INTRODUCTION

Bacillus cereus is a Gram-positive, aerobic spore-forming bacilli, commonly present in various natural environments and food matrices (Fiedoruk et al., 2017). B. cereus is responsible for causing diarrhea, emesis, fatal meningitis, and spoilage of different food products (Evreux et al., 2007).

Bacillus cereus food poisoning has two forms; the diarrheal form which is caused by ingesting large numbers of bacterial cells or their spores in a contaminated food, and the emetic form caused by ingesting food contaminated with the preformed toxin. The diarrheal type is primarily manifested by abdominal cramps and diarrhea following an 8 to 16 hrs incubation period (Murray et al., 2007). The emetic form is commonly caused by a toxin (cereulide), which is heat stable causing nausea and vomiting 1–5 hrs after consumption (Hoton et al., 2005). The similarities between the symptoms of the emetic disease and Staphylococcus aureus intoxication and the similarities between the diarrheal disease and that caused by Clostridium perfringens type A food poisoning create confusion to distinguish B. cereus food poisoning (Stenfors et al., 2008).

While there are various estimates of the number of B. cereus cells required to cause illness, there is a general agreement that foods containing <10³ CFU/g are safe for human consumption (Vilas-Boas et al., 2007). Meanwhile, it is reported that B. cereus should be 10⁴–10⁵ CFU/g in order to cause emetic or diarrheal illness (EFSA, 2005). Although, a small dose (<10⁴ CFU/g) may cause disease in susceptible individuals (Szabo et al., 1984). B. cereus food poisoning results from consumption of contaminated foods, as well as improper handling, storage and cooling of cooked foodstuffs (Schneider et al., 2004). Under improper storage conditions of food after cooking, the spores germinate and the vegetative cells multiply (Logan, 2011).

Presence of B. cereus in the dairy products is not only of concern as a public health hazard but also as a cause of economic losses through the reduction of shelf life and the spoilage of the contaminated products. It produces spoilage enzymes such as proteases, lipases, and lecithinases (Fagerlund et al., 2004), it causes bitter-rotten off-flavors due to the protease activity and fruity-rancid off-flavors due to the lipolytic activity (Stenfors et al., 2008).

B. cereus forms resistant spores that spread easily; therefore there is a risk in its transmission through processed, pasteurized, sterilized, and heat-treated food products (Kotiranta et al. 2000). Specific food types tend to be associated with illnesses caused by B. cereus. Desserts and dairy products are most frequently the vehicles for the transmission of the diarrheal form of the illness, whereas rice is the main vehicle of the emetic illness. The emergence of psychrotrophic and thermophilic species may mean that B. cereus will be of increasing concern to the food industry in the future (Griffiths and Schraft, 2017). It is believed that growth of psychrotrophic strains to high numbers in refrigerators is more significant than toxin production at low temperatures. Moreover, the psychrotrophic strains often limit the
keeping quality of pasteurized milk and its products (Adams and Moss, 2008).

Because of the ubiquitous distribution of B. cereus in the environment, the prevention of food contamination with the organism or its spores is almost impossible. However, high numbers of B. cereus are needed in the food for the food-borne disease to occur, this is frequently associated with holding food under conditions allowing active growth of the organism; it could be through poor refrigeration, slow or inadequate cooling, or holding food warm below 60°C. Therefore, control measures should be directed to prevent spore germination and to prevent the growth of vegetative cells, which might be the approach to effectively prevent and control the spread of this pathogen. Thorough cooking of foods will likely destroy the vegetative cells and spores, however, heat treatment below 100°C might not be effective against spores. Also, non-refrigerated storage of food should be avoided and it should be rapidly and efficiently cooled to less than 7°C (Griffiths and Schraft, 2017).

Since B. cereus has the ability to grow in wide varieties of food, capable of forming heat-resistant spores, and able to produce toxins adapting heat treatment and cold storage, thus posing risks to the consumers. Additionally, cases of B. cereus food poisoning through milk-based desserts have been reported by Johnson (1984); Meer et al. (1991); Andersson et al. (1995); Schoeni and Wang (2005). Because the dairy desserts are widely consumed in Egypt, this study was conducted to determine the occurrence of B. cereus and to evaluate its load in some milk-based desserts sold in Assiut city markets.

MATERIALS AND METHODS

I. Sampling
A total of 150 milk-based desserts samples were collected from different dairies and pastry shops in Assiut city, Egypt. The samples were rice with milk, pudding (Mahallabia in Arabic dialect), and custard (50 each). Each sample was obtained as sold to the public and dispatched directly to the laboratory with a minimum of delay. The preparation and handling of the samples were done according to APHA (1992).

