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Bacillus cereus Assessment in Dried Vegetables Distributed in Tehran, Iran

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HIGHLIGHTS

- Totally, 44 out of 140 (31.4%) dried vegetable samples were contaminated with *Bacillus cereus*.
- The *B. cereus* contaminations were found in 35.7 and 27.1% of open and packed dried vegetables, respectively.
- There was no significant difference between rate of *B. cereus* in open and packed dried vegetables.
- Rate of *B. cereus* was not significantly different among various kinds of vegetable samples.

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Acronyms and abbreviations
CFU=Colony Forming Unit

ABSTRACT

Background: *Bacillus cereus* is one of the important agents of the food-borne diseases worldwide. In the present study, the dried vegetable samples distributed in Tehran, Iran were evaluated in order to isolation, identification, and enumeration of *B. cereus*.

Methods: A total of 140 samples containing open and packed dried vegetables were randomly purchased from different areas of Tehran, Iran from March to August 2015. Dried vegetable samples were equally divided into seven groups, including dill, parsley, coriander, tarragon, mint, his, and pot roast. After culturing of samples, isolated *B. cereus* colonies were enumerated and identified using biochemical tests. The statistical tests were done by SPSS 16 (Chicago, IL, USA) software.

Results: Totally, 44 out of 140 (31.4%) dried vegetable samples were contaminated with *B. cereus*. The *B. cereus* contamination were found in 25 out of 70 (35.7%) and 19 out of 70 (27.1%) open and packed dried vegetable samples, respectively. There was no statistically significant difference (p>0.05) between contamination rate of *B. cereus* in open and packed dried vegetable samples. Also, contamination rate of *B. cereus* was not significantly different (p>0.05) among various kinds of vegetable samples.

Conclusion: Our study showed that dried vegetables sampled from Tehran, capital of Iran were contaminated with *B. cereus*. More researches are required in order to evaluate the prevalence of *B. cereus* contamination in raw and fresh vegetable samples consumed in the country.

Introduction

The reports of food-borne diseases associated with *Bacillus cereus* were described in 1950 for the first time. *B. cereus*, an aerobic spore-forming Gram-positive rod, is one of the important agents of the food-borne diseases worldwide (Clavel et al., 2007; Postollec et al., 2012; Tewari and Abdullah, 2015; Thorns, 2000). The

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bacterium is commonly present in soil, air, dust, water, dried, and processed foods especially in plant origin (Stenfors Arnesen et al., 2008). *B. cereus* spores are mainly isolated from different kinds of foods having considerable resistance to heat, dehydration, and radiation (Berthold-Pluta et al., 2015; Bottone, 2010). Endospores of *B. cereus* have ability of adhesion to the surfaces which is basically due to three features containing high hydrophobicity, spore formation, and low surface charge in spore (Husmark and Rönner, 1992; Tewari and Abdullah, 2015). Spores survive through cooking and pasteurization in high temperature; therefore, the control and preventing the food contamination is necessary (Ehling-Schulz et al., 2004; Glasset et al., 2016).

The minimal dose of *B. cereus* spores or vegetative cells that have been estimated to cause illnesses is around 10^5-10^8 Colony Forming Unit (CFU)/g of ingested food (Ehling-Schulz et al., 2004). *B. cereus* is an important cause of food-borne diseases (diarrheal type and emetic type) with short duration about 24-48 h (Forghani et al., 2015; Logan and Rodríguez-Díaz, 2006). The emetic type is mainly occurred due to the consumption of rice and pasta consumption; whereas the diarrheal type is often as a results of consumption of contaminated milk products, vegetables, and meat. Besides, *B. cereus* may also be etiologic agent of local and systemic infections such as endocarditis, endophthalmitis, and septicemia (Huseby et al., 2007; Kotiranta et al., 2000; Logan and Rodríguez-Díaz, 2006).

There is lack of data about contamination rate of *B. cereus* in various food products distributed in Iran. So, in the present study, the dried vegetable samples distributed in Tehran, Iran were evaluated in order to isolation, identification, and enumeration of *B. cereus*.

Materials and methods

Sampling

In this cross-sectional research, a total of 140 samples containing open (n=70) and packed dried vegetables (n=70) were randomly purchased from different areas of Tehran, Iran from March to August 2015. The dried vegetable samples were equally divided into seven groups, including dill, parsley, coriander, tarragon, mint, his, and pot roast.

Microbial analysis

Ten g dried vegetables were diluted in 90 ml sterile 0.1% peptone water. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were prepared in four tubes. In the next step, 1 ml suspensions was separately pour plated on mannitol-egg yolk-phenol red-polymyxin-agar (Scharlau, Spain) and incubated at 37 °C for 24 h. The pink colonies on each medium were subjected to Gram staining and biochemical tests. Standard plate count was performed on the plate with the lowest dilution after incubation. The pink colonies were assessed for nitrate reduction, catalase, hemolysis, sensitivity to penicillin, motility, and growth at 45 °C. The bacterial counts in dried vegetables were performed after identification by chemical tests.

