

Evaluation of microbial quality and safety of selected dairy products with special focus on toxigenic genes of *Bacillus cereus*

DOI: 10.15567/mljekarstvo.2021.0405

Adam A.H., Salwa A. Aly, Saad M.F.*

Cairo University, Faculty of Veterinary Medicine, Department of Food Hygiene and Control, Giza, Egypt

Received: 23.04.2021. / Accepted: 01.09.2021.

*Corresponding author: mena.saad@vet.cu.edu.eg

Abstract

Although dairy products play a significant role in human nutrition, they may cause a risk to the consumers. This study intends to determine the microbial contamination level in selected milk products concerning *Bacillus cereus* enterotoxigenic genes. Three hundred plant-origin flavoured cheese, flavoured yogurt, flavoured drinking yogurt, *kareish* (skim milk cheese), soft cheese, and Ras (Romy) cheese (50 samples for each) were randomly collected from retail markets at different districts in El Fayoum Governorate, Egypt. The microbiological analysis and the molecular identification of *Bacillus cereus* by multiplex PCR were applied and revealed potential risks associated with consumption of the tested milk products (white soft, Ras, and *kareish* cheeses). In all analysed cheeses (white soft, Ras and *kareish*) a high contamination level with coagulase positive staphylococci (20.0 %, 22.0 % and 20.0 %) and coliform bacteria (76.0, 86.0, and 88.0 %) was detected. The highest percent (78.0 %) of fungi was found in *kareish* cheese samples. *Bacillus licheniformis* was the most aerobic spore forming bacteria detected in *kareish* cheese (23.7 %) and flavoured drinking yogurt (17.0 %) samples. The highest prevalence (8.0%) of *Bacillus cereus* was recorded in the flavoured drinking yogurt and white soft cheese samples. The (*nhe*) was the most detected gene (100.0 %) in all of the examined samples. There was a significant difference ($p < 0.05$) between *nhe* and *ces* genes in all investigated samples except in flavoured drinking yogurt samples. *Salmonella sp.*, *Escherichia coli* and *Pseudomonas aeruginosa* were not detected. This survey is one of the very few surveys that describe the safety and quality status of plant-origin flavoured cheeses and flavoured drinking yogurt, particularly in Egypt.

Key words: *Bacillus cereus*; *E. coli*; CPS; dairy products; multiplex PCR; enterotoxigenic genes

Introduction

Milk products are vital components of the food articles as added-value products (Calcium-enriched) and dairy-based functional foods with added probiotics (Ozer and Kirmaci, 2010; Ibrahim et al., 2021). Around the world, yogurt consumption with different flavours increased due to its excellent acceptable taste, balanced nutrients, and therapeutic value. Drinking yogurt is an elevating interest's level due to its appropriateness, easily carried, and advantages to human health (Olugbuyiro, 2011; Bhattacharai and Das, 2016).

Worldwide, cheese is a popular product because of its health and flavour advantages. Besides, cheeses are satisfactory sources for dietary calcium, phosphorus, proteins, and decreasing the possibility of diabetes type 2 occurred. Based on the microbial content, the safety of food is represented with great attention to people, health authorities, and food manufacturers all over the world (Martínez-Ruiz et al., 2013; CDIC, 2014). Recently, the consumption of plant-origin analogous dairy products (cheese substitutes) has increased in Egypt, especially flavoured cheese, due to many benefits such as; lower cost-effectiveness and its simplicity of processing using cheaper vegetable products to use in the catering cheese sectors (Guinee, 2007).

Plant-origin cheese analogues are manufactured by blending various edible oils/fats, proteins, other ingredients, and water into a smooth homogeneous blend with the aid of heat, mechanical shear, and emulsifying salts. The proteolytic power of plant-origin coagulants as peppers and tomatoes are of higher specificity on α 1- and β -casein than the chymosin (Dublin, 2020; Sulejmani et al., 2021).

The pathogens occurrence in milk products is a significant concern in people's health in various world countries since dairy items can aid the food microbe growth. Food pathogens can contaminate the milk products from different sources and during manufacturing steps. *Salmonella* spp., *E. coli*, and Coagulase Positive Staphylococci (CPS) are the main pathogenic bacteria associated with food diseases due to milk items eating, and these are ubiquitous. Examinations for pathogens contamination are pertinent for the dairy industry because microbes' existence in the products causes economic damages due to non-agreement with legal specifications and negatively affecting public health (EFSA and ECDC, 2014; Choi et al., 2016).

Aerobic spore-forming bacteria include *Bacillus cereus* are a significant concern to the dairy industry, less for their pathogenicity but more for their spoilage-causing capabilities. Food safety and product quality are affected by different mechanisms; production of toxins, spoilage enzymes, and negatively affecting the milk products processing especially, cheeses and yogurt. A particular concern for these spore form bacteria, they withstand harsh environmental conditions (Postollec et al., 2012).

Based on the ubiquitous distribution of *Bacillus cereus*, it is virtually impossible to obtain products that are free

from *Bacillus cereus* spores (Griffiths and Schraft, 2017). However, high numbers in foods are responsible for disease occurring. Frequently this is associated with keeping foods under conditions permitting multiplication of *Bacillus cereus*, which can be through improper refrigeration, not enough cooling, or keeping foods warm at <60 °C. *Bacillus cereus* is responsible for 2 food poisoning models: the first, diarrheal syndrome and the second vomiting syndrome. The first syndrome occurs by heat-labile toxins in the small intestine like the enterotoxin (*nhe*) without haemolysis and cytotoxin K. The second syndrome has been produced by the toxin cereulide, which is heat resistant (Ehling-Schulz et al., 2015).

From 2010 to 2012, *Bacillus* outbreaks increased significantly (99 outbreaks in 2010; 220 outbreaks in 2011; 259 outbreaks in 2012) in Europe, also causing mastitis and meningitis (EFSA and ECDC, 2014; Nesma et al., 2020). *B. cereus* has emerged as the second foodborne pathogen after *S. aureus* and identified as a causative agent in 19 % of foodborne outbreaks was reported from 1998 to 2008 in the United States (Bennet et al., 2013; Glasset et al., 2016). WHO (2007) stated that *B. cereus* becomes a high spread in pasteurized dairy items.

Therefore, our study aimed to evaluate the microbial contamination levels of the selected milk products and investigate *B. cereus* virulence genes using PCR technique plus focusing on plant-origin flavored cheeses and flavored drinking yogurt, which have limited data around the world, especially in Egypt.

Materials and methods

Sample collection

A total of three hundred random samples of plant-origin flavoured (pepper and Tomato) cheese, flavoured yogurt (strawberry and banana), flavoured drinking yogurt (banana and strawberry), kareish, white soft, and Ras cheeses (representing 50 samples for each) obtained from retail markets at different districts in El Fayoum Governorate from November 2019 to January 2021. The quantity of each cheese type was 250 g, while yogurt and drinking yogurt samples, each package contained about 105 g and 440 mL, respectively. Samples were transported under completely aseptic conditions in a cool isolated icebox as soon as possible to the laboratory.