II. Isolation of B. cereus
An appropriate amount of each prepared sample was inoculated into brain heart infusion broth tubes, then was incubated for 24 h at 30°C. An aliquot from the incubated tubes was streaked onto a plate of MYP agar and incubated at 30°C for 24 h. Pink, lecithinase-positive colonies were transferred to nutrient agar slants and incubated at 30°C for 24 h for further identification.

III. Enumeration of B. cereus
Enumeration of B. cereus was done using the Most Probable Number technique (MPN) according to Tallent et al. (2012). Eleven grams of the prepared sample was aseptically transferred to 99 ml of sterile 0.1% peptone water and then thoroughly mixed to be emulsified completely to obtain a dilution 1/10, from which decimal dilutions were prepared. One ml from the previously prepared dilutions was added to tryptone soy polymyxin broth tubes (3 tubes for each dilution). The inoculated tubes were incubated at 30°C for 48 h. Loopfulls from the positive tubes were streaked onto Mannitol Egg Yolk Polymyxin (MYP) agar plates and incubated at 30°C for 24 h. The MPN of B. cereus/g of sample was obtained using the MPN table (Peeler et al., 1992).

IV. Identification of the isolates
The isolated organisms were identified microscopically and biochemically according to (Procop et al., 2017). The biochemical tests used for the identification were motility, catalase, nitrate reduction, citrate utilization, gelatin hydrolysis and indole tests. The identified B. cereus strains were large Gram-positive rods with non-swelling spore, produce lecithinase and do not ferment mannitol on MYP agar, reduce nitrate to nitrite and indole negative. They were motile, catalase positive, citrate utilization positive and gelatin hydrolysis positive.

RESULTS

Table 1: Incidence of B. cereus in the examined milk-based desserts samples.

<table>
<thead>
<tr>
<th>The examined samples</th>
<th>The No. of examined samples</th>
<th>The positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Rice with milk</td>
<td>50</td>
<td>31</td>
</tr>
<tr>
<td>Pudding</td>
<td>50</td>
<td>22</td>
</tr>
<tr>
<td>Custard</td>
<td>50</td>
<td>16</td>
</tr>
</tbody>
</table>

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DISCUSSION

Milk-based desserts are popular dairy food prepared from ingredients that milk is the basic constituent. They are palatable, nutritious and relatively inexpensive dairy food. These types of desserts are consumed in Egypt by a wide range of people of all ages and are usually served cooled (AL-Gendi, 2004). Rice with milk and pudding (Mahallabia) are the most widely consumed dairy desserts in Egypt (EL-Shaar, 1993). Milk-based products are good media for the growth of microorganisms because of their high nutritive value, almost neutral pH and long storage periods (Bell and Kyriakides, 1998). The bacteria most frequently found to contaminate milk-based desserts are members of the Enterobacteriaceae group, Pseudomonas spp., and Bacillus spp. (in particular B. cereus) (Lewis and Dale, 1994).

Dairy-based foods are important means for the transmission of different pathogens especially in places where the hygienic measures are not strictly adopted (Meyer-Broseta et al., 2003). Assessing the exposure to B. cereus is an important issue in estimating the risk for food-borne disease by this microorganism. The actual disease symptoms, however, are caused by toxins, either produced during growth in the gut; enterotoxins or during growth in food; emetic toxin (Adams and Moss, 2008).

Dairy desserts in Egypt are locally produced by traditional retail shops and the production takes place manually in stores. Custard is made from highly nutritive raw materials; milk and sugar and is easily spoiled by the multiplication of specific microbial contaminants (Arakawa et al., 2008).

The Most probable number (MPN) technique was used for enumeration of B. cereus in this study as it is described in reference methods and is recommended for routine surveillance of products in which small numbers of B. cereus are expected (Bennett et al., 2015).

The results recorded in Table 1 revealed that B. cereus was detected in 62%, 44% and 32% of the examined rice with milk, pudding, and custard samples, respectively. Nearly similar results were recorded by Al- Ashmawy et al. (1996); Reyes et al. (2007), while lower incidence for the rice with milk was recorded by Hassan and Afifi (2016); Mohamed et al. (2016). Also, higher incidence for pudding (Hussein et al., 2015) and lower incidence for custard (Van Netton et al., 1990) were recorded. The different results obtained by researchers can be due to regional, seasonal, sampling and methodological differences. Also, the degree of contamination depends on the precautions observed during processing.

