicrobials in treatment. The combination of heat and preservatives in low concentrations is recommended to local vendors for longer shelf life of food products and safeguard of consumer health.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

Aruwa C. E. carried out the research, financed, analysed data collected and prepared the article for publication. Akinyosoye F. A. supervised the project/study.

SIGNIFICANCE STATEMENT

Contaminated and improperly stored convenience foods account for a large number of food borne illnesses globally. Most approved food preservatives are usually not included in ready-to-eat food recipes. The research could aid relevant local and national food agencies put necessary public health policies in place for consumer protection. Information provided may also assist food hygiene and testing agencies work more efficiently; and health care providers correctly treat potential food borne illnesses/outbreaks.

REFERENCES

1. Aruwa, C. E. and Ogunlade, S. T. Classical Identification, 16S rDNA sequencing, and molecular characterization of *Bacillus* species from convenience foods. *British Journal of Applied Science & Technology*, 2016; 15(5): 1-11.
2. Tallent, S. M., KoTewicz, K. M., Strain, E. A. and Bennet, T. R. W. Food biological contaminants - efficient isolation and identification of *bacillus cereus* group. *Journal of AOAC International*, 2012; 95(2): 446-451.
3. World Health Organization (WHO) Background paper: Developing a food safety strategy. WHO Strategic Planning Meeting. Geneva, 2001.
4. Aruwa, C. E. and Akinyosoye, F. A. Microbiological Assessment of Ready-to-Eat Foods (RTEs) for the presence *Bacillus* species. *Journal of Advances in Biology and Biotechnology*, 2015; 3(4): 145-152.
5. Canadian Food Inspection Agency (CFIA). Canadian Food Inspection Agency, *Salmonella* Food Safety Facts. Preventing food borne illness, 2009.
6. Gould, G. W. Mechanisms of resistance and dormancy. In: Gould GW, Hurst A, editors. The bacterial spore 2. London: Academic Press, 1983; 173-209.
7. European Food Safety Authority (EFSA), EFSA Opinion. Reducing risk on *Bacillus cereus* to food chain, 2005– Internete: http://www.foodnavigator.com/Legislation/Reducin grisk- on-*Bacillus-cereus*-to-food chain-EFSA-opinion, 2005.
8. Oranusi, U. S. and Braide, W. A study of microbial safety of ready-to-eat foods vended on highways: Onitsha-Owerri, south east Nigeria. *International Research Journal of Microbiology*, 2012; 3(2): 066-071.
9. Chakravarty, I. and Canet, C. Street foods in Calcutta. *Pakistan Journal of Nutrition,* 2002; 8(10): 1657-1660.
10. Bonfoh, B., Roth, C., Traore´, A. N., Fane´, A., Simbe´, C. F., Alfaroukh, I. O., Nicolet, J., Farah, Z. and Zinsstag, J. Effect of washing and disinfecting containers on the microbiological quality of fresh milk sold in Bamako (Mali). *Food Control,* 2014; 17: 153-161. doi:10.1016/j.foodcont.2004.09.015.
11. Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M. and Mahillon, J. Fatal family outbreak of*Bacillus cereus* - associated food poisoning. *Journal of Clinical Microbiology*, 2005; 43: 4277-4279.
12. McCann, D., Barrett, A., Cooper, A., Crumpler, D., Dalen, L., Grimshaw, K., Kitchin, E. and Lok, K. "Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: A randomised, double-blinded, placebo-controlled trial". *The Lancet*, 2007; 370(9598): 1560–7.
13. Msagati, T. A. M. The Chemistry of Food Additives and Preservatives. Retrieved from http://www.eblib.com, 2012.
14. Byrne, B., Dunne, G. and Bolton, D. J. Thermal inactivation of *Bacillus cereus* and *Clostridium perfringens* vegetative cells and spores in pork luncheon roll. *Food Microbiology,* 2006; 23: 803-808. doi:10.1016/j.fm.2006.02.002
15. Anon. Food Preservatives - How Safe Are They? Courtesy www.medindia.com, 2013.
16. Allen, J. List of Chemical Preservatives. In www.eHOW.com © 1999-2013 Demand Media, Inc., 2013.
17. Schlegelova, J., Brychta, J., Klimova, E., Napravnikova, E. and Babak, V. The prevalence of andresistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs. *Journal of VeterinaryMedicine*, 2003; 48(11): 331-338.
18. Fawole, M. O. and Oso, B. A. Laboratory Manual of Microbiology. Spectrum books limited, Ibadan, Nigeria, 2001; 127.
19. Aruwa, C.E. and Akinyosoye, F.A. Microbiological assessment of ready-to-eat foods (RTEs) for the presence *Bacillus* species. *Journal of Advances in Biology and Biotechnology*, 2015; 3(4): 145-152.
20. Aruwa, C. E. and Olatope, S. O. A. Characterization of *Bacillus* Species from Convenience Foods with conventional and API Kit Method: A Comparative Analysis. *Journal of Applied Life Sciences International*, 2015; 3(1): 42-48.
21. Oyeleke, S. B., Daudu, B. E. N. and Boye, O. A. Antibiotic activity of *Ficus capensis*. *African Journal of Biotechnology*, 2008; 7(10): 1414-1417.
22. Davis, J. R., Lawley, R., Davis, J. and Curtis, L. The food safety hazard guidebook. Cambridge, UK: RSC Pub., 2008; 17.
23. Wood R., Foster, L., Damant, A. and Key, P. Analytical methods for food additives. CRC Press, Boca Raton Boston New York Washington, DC, Cambridge England, Woodhead Publishing Ltd., 2004; 278.
24. Ilesanmi, C. R., Aladesanmi, A. Z. and Adeoye, A. C. Preliminary studies on the cardiac effects of some