Microbiological examination

Preparation of dairy products homogenate and decimal dilutions

11 g of sample and sterile 99 mL of 2 % sodium citrate (Sigma-Aldrich) solution (cheese samples) or 99 mL of 0.1 % peptone (Himedia, RM001) water (yogurt samples) were mixed in a polyethylene bag and then placed into a Lab-Blender/400-Seward (Worthing UK) to obtain 1/10

dilution. 1 mL of the previously prepared homogenate and 9 mL of 0.1 % peptone water have mixed to prepare tenth fold decimal dilutions (APHA, 2015).

Enumeration of microorganisms in the examined samples

Coliforms count using three tubes (MPN/g) method based on (ISO, 4831: 2006). Count for Total Staphylococci and Coagulase Positive Staphylococci was done according to (BAM, 2016). Total yeasts and molds count was done based on (ISO, 21527-1: 2008).

Aerobic Spore Formers (ASF) was count according to (APHA, 2015). The homogenized samples were exposed to the shock of heat in a water bath at 80 ± 1 °C for 12 min. After heat treatment, the samples were cooled immediately in an ice bath. Then the samples were inoculated in a plate count (Himedia, M091A) agar supplemented with 0.1 % soluble starch, using the pour plate technique. The plates were incubated at 32 ± 1 °C for 48 h. The count of aerobic spore formers/g was calculated. Mannitol Egg Yolk-Polymyxin (MYP) agar (Himedia, M636F) plates were surface plated by spreading 0.1 mL of appropriate previously prepared serial dilutions and then incubated inverted at 32 °C for 24 h. In low count samples, 1 mL of the first dilution was distributed and spread on four plates of (MYP): 0.3 mL (3 plates) and 0.1 mL. *B. cereus* count was calculated after complete confirmation based on Amore *et al.* (2018).

Isolation and identification of the selected microorganisms in the examined samples

Isolation of *E. coli* was performed according to (SO 7251: 2005, *Salmonella* spp. according to ISO 6579-1: 2017, and *Pseudomonas aeruginosa* by *Pseudomonas* agar base (Himedia, M085) plus CetriNex supplement (FD029, Himedia) according to ISO 11059:2017).

For all isolates of ASF: ancillary biochemical tests (using Gram stain, Voges-Proskauer, dextrose fermentation anaerobically, reduction using nitrate broth, hydrolysis of tyrosine, lysozyme resistance, rhizoid growth, stained Crystal Protein, staining motility, haemolytic activity, growth at fifty degrees C, proteolytic activity on milk agar (Oxoid CM0021) and polysaccharide starch hydrolysis) were applied according to Bennett *et al.* (2015). Matrix-assisted laser desorption ionization-time off light mass spectrometry (MALDI-TOF MS) based on Yan *et al.* (2020) was done to confirm the examined isolates identification. Suspected isolates of *Salmonella* spp., *E. coli*, and *S. aureus* were identified according to De Vos *et al.* (2009).

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

Deoxyribonucleic acid from the identified *B. cereus* culture was extracted using a Gene JET purification kit (Thermo Fisher, K0721). DNA found in the supernatant is stored at -20 °C. *GyrB* gene detection was performed by multiplex PCR using the primer pair BC1F/BC2rR at 365bp, (ATT GGT

GAC ACC GAT CAA ACA: TCA TAC GTA TGG ATG TTA TTC), recognized as *B. cereus* using *B. cereus* positive control (ATCC® 10876™), and tested for enterotoxigenic genes (*bceT*, *nhe*, *hbl*, *cytK*, and *ces*).

The sequences (F:R) of primers were (TTACATTAC-CAGGACGTGCTT: TGTTTGTGATTGTAATTCAGG;AAG-CIGCTCTTCGIATTC:ITIGTTGAAATAA GCTGTGG;GTA-AATTAIGATGAICAATTC:AGAATAGGCATTCATAGATT; A CAGATATCGGICAAAATGC:CAAGTIACTTGACCIGTTGC; GGT-GACACATT ATCATATAAGGTG:GTAAGCGAACCTGTCTGTAA-CAACA) for *bceT*, *nhe*, *hbl*, *cytK* and *ces* genes at 428, 766, 1091, 421, 1271 bp respectively. The amplification cycles were achieved by a PT -100 Thermo-cycler (MJ Research, USA). PCR were done 2 times/isolate and visualization of products was achieved in 1.5 % (Tris Borate EDTA) agarose gels through UV. The molecular identification steps and sequences of primers used were according to Choma and Granum (2002); Ehling-Schluz *et al.*, (2006) and Park *et al.* (2020).

Statistical analysis

Chi-Square independence test and one-way ANOVA using Excel of Microsoft 365 enterprise was done to analyse the results of different parameters.

Results

Statistical analytical results of microbiological parameters of the examined dairy products

The highest mean counts for aerobic spore formers, *B. cereus*, Total Staphylococci, Coagulase positive Staphylococci, Coliforms (MPN/g) and Total yeast and mold were $1.9 \times 10^5 \pm 1.4 \times 10^6$, $1 \times 10^5 \pm 9.9 \times 10^4$, $2.6 \times 10^7 \pm 1.3 \times 10^7$, $6.6 \times 10^5 \pm 1.3 \times 10^4$, $4.1 \times 10^4 \pm 2.3 \times 10^4$ and $1.7 \times 10^6 \pm 6.4 \times 10^5$ CFU/g respectively in the white soft and kareish cheeses samples. The highest prevalence of ASF (88.0 %) was in the flavoured drinking yogurt samples, Coagulase Positive Staphylococci (22.0 %) in the Romy cheese, while the highest Coliforms prevalence (88.0%) and fungi (78.0 %) were in the kareish samples (Tables 1 and 2). Based on statistical results of flavoured products, there was a significant difference ($p < 0.05$) between all of them in aerobic spore formers count, while there was a significant difference ($p < 0.05$) between plant-origin flavoured cheese and flavoured drinking yogurt samples for the *B. cereus* and total Staphylococci counts. In unflavoured products, there was a significant difference ($p < 0.05$) between all of them for Coagulase +ve Staphylococci count, also there was a significant difference ($p < 0.05$) between kareish and soft cheeses and kareish and Ras cheeses samples for total yeast and mold count only.

Prevalence of Aerobic Spore Formers members in the examined samples

B. licheniformis was the most ASF in the investigated kareish and flavoured drinking yogurt samples in a percentage of 23.7 and 17.0 respectively, while *B. subtilis* was the most ASF in the surveyed Romy cheese (20.9 %) and plant-origin flavoured cheese (50.0 %). *B. pumilus* is the most species were detected in flavored yogurt samples with a percentage of 18.9 (Fig. 1).

Prevalence of toxigenic genes of *B. cereus* and its haemolytic activity in the examined dairy products

By testing suspected *B. cereus* isolates by PCR technique; the *gyrB* gene was found in 70.4 % that indicates *B. cereus* confirmation. The *nhe* was the most

detected gene in all (100.0 %) the examined isolates of *B. cereus*, followed by *cytK* (52.6 %) and *ces* (5.3 %) genes. The *hbl* and *bceT* genes could not be found in all the examined isolates (Fig. 2, 3, 4, 5). Based on statistical results of *nhe*, *cytK*, and *ces* genes, there was a significant difference ($p < 0.05$) between *nhe* and *ces* genes in all examined samples except in flavoured drinking yogurt samples.

