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Microbiological safety and quality survey of some dairy confectioneries with cream filling and topping

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Abstract

Background: Dairy confectionaries are recently categorized as an important part of different consumers' diets with increasing demand for cream-based cakes "Tourta" and gateau which are used as celebrating food for almost all occasions and celebrations.

Aim: The study was designed for evaluating general quality and safety of such products.

Methods: 100 cream-based (for topping and filing) samples; 50 cakes "Tourta" and 50 gateau, were purchased separately from several pastry shops covering international, national, and local brands in Great Cairo, Egypt, and subjected to microbiological analysis.

Results: Results showed that international brands were the best for gateau samples while national brands were the best quality for cake samples. Regardless of the brand, the general hygienic quality of the cake product "Tourta" was lower on an average total colony, coliforms, yeast, and mold counts as compared with the gateau product. Although coliforms were found in 100% of the examined samples with the highest mean value of $33 \times 10^2 \pm 7.0 \times 10^2$ CFU/g in local gateau samples, *Escherichia coli* and *Salmonella* spp. could not be detected in these samples. *Staphylococcus aureus* was isolated from 8% of the total examined samples with the highest incidence of (22.2%) and a mean count of $38 \times 10 \pm 6.0 \times 10$ CFU/g in local brand gateau samples. *Bacillus cereus* was extensively isolated with the highest incidence in cake samples (32%) and mean counts higher than 10^3 in all of the examined samples. *Bacillus cereus* strains have harbored more than one toxigenic gene. In this aspect, *nhe* gene was the most predominant one as it was detected in 100% of the examined isolates, followed by *cytK* gene in 80%, while*hbl* and *ces* genes could not be found. According to the Egyptian Standard specifications for cake (ES: 4037/2020), a higher acceptability degree was reported for the international brand gateau and cake "Tourta" samples. The incidence of using any preservative as an inhibitory substance was also analyzed generally using the *Bacillus subtilis* disk assay technique, but all samples were negative using this technique indicating the need for a more advanced technique for its detection such as using high-performance liquid chromatography.

Conclusion: Finally, it was concluded that; more attention is needed to cream-based cakes' quality and safety, with essential modification required in the standards of cakes in Egypt.

Keywords: *Bacillus cereus*, Cream-based cakes, Dairy confectionaries, Gateau, *B. cereus* toxigenic genes.

Introduction

Dairy confectionery products can be grouped into five categories: dried milk-based products, fermented products, acid-coagulated products, cereal-based desserts, and fat-rich products (Peter, 2008). Between these products; cakes are the most popular with different toppings and fillings especially those with cream. Filling cream is an important component of cakes, it is fat and sugar based to provide the desired texture, taste, and union of the baked cake besides its role as a dietary source of main nutrients; protein, carbohydrates and fat (Nicoletta *et al.*, 2015). The consistent safety and quality of the finished product are dependable on the quality of milk and the processing conditions which should be standardized (Peter, 2008).

It may touch on mind that there is a low incidence of microbial hazards in these products due to their exposure to elevated temperatures during the baking process, baking temperatures only kill vegetative forms of pathogens, while baked food is made of grains and powders, which are commonly contaminated with spore-forming organisms than vegetative form. During dough proofing, microbial growth of these spores occurs (Abd El-Rady *et al.*, 2016). Also, cakes are usually filled, layered, or covered with whipped and buttercream that is not conducted to heat treatment and they may contain pathogens and be excellent material for microbial growth and toxins production due to their high water activity, nearly neutral pH, and high nutrients (Sherif *et al.*, 2018). Cream-based cakes may be contaminated with pathogens from different sources as; improper handling

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during slicing, adding ingredients, and decoration especially with bad personal hygiene, followed by temperature abuse during storage. Application of one of the food safety management systems as hazard analysis critical control points (HACCP) for example can manage these risks (FSAI, 2018).

Food poisoning of cream-based cakes had been sporadically reported and had been linked to several pathogens including *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella* species, or *Escherichia coli* (EFSA and ECDC, 2014; Wiwanitkit, 2015; CDC, 2016), which may negatively affect the economic, public health and nonconformity with food safety standards and legal specifications (Choi *et al.*, 2016).

Staphylococcus aureus is one of the toxin-producing pathogen which hase concern, especially for chilled foods, however its importance is declared only when the produced food is stored in cooled conditions slowly or re-contaminated during storage (Brown, 2008). Wiwanitkit (2015) reported a case study of *Staphylococcus* toxin food poisoning due to the consumption of coconut cream-filled green tea cake, it was characterized by rapid acute abdominal pain after 4 hours of eating which furtherly progressed to severe diarrhea and vomiting.

After *S. aureus* the second emerged foodborne pathogen is *B. cereus* and it was identified as the causative agent responsible for about 19% of foodborne outbreaks which were reported in the United States from 1998 to 2008 (Bennet *et al.*, 2013). The foods most frequently linked to outbreaks are those that have been cooked or contain cooked ingredients, especially those high in protein or starch such as pudding, spores can survive cooking, and if cooling is insufficient; they can germinate and grow into vegetative cells (Bennett *et al.*, 2015b). *Bacillus cereus* produces about six types of toxins; including cereulide (an emetic toxin), a cytotoxin (*cytK*), and four enterotoxins (hemolysin *BL*, non-hemolytic enterotoxin, FM, and enterotoxins T). According to FDA (2012); cereulide toxin could be destroyed at 121°C for 30 minutes, resist proteolysis, has pH from 2 to 11 and is cold stable (4°C for 60 days), while *hbl* toxin suggested being the main virulence factor in diarrhea caused be *B. cereus* but it is sensitive to heat; as it could inactivate at 56°C for 5 minutes. (Logan and De Vos, 2009).