Nigerian Medicinal Plant In: The state of medicinal plant research in Nigeria. Abayomi Sofowora (Ed), 1986; 293-300.

25. Zanpantis, A., Lacy, M. and Hovart, R. Nationwide Antibiogram Analysis using NCCLS M39: A Guideline. *Journal of Clinical Microbiology*, 2008; 43: 2629-2634.
26. Grande, M. J., Lucas, R., Abriouel, H., Valdivia, E., Omar, N. B., Maqueda, M., Martı´nez-Bueno, M., Martı´nez-Can˜amero, M. and Ga´lvez, A. Inhibition of toxicogenic Bacillus cereus in rice-based foods by enterocin AS-48. *International Journal of Food Microbiology*, 2006; 106: 185-194. doi:10.1016/j.ijfoodmicro.2005.08.003.
27. Mols, M., Pier, I., Zwietering, M. H. and Abee, T. J. The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. *International Journal of Food Microbiology*, 2009; 135(3): 303-311.
28. Lovdal, I. S. Germination of *Bacillus* species which can lead to food poisoning. A doctoral thesis in the Norwegian School of Veterinary Science (NVH). Irene.s.lovdal@uis.no, 2012.
29. Curtis, L. *Bacillus* Species - Hazard Identification. http://www.foodsafetywatch.com, 2007.
30. Gopal, R. Food and Industrial Microbiology: Food as Substrate for Microorganisms. Dept. Microbiology Osmania University Hyderabad-7, 2013.
31. Oyawoye, O. M., Oyawoye, E. O., Tahir, F., Yusuf, I. Z. and Buhari, H. Effect of preservatives on the shelf life of locally produced cottage cheese. *Journal of Pure and Applied Science*, 1999; 2(1): 52-60.
32. Starosta, A. L., Lassak, J., Jung, K. and Wilson, D. N. The bacterial translation stress response. *FEMS Microbiology Review*, 2014; 38: 1172-1201. doi:10.1111/1574-6976.12083.
33. Augustin, J. C. Challenges in risk assessment and predictive microbiology of foodborne spore-forming bacteria. *Food Microbiology*, 2011; 28(2): 209-213.
34. Ogiehor I. S. and Ikenebomeh, M. J. Antimicrobial effects of sodium benzoate on the growth, survival, and aflatoxin production potential of some species of *Aspergillus* in Garri during storage. In: *Pakistan Journal of Nutrition*, 2004; 3(5): 300-303.
35. Lund, P., Tramonti, A. and De Biase, D. Coping with low pH: molecular strategies in neutralophilic bacteria. *FEMS Microbiology Review*, 2014; 38: 1091-1125. doi:10.1111/1574-6976.12076.
36. Jenson, I. and Moir, C. J. *Bacillus cereus* and other *Bacillus* species. Ch 14 In: Hocking AD (ed) Foodborne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, 2003; 445-478.
37. Rajkowski, K. T. and Bennett, R. W. *Bacillus cereus*. Ch 3 In: Miliotis MD, Bier JW (eds) International Handbook of Foodborne Pathogens. Marcel Dekker, New York, 2003; 27-39.
38. Cheesebrough, M. District laboratory practice in tropical countries. Cambridge University press, UK. Second low price edition, 2006; 434.
39. Ikeda, M., Yagihara, Y., Tatsuno, K., Okazaki, M., Okugawa, S. and Moriya, K. Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections. *Annals of Clinical Microbiology and Antimicrobials*, 2015; 14: 43.