By examination of *B. cereus* isolates for haemolytic activity, all isolates (100.0 %) were strong haemolytic (Fig. 6). More precisely, all strains (100.0 %) of *B. cereus* that carried *nhe* were able to show strong hemolysis.

Figure (7) is focused on the degree of acceptance of the examined milk products with the Egyptian Standards (ES). All the samples were complying with ES based on *E. coli* and *Salmonella* spp. results. According to other parameters; the degree of complying varied for each limit established by ES.

Table 1. Microbiological analysis of the examined flavored dairy products (CFU/g) (n=150)

Parameter	Plant-origin flavoured cheese (n= 50)					Flavoured yogurt (n= 50)					Flavoured drinking yogurt (n= 50)				
	No	%	Min	Max	Mean± S.E.M.	No	%	Min	Max	Mean± S.E.M.	No	%	Min	Max	Mean± S.E.M.
Aerobic spore formers	18	36	<10	2.2×10 ⁴	3.4×10 ^{2a} ±0.06×10 ²	33	66	<10	3×10 ⁵	1.3×10 ^{4b} ±6.6×10 ³	44	88	<10	1.2×10 ⁶	9.4×10 ^{4c} ±3.6×10 ⁴
<i>B. cereus</i>	2	4	<10	10 ²	3.7×10 ^{3a} ± 0.07×10	3	6	<10	2×10 ⁴	5.2×10 ^{2a} ±5.1×10 ²	4	8	<10	4×10 ⁵	8.0×10 ^{3b} ±1.6×10 ²
Total Staphylococci	15	30	<10	2.3×10 ⁵	5.6×10 ^{3a} ±1.12×10 ²	29	58	<10	1.1×10 ⁶	3.7×10 ^{4a} ±2.9×10 ⁴	21	42	<10	4.1×10 ⁷	8.2×10 ^{5b} ±1.2×10 ⁵
Coagulase +ve Staphylococci	0	0	<10 ^a			9	18	<10	3.5×10 ⁵	2.7×10 ^{3b} ±5.4×10	0	0	<10 ^a		
Coliforms (MPN/ g)	0	0	<3 ^a			32	64	<3	2.1×10 ⁴	8.5×10 ^{2a} ±5.9×10 ²	16	32	<3	4.3×10 ⁴	8.6×10 ^{2a} ±8.1×10 ²
Total yeast and mold	12	24	<10	2.7×10 ⁵	5.9×10 ^{3a} ±1.18×10 ²	15	30	<10	1.8×10 ⁵	7.4×10 ^{3a} ±4.9×10 ³	27	54	<10	1×10 ⁵	1×10 ^{4a} ±3.3×10 ³

N = total number of the examined samples; Min: Minimum; Max: Maximum; No.: number of positive samples

Different superscript letters in the same row indicate significance difference ($p < 0.05$).

Same superscript letters in the same row indicate non significance difference ($p > 0.05$).

Table 2. Microbiological analysis of the examined unflavoured dairy samples (CFU/g) (n=150)

Parameter	Kareish cheese (n= 50)					White soft cheese (n= 50)					Ras cheese (n= 50)				
	No	%	Min	Max	Mean± S.E.M.	No	%	Min	Max	Mean± S.E.M.	No	%	Min	Max	Mean± S.E.M.
Aerobic spore formers	38	76	<10	1.2×10 ⁶	1.2×10 ^{5a} ±3.9×10 ⁴	28	56	<10	7×10 ⁷	1.9×10 ^{6a} ±1.4×10 ⁶	43	86	<10	4.5×10 ⁶	2.1×10 ^{5a} ±1.0×10 ⁵
<i>B. cereus</i>	3	6	<10	7×10 ⁴	2.5×10 ^{3a} ±1.7×10 ³	4	8	<10	5×10 ⁶	1×10 ^{5a} ±9.9×10 ⁴	3	6	<10	5.0×10 ³	1.1×10 ^{2a} ±0.2×10
Total Staphylococci	50	100	8.4×10 ³	7×10 ⁹	2.6×10 ^{7a} ±1.3×10 ⁷	48	96	<10	2.4×10 ⁷	1.4×10 ^{6a} ±5.5×10 ⁵	50	100	2.3×10 ³	2.1×10 ⁷	2.1×10 ^{6a} ±5.9×10 ⁵
Coagulase Positive Staphylococci	10	20	1.6×10 ³	1.4×10 ⁸	6.6×10 ^{5a} ±1.3×10 ⁴	10	20	<10	4.9×10 ⁶	4.3×10 ^{4b} ±8.6×10 ²	11	22	5.0×10 ²	4.6×10 ⁶	4.6×10 ^{5c} ±9.2×10 ³
Coliforms (MPN/g)	44	88	<3	1.1×10 ⁶	4.1×10 ^{4a} ±2.3×10 ⁴	38	76	<3	4.6×10 ⁴	1.4×10 ^{3a} ±9.3×10 ²	43	86	<3	1.1×10 ⁵	3.1×10 ^{3a} ±2.2×10 ³
Total yeast and mold	39	78	<10	2.1×10 ⁷	1.7×10 ^{6a} ±6.4×10 ⁵	18	36	<10	1.7×10 ⁶	5.7×10 ^{4b} ±3.6×10 ⁴	18	36	<10	1.9×10 ⁶	8.8×10 ^{4b} ±4.7×10 ⁴

N = total number of the examined samples; Min: Minimum; Max: Maximum; No.: number of positive samples

Different superscript letters in the same row indicate significance difference ($p < 0.05$)

Same superscript letters in the same row indicate non significance difference ($p > 0.05$)

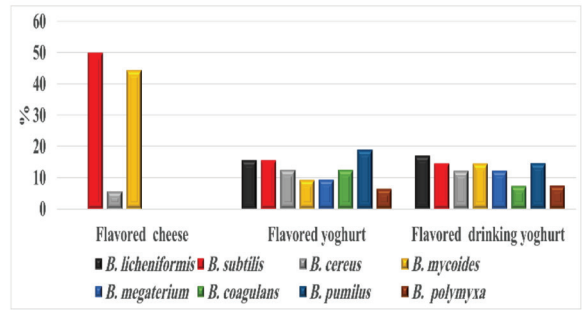
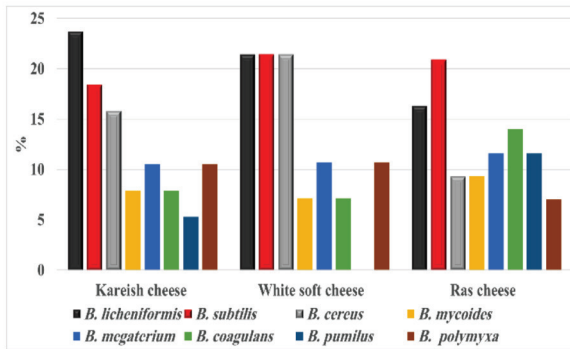


Figure 1. Percentages of identified ASF members obtained from the examined dairy products