The prevalence of *Salmonella* spp. in different bakery products has been interesting for many years. According to a case report delivered by Fielding *et al.* (2003) in September 2003, a *Salmonella typhimurium* outbreak related to cheesecake eating was recorded; the environmental inspection identified the cracked fecal contaminated eggs and mishandling from workers as the potential sources of *Salmonella* in this case. But nowadays, the use of pasteurized egg in cake industry had greatly reduced the incidence of *Salmonella* from such products. The Centre for Disease Control (CDC) estimates outbreaks due to *Salmonella* of about 1.35 million cases of infections, about 26,500 of them need hospitalizations, and 420 cases end with death in the United States each year related to food (CDC, 2022).

Escherichia coli is another one of the most important pathogens that cause food poisoning, it has been used as a fecal contamination indicator in fresh foods and its presence in high concentrations may occasionally connect with a higher likelihood of enteric pathogens presence (Kornacki *et al.,* 2015). A multistate *E. coli* outbreak connected to cake mix was reported by the CDC in July 2021; 16 persons became sick across 12 states linked to cake mix. *Escherichia coli* infections become more serious for children with the development of severe illness (CDC, 2021).

Microbial spoilage of cakes may occur by molds and yeasts as the main cause and by bacteria only occasionally, this is owing to reduced water activity (0.94) and pH (5.4–7.5) (Sherif *et al.*, 2018). Foods are rarely connected with infectious fungi, but some yeast can cause allergies, and some molds can infect people with compromised immune systems in addition to mycotoxins production (Da Silva *et al.*, 2018).

Re-formulation of cake recipes by manipulating water activity and/or pH of fillings is sometimes useful for extending their shelf life (Rodriguez-Garcia *et al.*, 2012). Certain synthetic chemicals could be used as food preservatives such as sorbic, acetic, ascorbic, and propionic acids, in addition to their salts in order to improve cake product's safety and quality (Saranraj and Geetha, 2012). Long-term consumption of these chemicals leads to several side reactions which can be either immediate or build up in the body over time (Sanjay, 2015).

Serving safe foods to consumers is mandatory to ensure public health (Shohana *et al.*, 2019). The aim of this research was to investigate the microbiological state of some dairy confectionaries (cream-based cakes "Tourta" and gateau) from different brands in Egyptian markets regarding the incidence of using inhibitory substances for preserving purposes. Several bacteria were isolated and identified based on the biochemical examination and *B. cereus* virulence genes were investigated using PCR technique which has limited data in Egypt.

Materials and Methods

Samples collection

Our study was directed in the period between November 2021 and July 2022, a total number of 100 commercially prepared cream-based confectioneries (50 cakes "Tourta" and 50 Gateau) were randomly obtained from different suppliers representing; (A) international brands chains, (B) national brands chains, and (C) local shops in different districts of great Cairo governments. Samples selection and collection was done based on an online survey by 1,300 participant from cake consumers in Egypt to select the most common cake brands, shops, types, and categories (Esraa *et al.*, 2022). Samples were transferred immediately to the laboratory in an insulated ice box

without delay. Collected samples were firstly subjected to complete chemical analysis, fatty acid profile, and nutritive value assessment, in addition to their compatibility to the Egyptian standards (Esraa *et al.*, 2023), and then the same samples were subjected to microbiological analysis.

Microbiological analysis

Preparation of samples and decimal dilution

From each sample, eleven grams were separately added to 99 ml of 0.1% sterile peptone water in a polyethylene bag and homogenized using a blender (Grindomix GM200, Retsch, Haan, Germany) for 30 seconds at room temperature, then 10-fold decimal dilutions were prepared in sterile 0.1% peptone in water (Taylor *et al.*, 2015).

Enumeration of microorganisms in examined samples

For determining the microbiological quality, samples were analyzed for total colony count according to (ISO, 4833-1: 2013) on Standard plate count agar medium and total yeast and mold count (ISO, 21527-1: 2008) on Sabaroud agar medium, Coliforms count using three tubes method (MPN/g) using Lauryl Sulphate broth, total *Staphylococci* count, *S. aureus* on Baired Parker agar and *B. cereus* counts on MYP medium using spreading technique were done according to APHA (2015).

Isolation and identification of specific pathogens from the examined samples

All samples were examined for the incidence of *B. cereus, S. aureus*, and *E. coli* (APHA, 2015) and *Salmonella* species (ISO, 6579-1: 2017) as main pathogens expected to be present in cream-based cakes "Tourta" and gateau samples.

- Biochemical confirmations of the *B. cereus* group including: anaerobic glucose fermentation, Voges-Proskauer, tyrosine decomposition, nitrate reduction, Rhizoid growth, lysozyme resistance, motility tests, and hemolytic activity were done on suspected isolates (Bennett *et al.*, 2015b).
- For *S. aureus*; coagulase, catalase, thermostable nuclease (thermonuclease), lysostaphin sensitivity, anaerobic utilization of glucose, and mannitol fermentation tests were done to confirm the examined isolates identification (Bennett *et al.*, 2015a).
- *Salmonella* species suspected isolates were confirmed biochemically using oxidase (Kovac's), H_2S (Triple Sugar Iron), lysine decarboxylase, Methyl red, Voges-Proskauuer, Motility, Nitrate reduction, Citrate utilization, and Indole production tests (Brenner and Farmer, 2005).
- For *E. coli* isolates confirmation, IMViC tests (indole, methyl red, Voges-Proskauer, and citrate) were done for all isolates (Da Silva *et al.*, 2018).