### Table 2: Frequency distribution of the positive samples based on their B. cereus count.

<table>
<thead>
<tr>
<th>Intervals</th>
<th>Rice with milk</th>
<th>Pudding</th>
<th>Custard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>3-&lt;10</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>10-&lt;10²</td>
<td>19</td>
<td>61.29</td>
<td>15</td>
</tr>
<tr>
<td>10²-&lt;10³</td>
<td>10</td>
<td>32.26</td>
<td>3</td>
</tr>
<tr>
<td>10³-&lt;10⁴</td>
<td>2</td>
<td>6.45</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>100</td>
<td>22</td>
</tr>
</tbody>
</table>

### Table 3: Microbial quality* of the examined samples based on their B. cereus count.

<table>
<thead>
<tr>
<th>The examined samples</th>
<th>Good &lt;10²</th>
<th>Acceptable 10²-&lt;10³</th>
<th>Unsatisfactory 10³-&lt;10⁴</th>
<th>Potentially hazardous ≥10⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No./50</td>
<td>%</td>
<td>No./50</td>
<td>%</td>
</tr>
<tr>
<td>Rice with milk</td>
<td>38</td>
<td>76</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Pudding</td>
<td>46</td>
<td>92</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Custard</td>
<td>49</td>
<td>98</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

* According to the public health guidelines (NSW Food Authority, 2009)
It is noticeable from Table 2 that most of the positive samples; 61.29% of the positive rice with milk samples, 68.18% of the positive pudding samples and 75% of the positive custard samples were in the range 10-<10^3 CFU/g. The obtained results are in agreement with that reported by Bryan et al. (1992) and Rosenquist et al. (2005).

The quality of the examined samples regarding the B. cereus count was estimated using the guidelines for the microbiological quality of ready-to-eat foods (NSW Food Authority, 2009), which shows four grades of the microbiological quality related to the B. cereus count; <10^7 CFU/g is considered good, 10^7-<10^8 CFU/g is considered acceptable, 10^8-<10^9 CFU/g is considered unsatisfactory and ≥10^9 CFU/g is considered unacceptable (potentially hazardous). It is clear from the results in Table 3 that 76% of the examined rice with milk samples, 92% of the examined pudding samples and 98% of the examined custard samples were considered good. Furthermore, 20%, 6% and 2% of the examined rice with milk, pudding, and custard samples, respectively were considered acceptable and only 4% of the examined rice with milk samples and 2% of the examined pudding samples were considered unsatisfactory. While, none of the examined samples was considered unacceptable (potentially hazardous). At the detected levels, B. cereus is not considered injurious to health, however, the risk will increase proportional to the detected levels and the likelihood of subsequent growth.

Since milk is the main constituent of the examined samples, it seems to be one of the sources of the contamination with the B. cereus. Soiling of cows’ udders is one of the main sources of contamination of milk with B. cereus. The control of B. cereus in the dairy industry is challenging owing to the numerous points at which milk could get contaminated, either during production or processing. The number of B. cereus or its spores may be limited in raw milk by proper cleaning of the udder and teats before milking. It is worth mentioning that pasteurization kills the vegetative bacteria, but the spores survive. Pasteurization might even activate some of the spores (heat activation), which might start germinating (Tewari and Abdullah, 2015). In general, the food production facilities must use heating methods that destroy B. cereus vegetative cells and most spores. Also, cooked food should not be stored at room temperature (Montanini et al., 2013). While, cold storage may be a suitable control measure for such products. However, up to 14% of B. cereus strains may be psychrotrophic (Granum, 1997), and so temperature alone is not a practical control measure. Milk-based desserts should be prepared in small batches, cooled rapidly, and stored at 4°C (Smith et al., 2003).

In conclusion, the examined rice with milk, pudding and custard samples were contaminated with B. cereus in different percentages but in low numbers. However, rice with milk was more contaminated than pudding and custard. The current B. cereus load is not threatening to health but further growth of the bacteria at the retail premises until selling is likely leading to health hazards. Therefore, it is recommended to adopt measures to minimize the contamination with B. cereus from the very outset, even before processing in order to avoid the growth of the organism to dangerous levels. Also, better hygienic practices are required in the production of such products.

REFERENCES


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تم جمع 150 عينة من الحلاوي لينة الأساس؛ الأرز باللبين، والمهلبي، والكسترد من مناطق مختلفة في مدينة أسوان، مصر. تم فحص العينات التي تم جمعها لعزل وعدد البالاسيل سيريس باستخدام tryptone MPN. وقد وجدت البالاسيل سيريس في 26%، 44% و 22% من عينات الأرز باللبين، والمهلبي، والكسترد على التوالي. وكان عدد البالاسيل سيريس في أغلب العينات التي تم فحصها في نطاق 10^3–10^6 CFU/g. وقد تم مقارنة خطرة تواجد البالاسيل سيريس.

الكلمات المفتاحية: البالاسيل سيريس، الأرز باللبين، المهلبي، الكسترد.