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A preliminary study on the effect of storage temperatures on the population of *Bacillus cereus* in dry ginger powder

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ABSTRACT: The formation of spores and increase in population of vegetative cells of *Bacillus cereus* associated with foodborne diseases is dependent on favourable temperature. In this study, *B. cereus* in a slant obtained from Microbiology Laboratory, University of Port Harcourt was confirmed based on morphological and biochemical tests and inoculated into dry ginger powder using a sterile pipette. The samples were stored at ambient temperature (25°C), refrigeration temperature (4°C) and freezer temperature (-18°C) for 21 days and the microbial counts of *B. cereus* was monitored at 7 days interval using *Bacillus cereus* agar base and standard microbiological methods. The result obtained showed that *B. cereus* steadily increased in population under the storage conditions. The highest microbial count recorded for *B. cereus* in dry ginger powder stored at 25°C, 4°C and -18°C was $7.15\times10^1 \pm 0.036$, $1.07\times10^2 \pm 0.061$ and $2.91\times10^2 \pm 0.070$ CFU/g, respectively. Although the values did not exceed 10^5 cells/g required to manifest foodborne illness in humans, it remains a risk to public health due to increasing population of *B. cereus* in dry ginger powder under the storage conditions.

Keywords: *Bacillus* species, endospores, foodborne disease, spices, *Zingiber officinale*.

INTRODUCTION

Bacillus cereus is a motile, Gram-positive, rod-shaped, aerobic-to-facultative anaerobic bacteria that typically forms endospores (Ölmez and Aran, 2005; Tewari and Abdullah, 2014). The genus *Bacillus* is widely distributed in nature and usually inhabits the soil, water bodies and foods. It can also survive extreme environments such as hot lakes where temperature exceed 60°C. *B. cereus* is ubiquitous and usually contaminate various food samples and commercial products alongside other spore-forming bacteria responsible for food spoilage and poisoning (Okanlawon et al., 2010; Berthold-Pluta et al., 2019). Among the species of *Bacillus*, only *B. anthracis* and *B. cereus* are implicated with foodborne illness. The toxins produced by *B. cereus* are responsible for food poisoning syndromes known as diarrhoea and emesis (Fogele et al., 2017; Aksu et al., 2000). Although the presence of *B. cereus* in food is regarded as a microbiological hazard, it has to exceed 10^5 cells/g before it can cause foodborne illness (Feijoo et al., 1997; Kwon et al., 2019).

Several *B. cereus* foodborne outbreaks involving different foods in different regions/countries have been reported (Pexara et al., 2010; Doren et al., 2013). Endospores produced by *B. cereus* exhibit strong resistance against enzymes, chemicals, wet or dry heat, high pressure, radiation, desiccation and extreme pH (Kwon et al., 2019). The survival of *B. cereus* reintroduced into food is fairly low because it lacks the ability to compete with other microorganisms. Therefore, *B. cereus* leverages on the endospores as the main transmission route in foods (Ubong et al., 2019). Spices, herbs and other types of foods mainly of plant origin harbour spores and vegetative forms of *Bacillus cereus* (Tewari and Abdullah, 2014).

Ginger (*Zingiber officinale* Roscoe) is a popular spice that originated from South-East Asia (Ugwoke and

Nzekwe, 2010; Guirguis, 2020). It is the rhizome of a perennial plant favoured by the climatic conditions in Asia, China, India, Africa, Jamaica, Mexico, Haiti and Australia (Mendi et al., 2009). Globally, Nigeria is rated 5th largest producer and consumer of ginger (Bag, 2018). Ginger either in a fresh paste or dry powder is consumed as a flavouring agent, spice, garnish, medicine and food preservatives (Bhatt et al., 2013). Its utilization could be in the form of dried ginger, ginger powder, ginger paste, ginger oil, ginger syrup, ginger oleoresin and herbal tea (Bag, 2018; Guirguis, 2020). Some of the health benefits associated with ginger include cardiovascular effect, hypotensive effect, anti-hypercholesteraemic, gastrointestinal effect, antimicrobial effect, anti-diabetic effect, anti-obesity effect and anti-inflammatory effect (Al-Awwadi, 2017; Singh et al., 2018; Senay, 2020). Studies have shown that ginger rhizomes and ginger powder harbours numerous microorganisms associated with cultivation, harvesting, processing and storage of the product (Mendi et al., 2009; Guirguis, 2020). Dry ginger powder is well appreciated for culinary purposes and preparation of seasoning ingredients. It has a longer shelf life than fresh ginger (Shahrajabian et al., 2019; Akter et al., 2020). The presence of *B. cereus* in powdered ginger and other spices sampled from different shops in Istanbul was reported by Aksu et al. (2000).