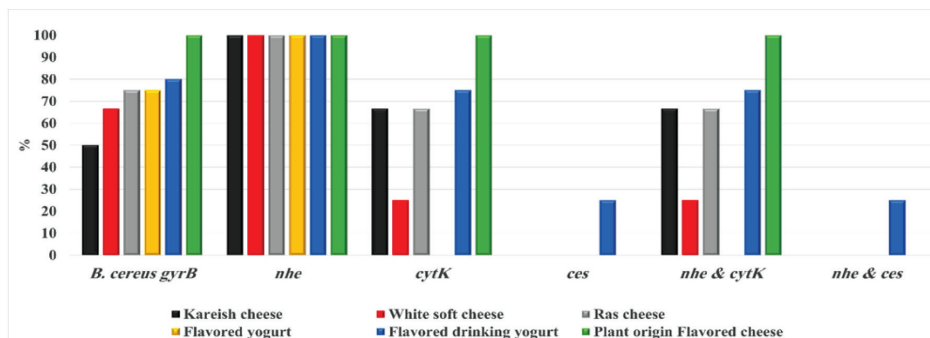


Figure 2. Prevalence of toxigenic *B. cereus* isolates from the examined dairy products samples *hbl* and *bceT* genes absence in all the examined isolates

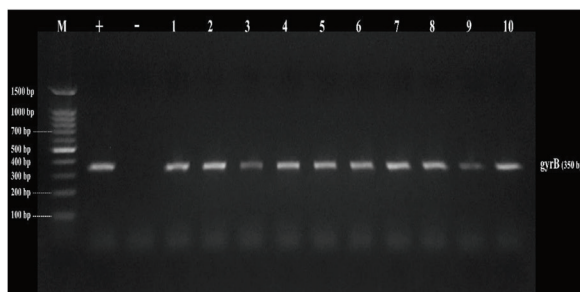


Figure 3. Amplicons of *B. cereus* isolates on agarose gel using *gyrB* (350bp). Lane (1-10) *B. cereus* isolates, and Lane (M) DNA ladder 100 bp molecular weight marker

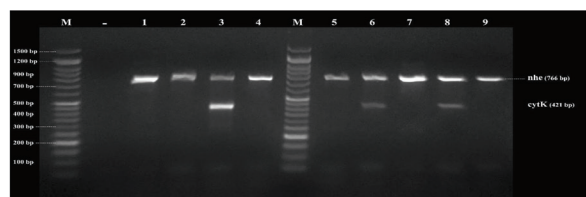


Figure 4. Agarose gel electrophoresis of PCR products of *B. cereus* genes found in examined samples by direct multiplex PCR. *nhe* (766bp) and *cytK* (421bp), Lane (1-9) *B. cereus* isolates, and Lane (M) DNA ladder 100 bp molecular weight marker

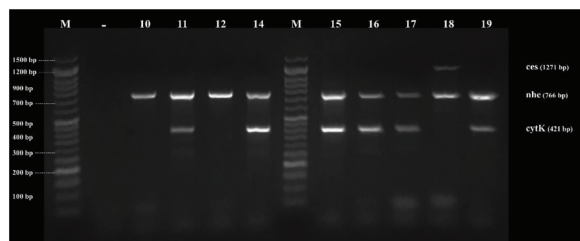


Figure 5. Agarose gel electrophoresis of PCR products of *B. cereus* genes found in examined samples by direct multiplex PCR. *ces* (1271bp) *nhe* (766bp) and *cytK* (421bp), Lane (10-19) *B. cereus* isolates, and Lane (M) DNA ladder 100 bp molecular weight marker.

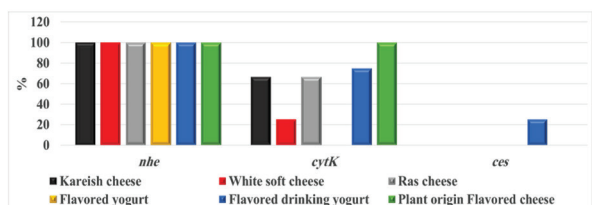


Figure 6. Relationship between haemolytic activities of *B. cereus* isolates obtained from different dairy products and the obtained toxigenic genes (No. =19).

All examined isolates were non weak haemolytic activity
No.: Number of the isolates harbouring *gyrB* gene

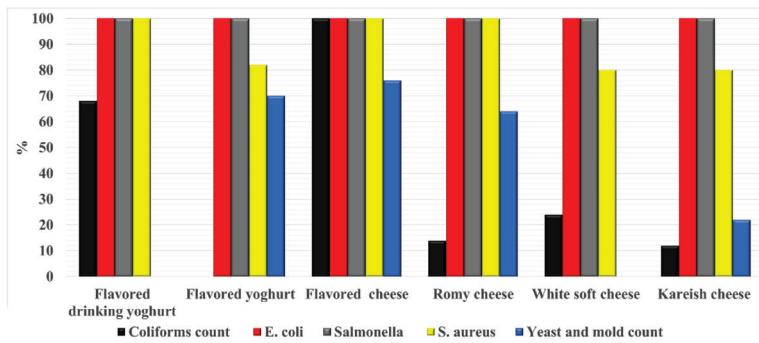


Figure 7. Degree of acceptance of each the examined product type according to the Egyptian standards (ES).

Drinking yogurt: (ES: 8042/2016): free from Coliforms, *E. coli*, *S. aureus*, *Salmonella* spp.

Flavored yogurt (ES: 8042/2016): free from *E. coli*, *S. aureus*, *B. cereus*, *Salmonella* spp., molds < 10 cells/g.

Flavored cheese of plant-origin: (ES: 1867/2005): Coliforms < 10 cells/g, *E. coli*, *S. aureus*, *Salmonella* spp., molds < 10 cells/g, Yeast < 400 cells/g.

Romy cheese (ES: 1007/4/2020): Coliforms < 10 cells/g, *E. coli*, *S. aureus*, *Salmonella* spp., molds < 10 cells/g, Yeast < 100 cells/g.

Soft cheese (ES: 8390/2020): Coliforms < 10 cells/g, *E. coli*, *S. aureus*, *Salmonella* spp., molds < 10 cells/g, Yeast < 400 cells/g.

Kareish cheese (ES: 1008/4/2005): Coliforms < 10 cells/g, *E. coli*, *S. aureus*, *Salmonella* spp., molds < 10 cells/g, Yeast < 400 cells/g.

The missing columns indicate: not mentioned in ES.

Discussion

The hygienic quality of cheeses is critical to prevent their microbial contamination due to their high protein content, and children with weak immunity eat these types of cheeses (Youssif et al., 2020). High counts of coliform bacteria in the investigated cheeses indicate contamination (direct and/or indirect), and reflect a bad hygiene that leads to an unsafe final product. The lower count of coliforms in surveyed plant-origin flavoured cheese may be due to bacteria of 2-Hydroxypropanoic Acid (LAB) which grow in unheated milk or cheese that makes the antagonism effect to undesirable microorganisms (Martínez-Ruiz et al., 2013). Coliforms were in 42.0 % (lower than our results) of the examined yogurt samples (Adebisi et al., 2017).

Heikal et al. (2014) found those coliforms and staphylococci 2.5×10^4 (higher than our results) and 2.3×10^4 (lower than our results) CFU/g respectively in the white soft cheeses, while *Staphylococcus aureus* was detected in 6.7 % of the examined cheese samples (lower than our results). A lower count for *S. aureus* was reported by Worku et al. (2015) in the surveyed flavoured yogurt samples. Ibrahim et al. (2015) found that *S. aureus* in a higher percentage (66.0 %) than our results in the kareish cheese samples with a mean of 1.9×10^5 CFU/g, also the mean of coliforms was 3.9×10^3 cells/g (similar to our obtained results).