Molecular identification of biochemically confirmed B. cereus isolates and detection of its virulence genes (Deoxyribonucleic acid extraction and multiplex PCR for virulence genes detection)

Deoxyribonucleic acid from the previously identified *B. cereus* cultures was extracted using a QIAamp DNA Mini kit (Catalogue no., 51304). GroEL gene detection was performed by Emerald AmpGT PCR master mix (Code No. RR310A) using Oligonucleotide primers sequences (Metabion, Germany) at 533 bp, (TGCAACTGTATTAGCACAAGCT: TACCACGAAGTTTGTTCACTACT), and recognized using *B. cereus* positive control as *B. cereus* (ATCC® 10876TM).

- Molecularly identified *B. cereus* cultures were analyzed for enterotoxigenic genes (*hbl*, *cytK*, nhe, and *ces*). The sequences (F: R) of primers were (GTA AAT TAI GAT GAI CAA TTTC: AGA ATA GGC ATT CAT AGA TT; AAG CIG CTC TTC GIA TTC: ITI GTT GAA ATA AGC TGT GG; ACA GAT ATC GGI CAA AAT GC: CAA GTI ACT TGA CCI GTT GC and GGTGACACATTATCATATAAGGTG: GTAAGCGAACCTGTCTGTAACAACA) for *hbl*, *nhe*, *cytK*, and *ces* genes at 1,091, 766, 421, 1,271 bp, respectively. PT-100 Thermo-cycler (MJ Research, USA) was used to achieve the amplification cycles. Polymerase chain reaction was done twice per isolate and to visualize the products; 1.5% (Tris Borate EDTA) agarose gels were used and examined via UV. The molecular identification materials used, steps, and primer sequences were applied according to Sambrook *et al.* (1989), Ehling-Schulz *et al.* (2006), and Das *et al.* (2013).

Detection of inhibitory substances in some of the examined cake samples

Sample preparation

Ten grams from 30 cake samples (with the lowest total colony counts) were presoaked overnight in 100 ml of sterile distilled water (SDW). The extract was filtered using thick filter papers (35 mm diameter) and centrifuged at 5,000 rpm for 25 minutes. After that, the supernatant was filtered by using a 0.2 µm bacteriological filter (Shandeep *et al.*, 2021).

Antimicrobial assay method for detection of inhibitory substances

Bacillus subtilis ATCC 6633 was grown in liquid brain-heart infusion media at 37°C for 6 hours. Mueller-Hinton agar (Oxoid) plates previously prepared and solidified were swapped with 0.1 ml inoculum of *B. subtilis* broth (10^6 cells/ml^{-1}). Each cake sample prepared filtrate was inoculated with a suitable amount in a circular well (10 mm diameter) in the agar plate containing *B. subtilis* after dryness. After that, the plates were incubated at 37°C ± 2°C for 24 hours, and the diameter of the zone of inhibition of the bacterial growth was measured in mm using a zone inhibition scale. The presence of an inhibition zone indicates the incompatibility of inhibitory substances and vice versa. A control negative well was inoculated with SDW and chloramphenicol 0.1% was inoculated in another well as a control positive. Each test was carried out three times for more confirmation of results (Mahantesh *et al.*, 2019).

Statistical analysis

One-way analysis of variance using Excel of Microsoft 365 enterprise was done to analyze the results of different parameters.

Ethical approval

Not needed for this study. All authors are committed to general research ethics and the journal ethics and rules.

Results

Results in Table 1 explored that the highest mean counts for total colony count, total mold, and yeast were 13 × 10^6, 10 × 10^5, and 46 × 10^5 CFU/g, respectively in national brand gateau samples, while the highest mean counts for total *Staphylococci*, Coliforms, *S. aureus*, and *B. cereus* were 23 × 10^4, 33 × 10^2, 38 × 10, and 55 × 10^2 CFU or MPN/g, respectively in local brand gateau samples. It was also clear that the highest prevalence of *S. aureus* was (22.2%) and *B. cereus* was (66.7%) in local brand gateau samples and it couldn't be isolated from international brand gateau samples and both organisms were isolated from one sample only (5%) of the national brand samples. On the other side, *Salmonella* spp. and *E. coli* couldn't be detected in all examined samples.

By examining the microbiological quality of cream based cake samples (Tourta) as shown in Table 2, the highest mean count for the total colony and total mold was 76 × 10^5 and 97 × 10^3 CFU/g, respectively in the international brand group, while the highest mean counts for total *Staphylococci*, coliforms, and yeast were 40 × 10^4, 25 × 10^2, and 49 × 10^4 CFU or MPN/g, respectively in local brand samples. The highest prevalence of *S. aureus* was (15%) and *B. cereus* was (55%) in local brand Tourta samples, while *S. aureus* couldn't be isolated from any of the international and local brand samples. The same as in gateau samples; *Salmonella* spp. and *E. coli* couldn't be detected in all examined cream-based cake samples.

After biochemical identification of *B. cereus* strains isolated from all examined samples, some isolates (five isolates) were subjected to molecular identification for more confirmation (Fig. 1). The incidence of toxigenic genes in these confirmed isolates have been examined and it was found that *B. cereus* harbored more than one toxigenic gene. The *nhe* gene was detected in all of the examined isolates (100%), followed by *cytK* gene (80%), while *hbl* and *ces* genes could not be found (Table 3 and Fig. 2). Also Figure 3 showed that there is a great relationship between hemolytic activities of *B. cereus* isolates obtained from examined samples and the obtained toxigenic genes.

According to the Egyptian Standard specifications for cake (ES: 4037/2020) the higher acceptability degree was reported for the international brand gateau (100% for all parameters) and cake "Tourta" samples (100% for all parameters, except for mold 75% and *B. cereus* 83.33%).

