Original Research Article

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Monitoring of *Bacillus cereus* in rice pudding

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Abstract

Bacillus cereus is considered to be the most common pathogenic *bacillus* species found in raw milk and at all stages of dairy processing. Cinnamon essential oil is considered a suitable source of antimicrobial and antifungal compounds; therefore, the objective of this study was to reveal microbiologically the presence of *Bacillus cereus* in dairy products by using HiCrome™ Bacillus Agar and evaluating the significance of added cinnamon on the growth of *Bacillus cereus* in prepared rice pudding. The mean values of *B. cereus* count in the examined 50 rice pudding was $2.1\times10^5 \pm 7.4\times10^4$ (cfu /g). The addition of cinnamon powder to rice pudding significantly affects the viability of *B. cereus* in investigated samples ($p \leq 0.05$), as the reduction % in the count of *B. cereus* at 0.5 and 1 % cinnamon concentrations after 12, 24, 36 and 48 hr. were 23.91, 82.22, 94.71, 24.14 %, and 60.87, 99.07, 99.99, 99.89 %, respectively.

Keywords: *Bacillus cereus*, Cinnamon, HiCrome™ Bacillus Agar, Rice pudding

Introduction

Worldwide, milk is a base for preparing numerous desserts including ice cream which is the most globally known dairy dessert. Regionally in Egypt, the most famous dairy desserts in addition to ice cream are mahallbia and rice pudding. Consumers of dairy desserts are of all ages including children of vulnerable age groups, as a sequence, it is required to be free from microbiological hazards (Warke et al., 2000 and Caglayanlar et al., 2009).

Bacillus cereus is a Gram-positive, rod-shaped, and is an important cause of food poisoning. (Hall, 2001). B. cereus is a facultatively anaerobic, ubiquitous, endospore-forming bacterium with a high frequency of isolation from various kinds of contaminated raw and processed food products, such as rice, spices, milk, dairy products, vegetables, desserts, and cakes. After cooking if food is not adequately refrigerated and in the absence of competitive flora, B. cereus grows well (Kramer, 1989).

Bacillus cereus is the causative agent of two different foodborne diseases one emetic (intoxication) due to a preformed small heat-stable cyclic peptide; cereulide encoded by (ces) gene (Arnesen et al., 2008) and one diarrhoeal (infection) due to enterotoxins production in intestines which are Hemolysin BL (Hbl), Nonhemolytic enterotoxin (Nhe) and Cytotoxin K (CytK) which are heat labile, acid sensitive and proteolysis (Lund et al., 2000) diarrheal syndrome develops 8-16 h after consumption of the contaminated food and characterized by abdominal pain and diarrhea. (Gilbert, 1979 and Agata et al., 1995).

B. cereus is undervalued as a foodborne pathogen, although, it has been implicated in various foodborne outbreaks worldwide (Bennett et al., 2013).

B. cereus is considered the main microbial cause of the spoilage of milk and milk products and the main reason for significant economic losses in the dairy industry (Brown ,2000).

Dairy plants laboratories can be advised to use the reference method employing MYP Agar medium, while the chromogenic medium HiCrome™ Bacillus Agar seems to be suitable especially for research purposes because it enables to distinguish the widest spectrum of biochemical activities in one step (Nemeckova et al., 2011).

Cinnamon essential oil (CEO) has been proposed as a suitable source of antimicrobial and antifungal compounds (Paudel et al., 2019). The antimicrobial and antioxidant activity of CEO is primarily related to cinnamaldehyde, eugenol and cinnamic acid (Siripatrawan, 2016). Several studies have demonstrated the efficacy of CEO against several foodborne pathogens in vitro and in milk (Cava-Roda et al., 2010 and Aliakbarlu et al., 2013).

Materials and Methods

Collection and Preparation of samples:

A total of fifty (50) samples of rice pudding were collected randomly from different dairy shops, pastry shops and supermarkets at different localities in Fayoum city, Egypt. The samples were delivered as soon as possible to the laboratory in an insulated ice box and examined at the same day.

11 ml/gm. of the well-mixed sample were added to 99 ml of sterile peptone water 0.1% to make a dilution of 1/10 from which 10-fold serial dilutions were made up (APHA, 1992).

Enumeration and Identification of *Bacillus cereus* (FDA, 2012):

0.1ml from each dilution was evenly spread over a dry surface of HiCrome™ *Bacillus* Agar plates based on the formulation of MYP Agar (Mossel *et al*.,1967) by using a sterile bent glass rod. Streaked plates as well as control ones were incubated at 30ºC for 24-48 hr. Suspected light blue, large, flat colonies with the blue center were counted, then the plates were re-incubated for another 24 hr. before being counted again for further growth.

Suspected colonies were picked up and streaked into nutrient agar slants which were incubated at 30 ºC for 24 hr. for further identification.

All *B. cereus* isolates were biochemically confirmed by Nitrate broth, Modified VP medium, Phenol red glucose broth, Tyrosine agar, Lysozyme broth and Hemolytic activity

Inoculation of rice pudding sample:

a. The prepared rice pudding was artificially inoculated by 2.5 - 3 x 10^5 cfu / g. of old culture of previously isolated and identified *B. cereus* strains, the concentration was adjusted by comparing with 1 McFarland standard.

b. The rice pudding was divided into 3 equal portions; the 1st was used as a positive control, and the 2nd and 3rd were used for adding varying concentrations of ground cinnamon 0.5 & 1 %, respectively.

c. The rice pudding was poured into sterile containers and stored in the refrigerator (temperature 4 ± 1°c).

Enumeration of *B. cereus* in artificially inoculated rice pudding:

The 3 portions of rice pudding were examined at intervals of 0, 12, 24, 36 and 48 hr. for *B. cereus* count by using the direct plating technique on HiCrome *Bacillus* Agar plates and the results were recorded.