The reduced power of hydrogen ion value of yogurt and flavoured drinking yogurt, participate in the multiplication reduction of organisms in samples with a low count of staphylococci (<10 CFU/g) and coliforms (<3 MPN/g) (Kailasapathy, 2006). Metwalli (2011) found mean counts of coliforms 1.5×10^5 CFU/g, which was lower than our results. *S. aureus* was detected in all kareish cheese samples with a mean of 0.2×10^6 CFU/g, which was similar to our results. The attributing reasons for the low enumeration of CPS (<10 CFU/g) was a decreasing of power of hydrogen ion of the examined drinking yogurt samples and the bacteria of 2-Hydroxypropanoic acid (Le Marc et al., 2009). Rahimzadeh et al. (2020) could not

detect the *S. aureus* in the yogurt samples, and this result was similar to our results.

The high enumeration of molds and yeasts in the surveyed cheeses and flavoured products may be attributed to improper hygienic measures in processing and/or adding poor quality flavoured substances as an origin of fungal pollution (Graciela et al., 2001). So, the manufacturers shall be careful when purchasing these substances. Metwalli (2011) found that fungi in all surveyed kareish cheese samples with a mean of 4×10^3 CFU/g, which was lower than our results. Heikal et al. (2014) found that fungi were present in 100.0 % of white soft cheese samples, with a mean of 1.9×10^3 CFU/g, which was lower than our results. Ibrahim et al. (2015) could detect fungi in all investigated kareish cheese samples with a mean count of 3.1×10^5 CFU/g. A nearly similar count of fungi ($\geq 10^2$ CFU/g) was reported by Worku et al. (2015) in the surveyed flavoured yogurt samples. Adebisi et al. (2017) found that fungi in 75.0 % of the examined yogurt samples, which was higher than our results.

B. licheniformis spores cause milk products spoilage and have a bad effect on their sensory and technological characteristics (Dhakal et al., 2014). *B. pumilus*, and *B. subtilis* are often isolated from animal surroundings and have been recorded to produce proteases and enterotoxin substances which damaging public health (Yoo et al., 2014). In yogurt samples, a decreasing value for the power of hydrogen ion participates in the multiplication reduction of ASF in low count samples (<10 CFU/g) (Kailasapathy, 2006).

Spores of ASF found in raw milk can resist pasteurization temperature and ultimately become incorporated into final products (Coorevits et al., 2008). Our results of ASF in flavoured drinking yogurt were mainly lower than the results obtained by Aly and Galal (2016). While the obtained results from Ras cheese samples were higher than those obtained by Khater and Abdella, (2017) and Nassib et al. (2018), white soft cheese results were higher than those obtained by Khater and Abdella (2017) and Nazem et al. (2020). *Bacillus cereus* was not detected in any cheese sample examined by Heikal et al. (2014) as well as in the study by Ibrahim et al. (2015).

The *bceT* gene of *B. cereus* does not cause diarrhoea and was not confirmed as a food-borne agent (Choma and Granum, 2002). *Bacillus cereus* strains have harboured more than one toxin. A total of 52.6 % of isolates have expressed 2 genes (*nhe* and *cytK*), while 5.3 % of isolates harboured 2 genes (*nhe* and *ces*). Our findings were almost like those of Zhao et al. (2020), who concluded that 94.4 % of *B. cereus* harboured *nhe* gene, while *cytK* gene was present in a higher percent (75.9 %) than our findings. Yibar et al. (2017) recorded that 42.3 % of *B. cereus* isolates from the examined different cheeses types harboured *nhe* gene. There was a significant difference ($p < 0.05$) between *nhe* and *ces* genes in all investigated samples except in flavoured drinking yogurt, which indicates a high association of these genes with these products.

The *B. cereus* prevalence in yogurt was detected in 3 (6.0 %) out of 50 examined samples. This result was lower than those recorded by Ayoub et al. (2003), who found that 20.0 % of examined yogurts was polluted with *B. cereus*, while Abdel-Khalek (2002) reported no contamination for yogurt. The lower prevalence of *B. cereus* in yogurt may be attributed to the restrained action of LAB and bacteriocins, organic acids, and hydrogen peroxide production. Badly, a lower count ($10^3/g$) may cause health problems in immunosuppressive people (Granum, 2005). EFSA (2016) reported that more than 10^4 *B. cereus* colony-forming unit/g leads to diarrheal toxins secretion in the gastrointestinal tract of people.

B. cereus multiplication is prevented under the power of hydrogen ion 4.5, as yogurt is not related to *B. cereus* food poisoning on condition storage below 4 °C. The investigated dairy products contain *B. cereus* with different enumeration, and most of the strains presented toxigenic power, so *B. cereus* may be considered as a hazard to people. However, the financial damages of *B. cereus* toxins shall not be ignored in the dairy industry. A famous technique of preservation to control *B. cereus* growth is acidification (Rosslund et al., 2003). The suggested reason for the presence of *B. cereus* in the investigated dairy products especially in the surveyed white soft cheese and flavoured drinking yogurt is the use of raw milk contaminated with spores which are resistant to pasteurization. The origin of these spore is most probably the soil present in due to bad environmental milking practice (Gopal et al., 2015).

The use of raw materials with reduced spore enumeration is a highly preventive way for poisoning by *B. cereus*. Spores may transmit to multiple milk products by un-heat-treated milk and spores attached by biofilms to dairy utensils (Caro-Astorga et al., 2020). The lethal poisoning, cellular haemolytic, necrotic effects, hepatic damage, and sugar disorders cause by cytotoxic gene (Vangoitsenhoven et al., 2014). The *nhe* toxin has cytotoxic characteristics, while *cytK* toxin is the reason for bloody diarrhea (Jessberger et al., 2020).

Based on the data in (Fig.6), this proved the complete association between harboring toxic gene (*nhe*) and exhibition of virulence feature (strong hemolysis). The beta-hemolytic *B. cereus* is the cause of food poisoning. More than 25.0 % of beta-hemolytic *B. cereus* isolates

were not produced emetic toxin (Jessberger et al., 2020). Counting of *B. cereus* and examination for its toxic genes are essential for the microbiological quality and safety of these dairy products. It is the main threat in the food industry both by deteriorating the products and by endangering people's life upon consumption. Applying excellent manufacturing principles and the HACCP plan are essential for elevating safe products (Hafiz et al., 2016; Ibrahim et al., 2020; Youssif et al., 2021).

E. coli, *Salmonella* spp., and *P. aeruginosa* could not be found in any of the investigated milk products. The *E. coli* absence in the investigated flavoured soft cheeses was attributed to inhibitive effect of the starter cultures, competition for nutrients actions and areas of colonization, as well as vacuum packaging that reduces the oxygen concentration (Ganesan et al., 2012).

None detected *Salmonella* and *E. coli* was reported by Worku et al. (2015) in the surveyed flavoured yogurt samples. Rahimzadeh et al. (2020) could not detect *E. coli* in the examined yogurt samples, similar to our results. El-Ziney (2018) failed to detect *Salmonella* in the tested hard cheeses due to the inability of *Salmonella* to survive at low water activity. Metwalli (2011) reported that *Salmonella* spp. was not present in all samples. The most suggested causes of *Pseudomonas aeruginosa* absence is the non-formation of biofilms in milk tanks as well as their removal through heat treatment (pasteurization or UHT) (Simoes et al., 2009).