Unfortunately, inhibitory substances could not be detected in any of the examined samples using the *B. subtilis* disc assay technique. That does not indicate the real absence of inhibitory substances from cream-based cakes, but it may indicate the low sensitivity and specificity of this technique.

Discussion

Cakes, especially the cream-based one "Tourta" with different ingredients of topping and filling, could be considered nowadays as the most popular confectionaries in our country. The cream-based cake is one of the milk-based confectioneries with high production and consumption rates. During the production of such products, several ingredients are used; as: milk, eggs, wheat flour, cream, fresh fruits, jelly, chocolate, and coloring and flavoring agents (Hassan *et al.*, 2018). The finished product properties, quality, and stability during shelf-life are dependable on these ingredients' quality (Hanee, 2013).

Thermal processing such as baking is the most widely used method to control pathogens in ready-to-eat cakes; however, the effectiveness of the thermal process depends primarily on the heat resistance of microorganisms and other factors including water activity (aw; above 0.94), pH (5.4–7.5), and fat content (30%–60%)of the final product (Vincenzo *et al.*, 2020). Contaminated processed food like cakes is a serious situation threatening public health. Thus, the continuous evaluation of final products before their declaration as compared with different food standard guidelines is mandatory (Shohana *et al.*, 2019), he added that the maximum permissible limits in ready-to-eat foods (as cakes) for total plate count is <10^5 CFU/g, yeast, and mold is <10^4 CFU/g, coliform bacteria <200 MPN/g with the absence of *E. coli*.

Cream-based cakes are sold in Egyptian markets in several forms; commonly the one-piece large size form (Tourta) and small pieces form (Gateau) with the same filling, topping, and decorations are extensively sold in different pastry shops of different brands (international, national, and local). The extensive use of these products by consumers from different categories on different occasions, together with the considerable lack of reported data on their microbiological quality and safety encouraged studying such products.

The most widely used general indication of bacterial populations in food is the total aerobic plate count. It indicates the product's hygienic quality, manufacturing procedures, raw materials, processing conditions, handling procedures, and shelf life, but it is a poor indicator of food safety (Da Silva *et al.*, 2018). The examined gateau samples from the national brand were of the lowest quality among brands as shown in Table 1; with a total colony, mold, and yeast counts mean values of 13 × 10^6 ± 3.0 × 10^6; 10 × 10^5, and 46 × 10^5 ± 13 × 10^5 CFU/g, respectively. These high counts could be linked to the high production with low release rates than other brands, together with the fact that in these brands, one factory manufactures and then distributes the cakes to several branches of the same brand which increase the contamination probability during transportation due to temperature abuse.

On the other side, low personal hygiene was very obvious in gateau samples from local brands; where

Table 1. Microbiological analysis of the examined cream-based "Gateau" samples from different brands (total number = 50).

Count	International (Group A) (*n* = 12)					National (Group B) (*n* = 20)					Local (Group C) (*n* = 18)					Average of 3 groups
	Incidence		Min.	Max.	Mean ± SEM	Incidence		Min	Max	Mean ± SEM	Incidence		Min	Max	Mean ± SEM	Mean ± SEM
	No.	%				No.	%				No.	%				
Total colony (CFU/g)	12	100	42×10^3	27×10^5	$61 \times 10^4 \pm 13 \times 10^4$	20	100	16×10^4	83×10^6	$13 \times 10^6 \pm 3.0 \times 10^6$	18	100	29×10^3	28×10^6	$53 \times 10^5 \pm 10 \times 10^5$	$72 \times 10^5 \pm 20 \times 10^5$
Total *Staphylococci* (CFU/g)	12	100	66×10^2	80×10^3	$55 \times 10^3 \pm 3.0 \times 10^3$	20	100	14×10^2	58×10^4	$80 \times 10^3 \pm 23 \times 10^3$	18	100	13×10^2	30×10^5	$23 \times 10^4 \pm 11 \times 10^4$	$13 \times 10^4 \pm 6.0 \times 10^4$
Coliforms (MPN/g)	12	100	40	11×10^3	$25 \times 10^2 \pm 7.0 \times 10^2$	20	100	40	9.3×10^2	$29 \times 10 \pm 3.5 \times 10$	18	100	40	11×10^3	$33 \times 10^2 \pm 7.0 \times 10^2$	$20 \times 10^2 \pm 6.0 \times 0^2$
Mold (CFU/g)	0	0		---		1	5	10×10^5		---	2	11.1	30×10^2	20×10^4	$10 \times 10^4 \pm 2.0 \times 10^4$	$40 \times 10^4 \pm 8.0 \times 10^4$
Yeast (CFU/g)	12	100	11×10^3	50×10^4	$15 \times 10^4 \pm 2.0 \times 10^4$	20	100	16×10^3	30×10^6	$46 \times 10^5 \pm 13 \times 10^5$	18	100	70×10^2	68×10^5	$12 \times 10^5 \pm 3.0 \times 10^5$	$23 \times 10^5 \pm 9.0 \times 10^5$
Coagulase positive *Staphylococcus aureus* (CFU/g)	0	0		---		1	5	23×10^2		---	4	22.2	10×10	10×10^2	$38 \times 10 \pm 6.0 \times 10$	$76 \times 10 \pm 13 \times 10$
Bacillus cereus (CFU/g)	0	0		---		1	5	40×10^2		---	12	66.7	10×10^2	45×10^3	$55 \times 10^2 \pm 18 \times 10^2$	$54 \times 10^2 \pm 17 \times 10^2$

(n): total number of the examined samples; (Min): minimum; (Max): maximum; (No.): number of positive samples; (CFU): colony forming unit; (MPN): most probable number. N. B.: Salmonella spp. and *E. coli* could not be detected in all examined samples.