The survival of *Bacillus cereus* endospores in some commercial products such as powdered ginger could be a threat to public health. There are several risk factors that lead to contamination of ginger and other spices by spore-forming bacteria such as *B. cereus.* Poor sanitary condition during harvesting of ginger; allowing the spice have direct contact with the soil during drying process are common practices which could result in contamination by bacterial spores (Mathot et al., 2020). *B. cereus* grows optimally within the temperature range of 30-37°C (Bae et al., 2012; Ubong et al. 2019). However, some strains of *B. cereus* can be found growing in food which had been cooked and allowed to cool <50°C. *B. weihenstephanensis* which belongs to *B. cereus* group is a psychrotolerant specie capable of growing below 7°C (Ubong et al., 2019). Since temperature is one of the physical parameters that influences microbial growth to a large extent, different storage temperatures could affect the growth and multiplication of *B. cereus* in dry ginger powder. Feijoo et al. (1997) studied the effect of temperature and ingredients in coffee creamers on the growth of *B. cereus*. They reported that no growth on the stored product occurred at 4 and 7°C. Similar studies involving other food materials such as ginger rhizome with high risk of being contaminated by *Bacillus cereus* endospores is limited. Therefore, this study is aimed at determining the effect of different storage temperatures on the population of *Bacillus cereus* inoculated into dry ginger powder.

MATERIALS AND METHODS

Fresh and mature ginger rhizomes were purchased from Choba market, Obio/Akpor L. G. A., Rivers State using a sterile polythene bag. The samples were taken to Food and Industrial Laboratory, University of Port Harcourt, for analysis.

Source of *Bacillus* test isolate and confirmation of the isolate

B. cereus in a slant was obtained from Microbiology Undergraduate Laboratory, University of Port Harcourt. The isolate was sub cultured in nutrient agar plates and incubated at 37°C for 24 hours. The pure culture was confirmed to be *Bacillus cereus* based on cultural and morphological characteristics followed by biochemical tests as described by Shoaib et al. (2020).

Preparation of dry ginger powder

Ginger rhizome was washed using potable water. Then, knife sterilized with 70% ethanol was used to peel the bark of ginger. Afterwards, the well peeled ginger were sliced into small pieces and dried using oven (Gulfex Medical and Scientific England) set at 55°C for 7 hours. Dried ginger slices were crushed into fine powder using an electric blender (Usha Mixer Grinder, India). Twenty grams (20 g) of dried ginger powder was weighed using an electronic balance (Metler MT-2000) and sterilized using an autoclave (Lincoln Mark Medical England, Model YX-280A) at 121°C, 15 psi for 15 minutes.

Preparation of inoculum

Inoculum of *Bacillus cereus* was prepared by aseptically transferring a loopful of the subculture into a 20 ml of nutrient broth. The optical density of the nutrient broths were determined using spectrophotometer at 600 nm. The cell density of *Bacillus cereus* in nutrient broth was 0.214 OD. Using standard microbiological methods which involves spread plate method, the inoculum size was determined and the result was expressed in CFU/g.

Preparation of dry ginger powder inoculated with *B. cereus*

Twenty milliliter (20 ml) of *Bacillus cereus* in nutrient broth was inoculated into 20 g of sterilized dry ginger powder using a sterile pipette and homogenized. This set up was done in triplicates.

Storage of dry ginger powder inoculated with *B. cereus* at different temperatures

The inoculated samples of dry ginger powder were stored at three (3) different temperatures- ambient temperature (25°C), refrigeration temperature (4°C) and freezer temperature (-18°C) for a period of 21 days.

Table 1. Morphological and metabolic characteristics of *Bacillus cereus* used as inoculum.

Isolate	Growth characteristics on *Bacillus cereus* selective agar	Gram reaction	Spore stain	Catalase	Oxidase	Sucrose	Glucose	Lactose	TSIA Slant	Agar butt	Gas	H_2S	Indole	MR	VP	Citrate	Motility	Probable organism
A	No distinct colonies; Zone of clearing observed around the growth which is milky in colour; Flat growth with crenated edges	+ Short rods in chains	+	+	-	+	+	-	B	A	-	+	-	-	+	+	+	*Bacillus cereus*

Key: Positive: +; Negative: -; Acidic: A; Basic: B; Methyl red: MR; Voges Proskauer: VP; Triple sugar iron agar: TSIA.

Enumeration of *B. cereus*

At 7 days interval, 1 g of dry ginger powder inoculated with *Bacillus cereus* stored at ambient temperature (25±2°C), refrigeration temperature (4°C) and freezer temperature (-18°C) were aseptically collected. A ten-fold dilution of the sample was carried out using sterile saline solution of NaCl (0.85%). By adopting the spread plate method, a sterile glass spreading rod was used to inoculate 0.1 ml of dilutions 10^{-3} and 10^{-4} of the sample into *Bacillus cereus* agar base (Biolab) plates prepared according to manufacturer's instruction. The inoculated plates were incubated at 37°C for 24 hours. The number of colonies on the plates were counted manually and the result was noted. The bacterial population was calculated using the formula stated below. The result is expressed as colony forming units per gram (CFU/g).

$$\mathrm{CFU/g} = \text{no. of colonies x } \frac{1}{dilution\ factor} x \frac{1}{volume\ plated}$$

RESULTS AND DISCUSSION

The cultural and morphological characteristics, Gram staining, spore staining, motility and biochemical tests carried out to confirm that the isolate is *Bacillus cereus* is presented in Table 1. The microbial population of *Bacillus cereus* in dry ginger powder inoculated with the vegetative cells during storage at different temperatures is shown in Table 2.