The reasons for unacceptable samples (Fig. 7) may be due to microbe species in un-heat-treated milk, degree of proper manufacturing, the degree of hygiene applied in dairy processing units, and milk handling practice during milk processing (Heikal et al., 2014). Finally, the ES needs to be more restricted and established the limit of parameters, which are not mentioned to ensure safe dairy product consumption.

Conclusions

Significant attention has been drawn to the use of flavoured yogurt, kareish, soft, and Romy cheeses with different pathogens types. Since it is of vital concern, it is recommended to adopt strict control measures during various stages of production and processing of such products. Finally, proper consumer handling and storage practices have affecting tools to control pathogens and protect milk products from deterioration.

Ispitivanje mikrobiološke kvalitete i sigurnosti odabranih mliječnih proizvoda s posebnim naglaskom na toksigene gene *Bacillus cereus*

Sažetak

Iako mliječni proizvodi igraju značajnu ulogu u prehrani ljudi, mogu predstavljati rizik za potrošače. Cilj ovog istraživanja bio je utvrditi razinu mikrobiološke kontaminacije u odabranim mliječnim proizvodima, prije svega prisutnost enterotoksigenih gena *Bacillus cereus*. Tristo sireva obogaćenih dodacima biljnog podrijetla, aromatiziranih jogurta, aromatiziranih jogurta za piće, kareish (sir od obranog mlijeka), mekani sir i sir Ras (Romy) (od svake vrste 50 uzoraka) nasumce je sakupljeno s maloprodajnih tržnica u različitim okruzima u gubernorati El Fayoum, Egipat. Mikrobiološka analiza i molekularna identifikacija *Bacillus cereus* multiplex PCR-om otkrila je potencijalni rizik povezan s konzumacijom ovih mliječnih proizvoda (bijeli mekani, Ras i kareish sirevi). U svim analiziranim uzorcima sira (meki sir, Ras i kareish) utvrđena je visoka razina kontaminacije koagulaza pozitivnim stafilokokima (20,0, 22,0 i 20,0 %) i koliformnim bakterijama (76,0, 86,0 i 88,0 %). Najveći postotak (78,0 %) plijesni utvrđen je u uzorcima sira kareish. *Bacillus licheniformis* najjaerobniji je tvorilac spora iz kareish sira (23,7 %) i aromatiziranog jogurta (17,0 %). Najveći broj (8,0 %) *Bacillus cereus* zabilježen je u uzorcima aromatiziranog jogurta i mekog sira. U svim ispitivanim uzorcima najzastupljeniji gen bio je gen (*nhe*) (100,0 %). U svim ispitivanim uzorcima postojala je značajna razlika ($p < 0,05$) između gena *nhe* i *ces*, osim u uzorcima aromatiziranog jogurta. Niti u jednom uzorku nisu detektirani *Salmonella sp.*, *Escherichia coli* i *Pseudomonas aeruginosa*. Ovo je jedno od rijetkih istraživanja koje opisuje sigurnost i kvalitetu sireva obogaćenih dodacima biljnog podrijetla i aromatiziranih jogurta, posebno u Egipat.

Ključne riječi: *Bacillus cereus*; *E. coli*; CPS; mliječni proizvodi; multipleks PCR; enterotoksigeni geni

References

1. Abdel-Khalek A. (2002): Incidence and characterization of enterotoxigenic *Bacillus cereus* in some dairy products. *SCVMJ* 5 (1), 1-10.
2. Adebisi, O.O., Oyetayo, V.O., Ajayi, K.O. (2017): Microbial quality and sensory assessment of yogurt marketed in Akure Metropolis, Nigeria. *International Journal of Scientific and Research Publications* 7 (11), 704-710. <http://www.ijrsp.org/research-paper-1117.php?rp=P716991>
3. Aly, S.A., Galal, E.A. (2016): Microbiological quality aspect associated with plain and flavored drinking yogurt. *International Journal of Perceptions in Public Health* 1 (1), 66-80.
4. Amore, M.G., Siala, M., Zayani, M., Grosseet, N., Smaoui, S., Messadi-Akrout, F., Baron, F., Jan, S., Gautier, M., Gdoura, R. (2018): Isolation, identification, prevalence, and genetic diversity of *Bacillus cereus* group bacteria from different foodstuffs in Tunisia. *Front Microbiol* 12, 9-447. <https://dx.doi.org/10.3389/fmicb.2018.00447>
5. APHA (2015): American Public Health Association. *Compendium of methods for the microbiological examination of foods*, 5th Ed., Washington, DC., USA.
6. Ayoub, M.A., El-Shayeb, T.M., Zaki, M.S.A. (2003): Characterization of *Bacillus cereus* isolated from raw milk and some dairy products. *SCVMJ* 6, 123-133.
7. BAM (2016): *Bacteriological Analytical Manual Online*. Chapter 12, *Staphylococcus aureus*. U.S. Food & Drug Administration Center for Food Safety & Applied Nutrition.
8. Bennet, S.D., Walsh, K.A., Gould, L.H. (2013): Foodborne disease outbreaks caused by *Bacillus cereus*, *Clostridium perfringens*, and *Staphylococcus aureus* -United States, 1998-2008. *Clin Infect Dis* 5, (3) 425-33. <https://doi.org/10.1093/cid/cit244>
9. Bennett, R.W., Tallent, S.M., Hait, J.M. (2015): *Bacillus cereus*. In: Salfinger, Y., Tortorello, M.L., (eds) *Compendium of Methods for the Microbiological Examination of Foods*. 5th edition. American Public Health Association, Washington, DC, USA. Chapter 31, 375-390.