Table 2. Microbiological analysis of the examined cream-based cake "Tourta" samples from different brands (total number = 50).

Count	International (Group A) (*n* = 12)					National (Group B) (*n* = 20)					Local (Group C) (*n* = 18)					Average of 3 groups
	Incidence		Min.	Max.	Mean ± SEM	Incidence		Min.	Max.	Mean ± SEM	Incidence		Min.	Max.	Mean ± SEM	Mean ± SEM
	No.	%				No.	%				No.	%				
Total colony (CFU/g)	12	100	18×10^4	80×10^6	$76 \times 10^5 \pm 33 \times 10^5$	20	100	12×10^3	58×10^5	$48 \times 10^4 \pm 18 \times 10^4$	18	100	39×10^3	39×10^6	$47 \times 10^5 \pm 14 \times 10^5$	$37 \times 10^5 \pm 18 \times 10^5$
Total *Staphylococci* (CFU/g)	12	100	20×10^3	54×10^4	$15 \times 10^4 \pm 2.0 \times 10^4$	20	100	16×10^2	16×10^4	$23 \times 10^3 \pm 5.0 \times 10^3$	18	100	30×10^2	36×10^5	$40 \times 10^4 \pm 12 \times 10^4$	$19 \times 10^4 \pm 7.0 \times 10^4$
Coliforms (MPN/g)	12	100	40	93×10	$25 \times 10 \pm 3.0 \times 10$	20	100	40	15×10^2	$47 \times 10 \pm 7.0 \times 10$	18	100	40	11×10^3	$25 \times 10^2 \pm 6.0 \times 10^2$	$12 \times 10^2 \pm 4.0 \times 10^2$
Mold (CFU/g)	3	25	10×10^2	19×10^4	$97 \times 10^3 \pm 14 \times 10^3$	3	15	10×10	10×10^4	$37 \times 10^3 \pm 8.0 \times 10^3$	8	44.4	10×10	10×10^4	$18 \times 10^3 \pm 5.0 \times 10^3$	$34 \times 10^3 \pm 9.0 \times 10^3$
Yeast (CFU/g)	12	100	58×10^3	17×10^5	$47 \times 10^4 \pm 7.0 \times 10^4$	20	100	40×10^2	31×10^5	$23 \times 10^4 \pm 10 \times 10^4$	18	100	11×10^3	13×10^5	$49 \times 10^4 \pm 6.0 \times 10^4$	$38 \times 10^4 \pm 8.0 \times 10^4$
Coagulase positive *Staphylococcus aureus* (CFU/g)	0	0		---		3	15	30×10	20×10^2	$14 \times 10^2 \pm 1.0 \times 10^2$	0	0		---		$14 \times 10^2 \pm 14 \times 10$
Bacillus cereus (CFU/g)	2	16.7	10×10^2	3×10^3	$20 \times 10^2 \pm 2.0 \times 10^2$	11	55	10×10^2	30×10^4	$44 \times 10^3 \pm 12 \times 10^3$	3	16.7	40×10^2	10×10^3	$63 \times 10^2 \pm 5.0 \times 10^2$	$32 \times 10^3 \pm 10 \times 10^3$

(n): total number of the examined samples; (Min): minimum; (Max): maximum; (No.): number of positive samples; (CFU): colony forming unit; (MPN): most probable number. N.B: *Salmonella* spp. and *E.coli* could not be detected in all examined samples.

there is more intensive contact between the staff and the product with little or no awareness about the hygiene concept or management system. Data in Table 1 showed that the highest mean count of total *Staphylococci* was $23 \times 10^4 \pm 11 \times 10^4$ CFU/g and incidence of *S. aureus* in 22.2% of the samples with a mean count of $38 \times 10 \pm 6.0 \times 10$ CFU/g in this group.

In the field of confectionaries, especially cream-based cakes, regarding food safety and quality, the ICMSF (2003) recommended several steps for improving the final product quality including: using pasteurized eggs and dairy products, the separation between cooked and raw materials, limiting the aerosols, and dust in food preparation and displaying area, efficient cleaning, product holding at 5°C or below and using well-trained food handlers.

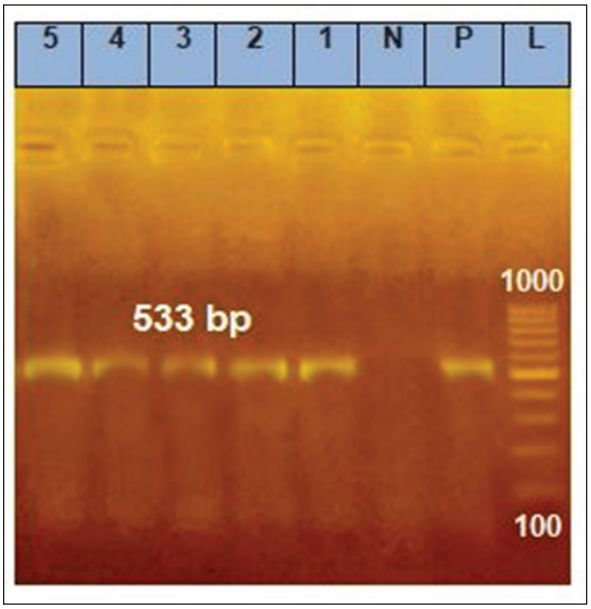

Fig. 1. Amplicons of *B. cereus* isolates on agarose gel using groEL (533 bp). Lane (1–5) *B. cereus* isolates, and Lane (L) DNA ladder 100 bp molecular weight marker.