Statistical analysis

Chi-square test was used to compare differences for the qualitative variables such as contamination rate and type of package (open or packed). One-way ANOVA test was also used to compare differences between type of dried vegetables and contamination rate. The statistical tests were carried out by SPSS version 16.0 (Chicago, IL, USA) software. The $p<0.05$ were considered as significant.

Results

Totally, 44 out of 140 (31.4%) dried vegetable samples were contaminated with *B. cereus*. The *B. cereus* contamination were found in 25 out of 70 (35.7%) and 19 out of 70 (27.1%) open and packed dried vegetable samples, respectively. Contamination rates of *B. cereus* in various kinds of vegetables are illustrated in Figure 1. Also, the mean *B. cereus* count is shown in Table 1.

There was no statistically significant difference ($p>0.05$) between contamination rate of *B. cereus* in open and packed dried vegetable samples. Also, contamination rate of *B. cereus* was not significantly different ($p>0.05$) among various kinds of vegetable samples.

Discussion

Few researches have been conducted for characterization of *B. cereus* isolates from vegetables up now. Almost, outbreaks of *B. cereus* have been related to rice and/or other grains and vegetables (Kim et al., 2017; Pirhonen et al., 2005; Valero et al., 2002). In the present study, considerable *B. cereus* count was reported in open and packed dried vegetable samples, indicating potential risk of these food products. Although contamination rate between open and packed dried vegetables was not statistically different, but the mean *B. cereus* count was higher in open dried vegetables comparing to the packed ones which might be due to aerobic condition in open dried vegetable samples.

According to findings of Choma et al. (2000), *B. cereus* was detected in 30% of raw vegetable samples sold in France which is somewhat similar to our results. In another study, number of *Bacillus* species ranged from

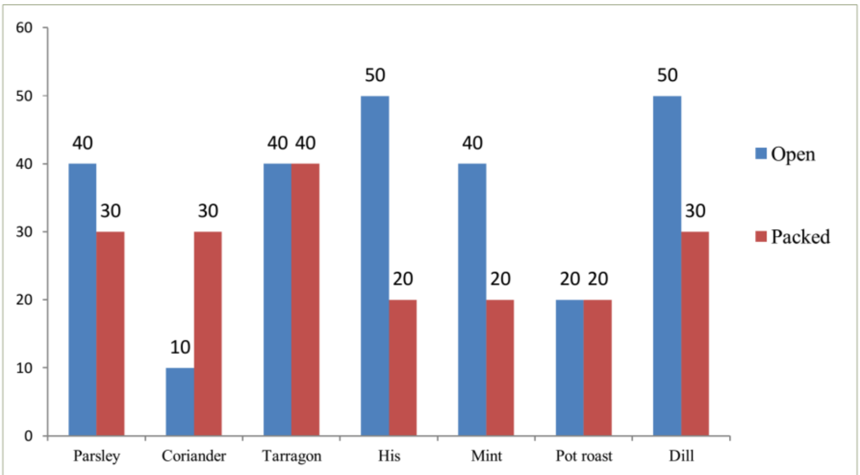

Figure 1: Contamination rate (%) of *Bacillus cereus* in open and packed vegetable samples

Table 1: Mean *Bacillus cereus* count in various kinds of vegetable samples

	Open		Packed	
	Sample size	Count (CFU/g)	Sample size	Count (CFU/g)
Parsley	10	4×10^4	10	3×10^3
Coriander	10	1×10^6	10	2×10^3
Tarragon	10	3×10^4	10	2×10^3
His	10	3×10^3	10	2×10^2
Mint	10	3×10^3	10	3×10^2
Pot roast	10	5×10^3	10	3×10^3
Dill	10	3×10^4	10	3×10^3

10^3 to 10^6 CFU/g in organic vegetable samples distributed in Ghana (Kudjawu et al., 2011). Flores-Urbán et al. (2014) found *B. cereus* in 32-84% of various vegetable samples, including broccoli, coriander, carrot, and lettuce sold in Mexico City. According to a similar survey in Iran, *B. cereus* was reported in 46% different food samples, including meat products, dairy products, rice, and salad samples (Deilami and Nasiri, 2016). Also, Sagoo et al. (2009) stated that 1% dried vegetable and spice samples from United Kingdom were contaminated to *B. cereus*. The wide variations of *B. cereus* contamination rates in vegetable samples from all over the world may be due to differences in sampling method, climate and agricultural condition, analytical assay, sanitation level, etc.

Conclusion

Our study showed that dried vegetables sampled from Tehran, capital of Iran were contaminated with *B. cereus*. More researches are required in order to evaluate the prevalence of *B. cereus* contamination in raw and fresh vegetable samples consumed in the country.

Conflicts of interest

There is no conflict of interest in this research.

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