10. Bhattarai, R.R., Das, S.K.L. (2016): Evaluation of microbiological quality of indigenous Dahi from Eastern Nepal. *Sunsari Technical College Journal* 2 (1), 23-26.
<https://doi.org/10.3126/stcj.v2i1.14793>
11. Caro-Astorga, J., Frenzel, E., James, R. Perkins, Alvarez-Mena, A., De Vicente, A., Ranea, J.A.G., Kuipers, O.P., Romero, D. (2020): Biofilm formation displays intrinsic offensive and defensive features of *Bacillus cereus*. *Biofilms and Microbiomes* 6 (3), 1-15.
<https://doi.org/10.1038/s41522-019-0112-7>
12. CDIC (2014): Canadian Dairy Information Centre Global consumption per capita of dairy products: Total cheese consumption.
13. Choi, K.H., Lee, H., Lee, S., Kim, S., Yoon, Y. (2016): Cheese microbial risk assessments - A review. *Asian-Australasian Journal of Animal Sciences* 29, 307-314.
<https://doi.org/10.5713/ajas.15.0332>
14. Choma, C., Granum, P.E. (2002): The enterotoxin T (BcET) from *Bacillus cereus* can probably not contribute to food poisoning. *FEMS Microbiology Letters* 217, 115-119.
<https://doi.org/10.1111/j.1574-6968.2002.tb11464.x>
15. Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., De vos, P., Heyndrickx, M. (2008): Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. *Syst Appl Microbiol* 31, 126-140.
<https://doi.org/10.1016/j.syapm.2008.03.002>
16. De Vos P, Garrity GM, Jones D, Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K-H., Whitman, W.B. (2009): *Bergey's manual of systematic bacteriology*. 2nd Ed. 3, 392-426.
17. Dhakal, R., Seale, R.B., Deeth, H.C., Craven, H., Turner, M.S. (2014): Draft genome comparison of representatives of the three dominant genotype groups of dairy *Bacillus licheniformis* strains. *Appl Environ Microbiol* 80, 3453-3462.
<https://dx.doi.org/10.1128%2FAEM.00065-14>
18. Dublin (2020): Plant based cheese market forecast, trend analysis & competition tracking - global market insights 2019 to 2027. Report code: FACT4549MR, 170.
19. EFSA and ECDC (2014): The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012. *EFSA Journal* 12, 312.
<http://dx.doi.org/10.2903/j.efsa.2014.3547>
20. EFSA (2016): European Food Safety Authority. Risks for public health related to the presence of *Bacillus cereus* and other *Bacillus* spp. including *Bacillus thuringiensis* in foodstuffs. *EFSA Journal* 14, 4524.
<https://doi.org/10.2903/j.efsa.2016.4524>
21. Egyptian Standards (2005): Cheeses of plant origin. *Egyptian Organization for Standardization*. (ES: 1867/2005).
22. Egyptian Standards (2005): Kareish cheeses. *Egyptian Organization for Standardization*. (ES: 1008/4/2005).
23. Egyptian Standards (2016): Fermented drinking yogurt. *Egyptian Organization for Standardization*. (ES: 8042/ 2016).
24. Egyptian Standards (2020): Romy cheeses. *Egyptian Organization for Standardization*. (ES: 1007/4/2020).
25. Egyptian Standards (2020): Soft cheeses. *Egyptian Organization for Standardization*. (ES: 8390/2020).
26. Ehling-Schulz, M., Guinebretière, M-H., Monthán, A., Berge, O., Fricker, M., Svensson, B. (2006): Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. *FEMS Microbiol. Lett* 260, 232-240.
<https://doi.org/10.1111/j.1574-6968.2006.00320.x>
27. Ehling-Schulz, M., Frenzel, E.Gohar, M. (2015): Food-bacteria interplay: pathometabolism of emetic *Bacillus cereus*. *Front Microbiol* 6, 704.
<http://dx.doi.org/10.3389/fmicb.2015.00704>
28. El-Ziney, M.G. (2018): Evaluation of microbiological quality and safety of milk and dairy products with reference to European and Gulf Standards. *Food and Public Health* 8, 47-56.
<https://doi.org/10.5923/j.fph.20180802.03>

29. Ganesan, B., Irish, D.A., Brotherson, C., McMahon, D.J. (2012): Evaluation of microbial survival post-incidence on fresh mozzarella cheese, *Journal of Dairy Science* 95, 6891-6896.
<https://doi.org/10.3168/jds.2012-5390>
30. Glasset, B., Herbin, S., Guillier, L. (2016): *Bacillus cereus*-induced food-borne outbreaks in France, 2007 to 2014: epidemiology and genetic characterization. *Euro Surveill* 21, 30413.
<https://doi.org/10.2807/1560-7917.es.2016.21.48.30413>
31. Gopal, N., Hill, C., Ross, P.R. (2015): The prevalence and control of *Bacillus* and related spore-forming bacteria in the dairy industry. *Front Microbiol* 6, 1-18.
<https://doi.org/10.3389/fmicb.2015.01418>
32. Graciela, L., Garrote, N., Graciela, D. (2001): Chemical and microbiological characterization of fermented milks. *J Dairy Res* 68, 639-652.
33. Granum, P.E. (2005): *Bacillus cereus*. In Food Borne Pathogens: *Microbiology and Molecular Biology* (Fratamico, P.M., Bhunia, A.K., Smith, J.L. eds.) 409-419. Caister Academic Press, Norwich, England.
34. Griffiths, M.W., Schraft, H. (2017): *Bacillus cereus* Food Poisoning (eds) *Foodborne Diseases*. 3rd Ed. Chapter 20, 395-405.
<http://dx.doi.org/10.1016/B978-0-12-385007-2.00020-6>
35. Guinee, T.P. (2007): Cheese-like products. In: *Cheese problems solved*. 1st ed. Woodhead Publishing limited and CRC Press LLC. 384-388.
36. Hafiz, N.M., Saad, M.F., Hanafy, M.H., Abdel-Latif, E.F. (2016): Detection of *Mycobacterium avium subsp. paratuberculosis* in raw buffalo's milk. *International Journal of ChemTech Research* 9, 123- 128.
37. Heikal, G.I., Khater, D.F., Al-Wakeel, S.A. (2014): Bacteriological hazard of white cheese processed in some small primitive plants (dairy shops) In Tanta City. *Benha Veterinary Medical Journal* 26, 185-194.
38. Ibrahim, G.A., Sharaf, O.M., Abd El-Khalek, A.B. (2015): Microbiological quality of commercial raw milk, domiati cheese and kareish cheese. *Middle East Journal of Applied Sciences* 5, 171-176.
39. Ibrahim, A.S., Saad, M.F., Hafiz, N.M. (2020): Toxic elements in dried milk and evaluation of their dietary intake in infant formula. *International Journal of Veterinary Science* 9, 563-567.
<http://dx.doi.org/10.37422/IJVS/20.070>
40. Ibrahim, A.S., Saad, M.F., Hafiz, N.M. (2021): Safety and quality aspects of whole and skimmed milk powders. *Acta Scientiarum Polonorum Technologia Alimentaria* 20, 165-177.
<https://doi.org/10.17306/J.AFS.2021.0874>
41. International Organization for Standardization (2005): ISO 7251:2005. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of presumptive *E. coli*. ISO, Geneva, Switzerland.
42. International Organization for Standardization (2006): ISO 4831:2006 Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of Coliforms - Most probable number technique. ISO, Geneva, Switzerland.
43. International Organization for Standardization (2008): ISO 21527-1:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0.95, by means of the colony count technique at 25 °C plus or minus 1 °C. ISO, Geneva, Switzerland.
44. International Organization for Standardization (2017): ISO 6579-1:2017. *Microbiology of the food chain* - Horizontal method for the detection, enumeration of *Salmonella* - Part 1: Detection of *Salmonella* spp. ISO, Geneva, Switzerland.
45. International Organization for Standardization (2017): ISO 11059- 2017. Milk and milk products - Method for the enumeration and isolation of *Pseudomonas* spp. in milk and milk products. ISO, Geneva, Switzerland.
46. Jessberger, N., Dietrich, R., Einar Granum P., Märtilbauer E. (2020): The *Bacillus cereus* Food Infection as Multifactorial Process. *Toxins* 12, 701-738.
<http://dx.doi.org/10.3390%2Ftoxins12110701>