Although Jay (2000) noted that cakes of all types rarely undergo bacterial spoilage due to their unusually high concentrations of sugars, which restrict the availability of water, this state was not the same in the case of cream-based cake samples as shown in Table 2. Results for cream-based cake samples "Tourta" from the international brand were shocking; as it showed the highest mean values for the total colony, mold, and yeast counts $76 \times 10^5 \pm 33 \times 10^5$; $97 \times 10^3 \pm 14 \times 10^3$ and $47 \times 10^4 \pm 7.0 \times 10^4$ CFU/g, respectively. While on the other side, samples from national brands showed better quality between brands with the lowest values for different examined organisms (Table 2).

These results are in match with those obtained from a survey of cakes by Sherif *et al.* (2018), where 50% of examined samples had aerobic microbial counts of $1–5 \times 10^6$ CFU/g, 16% were more than 10^6 CFU/g, 6.5% were more than 10^7 CFU/g.

Our obtained results were higher than those obtained by Visan and Bara (2010) who examined chocolate cake quality and found that TCC were varied between 6.8×10^3 and 1.4×10^4CFU/g. Also El-Fadaly *et al.* (2016) studied 30 cake samples with microbial quality and found that the contamination rate with mesophilic bacteria ranged between 1.2×10^2 and 28.6×10^4 CFU/g.

In comparison with our results, Sharifzadeh *et al.* (2016) examined 228 cream-based pastries and the microbial tests showed that 33.33% of all samples were contaminated by microbial agents with a high contamination rate with coliforms (61.84%), *Staphylococci* (48.68%), and yeast (27.63%).

Data presented in Tables 1 and 2 showed that regardless the brand; the general hygienic quality of cake "Tourta" product is better than gateau, where the cake showed lower average total colony, coliforms, yeast, and mold counts of $37 \times 10^5 \pm 18 \times 10^5$, $12 \times 10^2 \pm 0.4 \times 10^2$, $34 \times 10^3 \pm 9.0 \times 10^3$, and $38 \times 10^4 \pm 8.0 \times 10^4$ CFU or MPN/g, respectively. This fact may be attributed to several factors such as; its high price which provides the use of high-quality raw materials, low production rate (produced by piece), specific displaying refrigerator, and the dependence on specific handlers even during processing.

Table 3. Molecular identification and incidence of toxigenic genes in 5 *B. cereus* isolates isolated from the examined samples.

***B*acillus *cereus* isolate**	**Genes results**				
	groEL	***hbl***	***ces***	***cytK***	***nhe***
1	+ve	−ve	−ve	+ve	+ve
2	+ve	−ve	−ve	+ve	+ve
3	+ve	−ve	−ve	+ve	+ve
4	+ve	−ve	−ve	−ve	+ve
5	+ve	−ve	−ve	+ve	+ve
Total number of +ve isolates	5	0	0	4	5
Incidence %	100%	0%	0%	80%	100%

(−ve): absence of the gene; (+ve): presence of the gene.

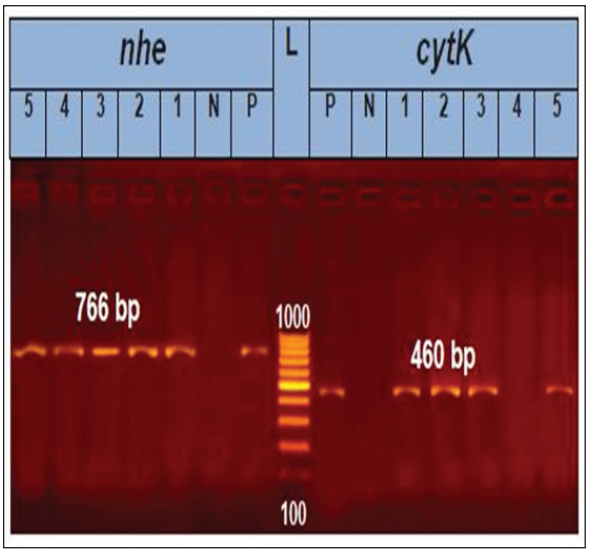

Fig. 2. Agarose gel electrophoresis of PCR products of B. cereus genes found in examined samples by direct multiplex PCR. nhe (766 bp) and cytK (460 bp), Lane (1–5) *B. cereus isolates,* and Lane (L) DNA ladder 100 bp molecular weight marker.

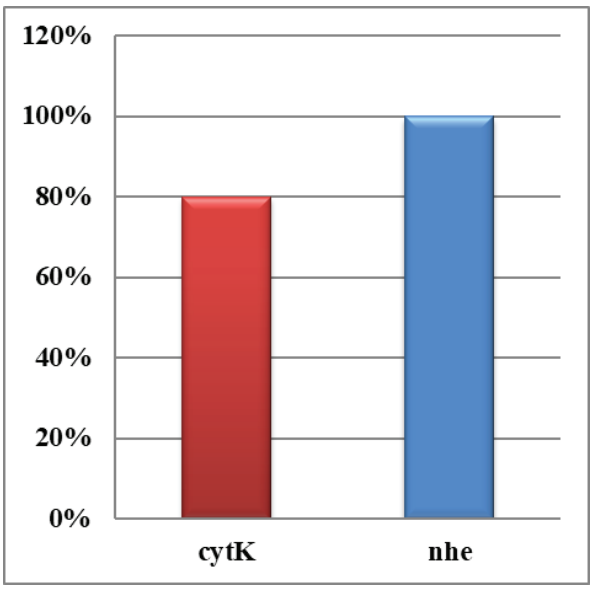

Fig. 3. Relationship between hemolytic activities of *B. cereus* isolates obtained from examined samples and the obtained toxigenic genes *(No. = 5;* the isolates harboring *gro EL* gene*)*. * All examined isolates showed strong hemolytic activity. * *hbl* and *ces* genes were absent in all the examined isolates.