The result obtained from this study shows that the population of *B. cereus* in the dry ginger powder stored at freezer temperature (-18°C) at Week 1 is lower than the values reported for the samples stored at higher temperatures (4 and 25°C) monitored at 7 days interval for 21 days. A related study which evaluated the effect of temperature (4 and 7°C) and ingredients in coffee creamer inoculated with spores and vegetative cells of *B. cereus* on the population of the organism reported no growth at both temperatures (Feijoo et al., 1997). The report is not in agreement with the result obtained in this study with regards to dry ginger powder inoculated with *B. cereus* and stored at refrigeration temperature (4°C).

Worthy to note is the increase in microbial count reported in dry ginger powder inoculated with *B. cereus* which were stored at -18°C, 4°C and 25°C monitored at Day 7, 14 and 21. The microbial population of *B. cereus* in the dry ginger powder were significantly different ($p<0.05$) with some exceptions. This could be attributed to the ability of endospores and some vegetative cells of *B. cereus* to survive unfavourable environmental conditions with regards to relatively low temperature. Probably, the population of *B. cereus* that persisted in dry ginger powder stored at -18°C are psychrotolerant species that belong to the *B. cereus* group (Ubong et al., 2019).

Several researchers have consistently stated that endospore formation of *B. cereus* gives it the ability to withstand hostile environmental conditions such as extremely low temperature (Duport et al., 2016; Ubong et al., 2019). Although the antimicrobial properties of ginger could also be a contributory factor towards suppressing the

Table 2. Microbial population (CFU/g) of *Bacillus cereus* in dry ginger powder inoculated with the bacteria during storage at different temperatures.

Storage condition	Week 1	Week 2	Week 3
Freezer temperature (-18°C)	$1.1 \times 10^{1} \pm 0.056^{a}$	$3.75 \times 10^{1} \pm 0.026^{b}$	$7.15 \times 10^{1} \pm 0.036^{c}$
Refrigeration temperature (4°C)	$9.5 \times 10^{1} \pm 0.040^{a}$	$1.01 \times 10^{2} \pm 0.036^{b}$	$1.07 \times 10^{2} \pm 0.061^{b}$
Ambient temperature (25°C)	$2.65 \times 10^{2} \pm 0.046^{a}$	$2.66 \times 10^{2} \pm 0.056^{a}$	$2.91 \times 10^{2} \pm 0.070^{b}$

Each value is expressed as mean ± standard error (n=3). The same superscript along the row is not significantly different at p>0.05 according to Turkey HSD Statistics.

growth and multiplication of *Bacillus cereus* in dry ginger powder stored at freezer (-18°C) and refrigeration (4°C) temperature, the result obtained in this study suggests that antimicrobial compounds in ginger was not effective in suppressing the growth and multiplication of *B. cereus* in the samples. Zerumbone, zingerone, paradol, gingerol and gingerol have been identified as active compounds of ginger responsible for antibacterial activity (Singh et al., 2018). According to Senay (2020), 5% concentration of ginger extract is capable of inhibiting the growth of *Bacillus cereus*.

The increase in storage temperature from -18°C to 4°C could have influenced the increase in population of *B. cereus* in the samples of dry ginger powder. A considerable increase in population of *B. cereus* inoculated into the dry ginger powder stored at refrigeration temperature (4°C) compared with the value recorded for the sample stored at freezing temperature (-18°C) could be attributed to the ability of the bacterium to withstand relatively low temperature usually unfavourable for growth and multiplication of microbial cells. The increase in storage temperature from 4 to 25°C could have influenced the increase in microbial population of *B. cereus* in dry ginger powder.

High population of *B. cereus* in the dry ginger powder stored at 25°C compare with the result recorded for samples stored at lower temperatures (-18 and 4°C) is an indication that relatively high temperature is favourable for the germination of *B. cereus* spores. Moore et al. (2019) reported that pH, available water content, ambient temperature and favourable condition of food stuffs could lead to the germination of *Bacillus cereus* spores. In a related study, Ubong et al. (2019) reported that vegetative *B. cereus* in chocolate milk had a slower growth at 25°C compare with growth of the microbial cells observed at a higher temperature. Although, a steady increase in population of *B. cereus* in dry ginger powder stored at freezer, refrigeration and ambient temperature was observed at week 2 and 3, it did not exceed the infective dose (10^{5} cells/g) required for the pathogen to cause foodborne disease in humans (Feijoo et al., 1997; Kwon et al., 2019).

Conclusion

This study revealed that dry ginger powder inoculated with *Bacillus cereus* stored at freezer temperature (-18°C) experienced slower increase in population of the microbial cells compared with the results recorded for samples stored at 4 and 25°C.

Recommendation

In order to prevent outbreak of foodborne diseases in humans as a result of consuming food containing ginger powder contaminated with *B. cereus*, the ginger rhizomes used in preparing the powdered product should be subjected to high temperature sufficient to kill spores and vegetative cells of the bacterium.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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