Statistical analysis:

Data for each treatment parameter were obtained from three experimental repeats and presented as mean ± standard error of the mean (SEM). The statistical analysis was performed with the Minitab software for windows (version 18). Two-way analysis of variance (ANOVA) was used to determine significant differences in the measured attributes at($P \leq 0.05$).

Results

Table 1. Statistical analytical results of B. cereus count (cfu / g.) in the examined samples

Product	No. of samples	Positive samples		Min.	Max.	Mean ± SEM
		NO.	%			
Rice pudding	50	42	84	1.5×10^2	2.8×10^6	2.1×10^5 ± 7.4×10^4

Table 2. Effect of cinnamon on the survival of B. cereus in the inoculated rice pudding

Intervals	cfu / g.		
	Positive control	0.5 % cinnamon	1 % cinnamon
0 time	3×10^5	3×10^5	3×10^5
12 hr.	4.6×10^5	3.5×10^5	1.8×10^5
24 hr.	2.7×10^6	4.8×10^5	2.5×10^4
36 hr.	3.4×10^7	1.8×10^6	2.1×10^3
48 hr.	2.9×10^6	2.2×10^6	3.3×10^3

Table 3. Reduction % in the count of B. cereus following treatment of the inoculated rice pudding with different concentrations of cinnamon

Reduction %	0.5 % cinnamon	1 % cinnamon
0 time	0	0
12 hr.	23.91	60.87
24 hr.	82.22	99.07
36 hr.	94.71	99.99
48 hr.	24.14	99.89

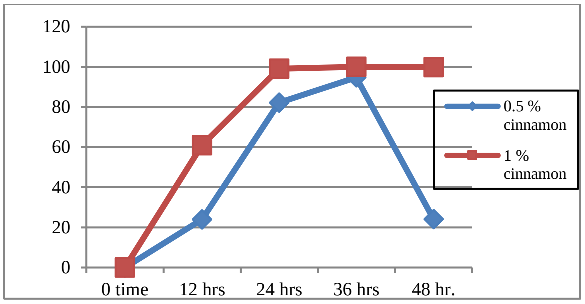

Figure 1. Reduction % in the count of B. cereus following treatment of the inoculated rice pudding with different concentrations of cinnamon

Discussion

Prevalence of *B. cereus*:

Inspection of Table (1) shows that the *B. cereus* existed in 42 (84%) of investigated rice pudding samples in counts ranging from 1.5×10^2 to 2.8×10^6 cfu /g. with a mean of $2.1 \times 10^5 \pm 7.4 \times 10^4$ cfu /g. The highest frequency distribution of the examined samples was 26% which were situated within the ranges 10^4 - $<10^5$.

Lower results of the prevalence of *B. cereus* in rice pudding samples were reported by Mohamed et al., (2016), Amin (2017) and El-Sherif *et al.*, (2021). While, the higher results were postulated by Hussein *et al.*, (2015), El-Karamani (2017), El-Zmakan and Mubarak (2017) and Ahmed (2018).

Bacillus food-poisoning usually occurs because spores survive cooking or pasteurization and then germinates and multiplies when food is inadequately refrigerated **(Granum, 2001).**

Effect of cinnamon on the survival of *B. cereus* in the inoculated rice pudding:

The results recorded in Table (2) showed that the counts of *B. cereus* in control prepared rice pudding samples were 3×10^5, 4.6×10^5, 2.7×10^6, $3.4 \times 10^7$and 2.9×10^6cfu/g at 0 time and then after 12, 24, 36 and 48 hr., respectively. The counts of *B. cereus* after adding 0.5 % cinnamon were 3×10^5, 3.5×10^5, 4.8×10^5, 1.8×10^6 and 2.2×10^6cfu/g, while after adding 1 % cinnamon were 3×10^5, 1.8×10^5, 2.5×10^4, 2.1×10^3 and 3.3×10^3cfu/g at 0 time and then after 12, 24, 36 and 48 hr., respectively.

Statistical analysis (analysis of variance) of the results in Table (3) revealed that the storage period (0 – 48) hours has no significant effect on *B. cereus* count reduction. On the other hand, Cinnamon concentration 0.5 and 1 % significantly affects the count of *B. cereus* in the investigated samples.

The addition of cinnamon powder to rice pudding significantly affects the count of *B. cereus* in investigated samples. There was a significant difference ($p \leq 0.05$) between the count of *B. cereus* in control samples and rice pudding samples treated with cinnamon 0.5 and 1 %, as well as the concentrations of 1% cinnamon had a more inhibitory effect on the growth of *B. cereus* than the concentrations of 0.5%. The reduction % in count of *B. cereus* at 0.5 and 1 % cinnamon concentrations after 12, 24, 36 and 48 hr. were 23.91, 82.22, 94.71, 24.14 %, and 60.87, 99.07, 99.99, 99.89 %, respectively. Table (3) and Figure (1).

Cinnamaldehyde, linalool, cinnamaldehyde para-methoxy and eugenol were the major chemical constituents of cinnamon essential oil which showed strong antimicrobial activity against some food-borne pathogens (*Escherichia coli*, *Candida albicans* and *Salmonella typhimurium*), so cinnamon oil may be considered as a natural preservative in food industry (Vazirian *et al.*, 2015).

Conclusion

The addition of cinnamon powder to rice pudding significantly affects the count of *B. cereus* in this study. as well as the concentrations of 1% cinnamon had a more inhibitory effect on the growth of *B. cereus* than the concentrations of 0.5%, so the addition of cinnamon to dairy product may be an effective method for inhibition of *B. cereus* growth and increase the shelf life of the dairy product.

Conflict of interest

The authors haven't conflict of interest to declare.

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