47. Kailasapathy, K. (2006): Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yogurt. *LWT - Food Science and Technology* 39, 1221-1227.
<https://doi.org/10.1016/j.lwt.2005.07.013>
48. Khater, K.A., Abdella, S.A. (2017): Prevalence and characterization of aerobic spore forming bacteria in raw milk and some cheeses. *Journal of Food and Dairy Sciences* 8, 213-216.
<https://dx.doi.org/10.21608/jfds.2017.38222>
49. Le Marc, Y., Valik, L., Medveova, A. (2009): Modelling the effect of the starter culture on the growth of *Staphylococcus aureus* in milk. *Int J Food Microbiol* 129, 306-311.
<https://doi.org/10.1016/j.ijfoodmicro.2008.12.015>
50. Martínez-Ruiz, N.D.R., Sandra, F.E., Vázquez-Nájera, R.E., López-Díaz, J.A. (2013): Microbiological quality of Asadero cheese manufactured with a plant-based coagulant from *Solanum elaeagnifolium*. *Food and Nutrition Sciences* 4, 75-81.
<http://dx.doi.org/10.4236/fns.2013.47A009>
51. Metwalli, S.A.H. (2011): Extended shelf life of kareish cheese by natural preservatives. *Egypt. Egyptian Journal of Agricultural Research* 89, 639-649.
52. Nassib, T. A., Darwish, M.S., Abdelbaky, I., Motee, D. (2018): Prevalence of Spore-Forming Bacteria Associated with the Processing of Milk and Dairy Products Processing in Mansoura City, *Journal of Food and Dairy Science* 9, 257-261.
<https://dx.doi.org/10.21608/jfds.2018.36015>
53. Nazem, A.M., Awad, S., Abo Shaala, E.K. (2020): Low salt soft cheese; compositional quality and incidence of aerobic spore forming bacteria, *Alexandria Journal of Veterinary Sciences* 66, 48-54.
<http://dx.doi.org/10.5455/ajvs.126240>
54. Nesma, H. Y., Nagah, M. H., Halawa, M. A., Saad, M. F. (2020): Influence of some hygienic measures on the prevalence of subclinical mastitis in a dairy farm. *International Journal of Dairy Science* 15, 38-47.
<https://dx.doi.org/10.3923/ijds.2020.38.47>
55. Ozer, B.H., Kirmaci, H.A. (2010): Functional milks and dairy beverages. *International Journal of Dairy Technology* 63, 1-15.
<https://doi.org/10.1111/j.1471-0307.2009.00547.x>
56. Olugbuyiro, J.A. (2011): Physico-chemical and sensory evaluation of market yoghurt in Nigeria. *Pakistan Journal of Nutrition* 10, 914-918.
<https://dx.doi.org/10.3923/pjn.2011.914.918>
57. Park, K.M., Kim, H.J., Jeong, M., Koo, M. (2020): Enterotoxin genes, antibiotic susceptibility, and biofilm Formation of low-temperature-tolerant *Bacillus cereus* isolated from green leaf lettuce in the cold chain. *Foods* 9, 249-263.
<https://doi.org/10.3390/foods9030249>
58. Postollec, F., Mathot, A.-G., Bernard, M., Divanac'h, M.-L., Pavan, S., Sohier, D. (2012): Tracking spore-forming bacteria in food: from natural biodiversity to selection by processes. *Int J Food Microbiol* 158, 1-8.
<https://doi.org/10.1016/j.ijfoodmicro.2012.03.004>
59. Rahimzadeh, G., Tay, A., Regenstein, J.M., Rokhzadi, A., Dabiri, H. (2020): Evaluation of microbial and sensory properties of flavored yogurt drink produced by *Noanea mucronata* and liquid smoke treatment. *Infectious Diseases and Herbal Medicine* 1, 77.
<https://doi.org/10.4081/idhm.2020.77>
60. Rosslund, E., Andersen Borge, G.I., Langsrud, T., Sorhaug, T. (2003): Inhibition of *Bacillus cereus* by strains of *Lactobacillus* and *Lactococcus* in milk. *Int J Food Microbiol* 89, 205-212.
[https://doi.org/10.1016/s0168-1605\(03\)00149-1](https://doi.org/10.1016/s0168-1605(03)00149-1)
61. Simoes, M., Simoes, L.C., Vieira, M.J. (2009): Species association increases biofilm resistance to chemical and mechanical treatments. *Water Res* 43, 229-237.
<https://doi.org/10.1016/j.watres.2008.10.010>
62. Sulejmani, E., Beqiri, L., Popeski-Dimovski, R. (2021): Effect of vegetable fat on the texture, colour and sensory properties of Macedonian white brined cheese. *Mljekarstvo* 71 (1), 25-34.
<https://doi.org/10.15567/mljekarstvo.2021.0103>

63. Vangoitsenhoven, R., Rondas, D., Crevecoeur, I., D'Hertog, W., Baatsen, P., Masini, M., Overbergh, L. (2014): Foodborne cereulide causes beta-cell dysfunction and apoptosis. *PLoS One* 9, e104866.
<https://doi.org/10.1371/journal.pone.0104866>
64. Yan, W., Qian, J., Ge, Y., Ye, K., Zhou, C., Zhang, H. (2020): Principal component analysis of MALDI-TOF MS of whole-cell foodborne pathogenic bacteria, *Analytical Biochemistry* 592, 113582.
<https://doi.org/10.1016/j.ab.2020.113582>
65. WHO (2007): World Health Organization Food Safety & Food-borne Illness. Fact Sheet No. 237, World Health Organization, Geneva, Switzerland.
66. Yibar, A., Cetinkaya, F., Soyutemiz, E., Yaman, G. (2017): Prevalence, enterotoxin production and antibiotic resistance of *Bacillus cereus* isolated from milk and cheese. *Kafkas University journal of the faculty of veterinary medicine* 23, 635-642.
<https://doi.org/10.9775/kvfd.2017.17480>
67. Yoo, J.G., Chang, J.-H., Kim, S.-Y., Ji, J.-Y., Hong, S.-W., Park, B.-Y., Oh, M.-H (2014): Analysis of emetic toxin production by *Bacillus* species using cellular cytotoxicity, molecular, and chromatographic assays. *Biotechnology and Bioprocess Engineering* 19, 978-983.
<https://doi.org/10.1007/s12257-014-0574-7>
68. Youssif, N.H., Hafiz, N.M., Halawa, M.A., Aziz H.M., Saad, M.F. (2020): Impact of subclinical mastitis on milk quality in different seasons. *International journal of veterinary science* 9, 313-31.
<https://doi.org/10.37422/IJVS/20.020>
69. Youssif, N.H., Hafiz, N.M., Halawa, M.A., Saad, M.F. (2021): Association of selected risk factors with bovine subclinical mastitis. *Acta Veterinaria Brasilica* 15, 153-160.
<https://doi.org/10.21708/avb.2021.15.2.9785>
70. Worku, K.F., Tefera, A.T., Tuji, F.A. (2015): Comparative analysis of microbial load of commercially prepared and traditionally homemade yoghurt (*Ergo*) retailed in Addis Ababa. *Advances in Life Sciences* 5, 58-63.
<https://doi.org/10.5923/j.als.20150503.02>
71. Zhao, S., Chen, J., Fei, P., Feng, H., Wang, Y., Ali, M.A., Li, S., Jing, H., Yang, W. (2020): Prevalence, molecular characterization, and antibiotic susceptibility of *Bacillus cereus* isolated from dairy products in China. *J Dairy Sci* 103, 3994-4001.
<https://doi.org/10.3168/jds.2019-17541>