Food poisoning from eating contaminated cakes has been sporadically reported (Wiwanitkit, 2015). The accidental contamination of food from unhygienic food handler is the main cause of Staphylococcal food intoxication, especially after the baking process, as the baking process has the ability to get rid such organism completely. *Staphylococci* overgrowth commonly occurs due to poor refrigeration preservation before eating (Pereira *et al.*, 1994; Anunciaçao *et al.*, 1995). Staphylococcal food poisoning can be caused by as little as 20–100 ng of enterotoxin (Asao *et al.*, 2003).

Staphylococcus aureus was isolated from 8% of all examined samples with the highest incidence (22.2%) and a mean count of ($38 \times 10 \pm 6.0 \times 10$ CFU/g) in local brand gateau samples. These samples could increase the probability of *S. aureus* food poisoning as it exceeds the guideline limit (20 – <100 CFU/g) reported by FSAI (2011). The rapid cooling of produced cake in refrigerator temperature <6°C is the main factor controlling the growth of *S. aureus* and the incidence of toxin production, as 7°C is the minimum temperature allowing growth of coagulase-positive *Staphylococci*, while the minimum temperature for staphylococcal enterotoxins production is 10°C (FSAI, 2011).

These results were in agreement with El-Fadaly *et al.* (2016) who isolated *S. aureus* from 9 samples only out of 30 samples with a count ranging between 3.7×10 and $>1 \times 10^3$ CFU/g, while higher figures were found by Sherif *et al.* (2018) in cake samples collected from Damietta and Dakahlia governorates as the *S. aureus* was presented in nearly 77% of the tested samples with the highest value of 35×10^2 CFU/g.

WHO (2007) stated that *B. cereus* is highly spreadable in pasteurized dairy products including cream. Foodborne diseases resulting from *B. cereus* are two types: diarrheal and emetic syndromes. The diarrheal type occurs when consuming food contaminated with 10^4 to 10^9 cells of *B. cereus* per gram of food, followed by enterotoxin production in the small intestine, compared with consuming food with toxin in the emetic form, which occurs when *B. cereus* reaches a population of 10^5–10^8 cells per gram of food (Logan and De Vos, 2009).

Bacillus cereus was extensively isolated from the examined samples from different brands with a higher incidence in "Tourta" samples (32%) than in gateau samples (26%). The mean count in all types of samples was higher than 10^3, and the highest mean values were reported for local brand gateau samples ($55 \times 10^2 \pm 18 \times 10^2$ CFU/g) and national brand Tourta samples ($44 \times 10^3 \pm 12 \times 10^3$ CFU/g) as shown in Tables 1 and 2. This count according to Logan and De Vos (2009) is risky with a high incidence of toxin production and food poisoning, so further molecular identification and incidence of toxigenic genes in the isolated strains were done for risk assessment. Similar figures were reported by El-Fadaly *et al.* (2016) and Sherif *et al.* (2018).

High microbial contamination level in examined samples indicates low-quality raw materials and poor production hygienic levels during cream-based cake processing. *Bacillus cereus* is a spore-forming microorganism which makes it easily spreadable in heat-treated, sterilized, pasteurized and processed

dairy-based ready to eat food products with high risk (Kotiranta *et al.*, 2000). The impact of *B. cereus* presence in food is not only on public health but also on the economic aspects through the final product shelf life reduction and spoilage via spoilage enzymes as: lipase, lecithinase, and protease, with subsequent losses (Wallaa, 2018).

By testing five of biochemically identified and confirmed *B. cereus* isolates that showed complete hemolytic activity using the PCR technique; the *groEL* gene was found in all of the examined isolates (100%) that confirm the incidence of *B. cereus* identified biochemically from different samples (Fig. 1). *Bacillus cereus* isolates have harbored more than one toxigenic gene; *nhe* gene was the most detected gene (100%), followed by *cytK* gene (80%), while *hbl* and *ces* genes could not be found in any of examined isolates (Figs. 2 and 3). Our results about *B. cereus* toxigenic genes incidence were similar to those recorded by Zhao *et al.* (2020) and Adam *et al.* (2021). *Bacillus cereus* with different counts and high toxigenic power is considered a hazard to consumer and increase the risk of cream-based cake products sold in Egyptian markets from national and local brands.

Although coliforms were detected in all examined samples (100%), it was found in low counts with the highest mean values of $33 \times 10^2 \pm 7.0 \times 10^2$ and $25 \times 10^2 \pm 6.0 \times 10^2$ MPN/g in local gateau and cake "Tourta" samples, respectively. The presence of Coliforms in different dairy desserts may be due to insufficient heat treatment, contaminated water, and contamination during serving, or poor sanitary practices of handlers (Shohana *et al.,* 2019). Lower results were reported by Sherif *et al.* (2018) as only 63% of the total examined cake samples were positive for Coliforms.

Salmonella sp. and *E. coli* could not be detected in all of the examined samples from different brands. Several cake studies couldn't also isolate *E. coli* from their samples (El-Fadaly *et al.*, 2016; Sherif *et al.*, 2018; Shohana *et al.*, 2019).

By comparing the obtained results with the Egyptian Standard specifications for cake (ES: 4037/2020), the highest acceptability degree was reported for the international brand gateau and "Tourta" samples (Fig. 4). The local brand gateau samples were the most unacceptable brand for several unsatisfactory parameters including mold presence and high incidence of *S. aureus* and *B. cereus*; on the other hand, for the cake samples, the national group samples showed the lowest acceptability level especially due to the presence of *B. cereus* organism with a high incidence of about 55% of the examined samples, which were unacceptable as matched with the Egyptian Standards. Higher unacceptable levels due to microbial contamination of cream-based cakes from Egyptian markets were reported by El-Fadaly *et al.* (2016) and Sherif *et al.* (2018), while lower levels of unacceptability were recorded by Hassan *et al.* (2018), in Iran.

In a study done by Abd El-Rady *et al.* (2016), dried milk powder was defined as the most potential hazard raw material for safe filling cream production. When HACCP system was applied to different production processes; whipping step was identified as the critical control point in this process. The study suggested variable control measures such heat treatment, natural preservatives application, with reduction of pH to guarantee safety of such product or even other food products containing filling cream as cakes.

Preventing the growth of microorganisms in the world of dairy confectionaries could be easily applied by using easy and cheap permitted chemical preservatives such aspropionic, sorbic, and citric acids or their salts with little concentrations ranging between 0.001% to 0.3% (Marin *et al.*, 2003). Although permitted; long-term consumption of some other chemical preservatives leads to several side reactions which can be either

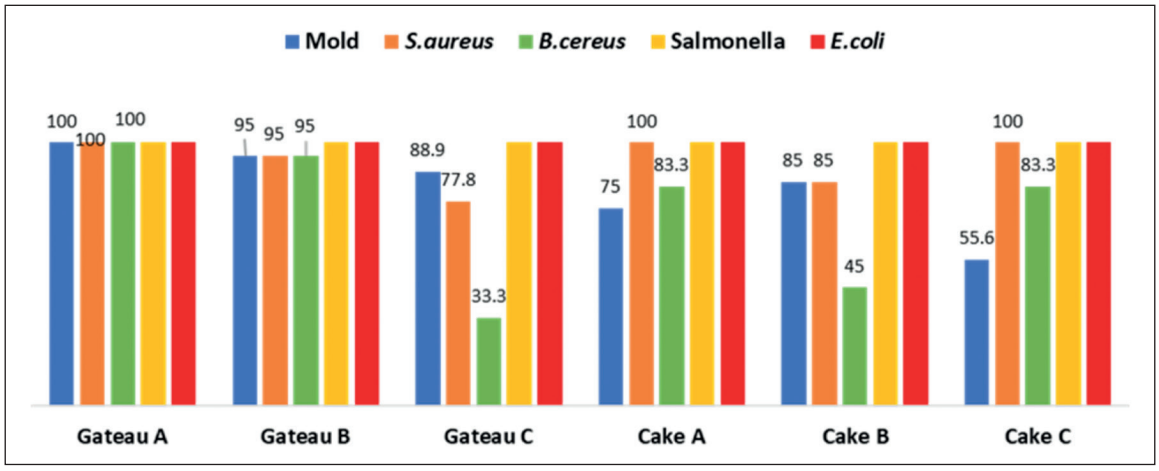

Fig. 4. Degree of acceptance of the examined cream-based cake "Tourta" and Gateau samples according to the Egyptian Standards (ES: 4037, 2020); it should be free from *E. coli*, *S. aureus*, *B. cereus*, *Salmonella* spp. and mold. (A): international brand; (B): national brand; (C): local brand.

immediate or build up in the body over time, such as; headaches, palpitations, allergies, and even cancer. Allergies are recently linked to benzoates consumption (Sanjay, 2015).

Therefore, producers and researchers had extensively searched for natural and safe replacers to improve the final cake product quality, prolong its shelf life and guarantee human safety, for example, the use of alcohol (Cauvain and Young, 2006), which is not accepted in Islamic countries**,** bacteriocins (Carla and Maria, 2009), ginger and turmeric (Muhammad *et al.*, 2014).

The persistence of cake products in markets for several days with constant quality indicates indeed the use of inhibitory substances during processing as a chemical preservative. Unfortunately, none of the examined samples in our study showed positive results using the modified *B. subtilis* microbial assay method, which may be attributed to lower sensitivity and specificity of this technique. Therefore, further studies are needed to find other more reliable, sensitive, and robust methods such as using chromatographic techniques; such as high-performance liquid chromatography; in order to detect or even determine the level of specific chemical substances in such products (Mahantesh *et al.*, 2019).

Conclusion

Our findings in this study have shown that cream-based dairy confectioneries, especially "Gateau" products from local shops, are considered a source of hazards as they can transmit pathogens and toxins to the consumer, especially the toxigenic strains of *B. cereus*. It is significant to adopt strict control measures during various stages of processing of such products starting from raw material selection until serving the final product to the consumer. The high incidence of pathogens with the ability of toxin production (*B. cereus* and *S. aureus*) points out the need for preventing post-baking contamination which requires careful attention to Good hygienic and manufacturing practices. The Egyptian standard specifications of cake ES: 4037/2020 do not include total colony and coliform counts in its parameters and it is recommended to be added. It is strongly recommended that all the principles of food hygiene be observed by the confectioneries producers in Egypt. Further studies are needed to access the most suitable method to determine the limit and detect the type of inhibitory substance/s added to such products.

Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

All authors contributed to the aim and design of work; Ashraf Moawad planned the study conception and design. Material preparation, data collection, and analysis were performed by Ayah Abdel-Salam and Esraa Owais. Statistical analyses were performed by Esraa Owais. The first draft of the manuscript was written by Ayah Abdel-Salam and Esraa Owais. All authors commented on previous versions of the manuscript. Then the final manuscript was revised by Ashraf Moawad. All authors read and approved the final version of the manuscript.

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Availability of data

All data supporting the findings of this study are available within the manuscript. Any extra data needed are available from the corresponding author upon reasonable request.

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