Received: 11 March 2023, Accepted: 14 April 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 3, 412-420

Behavioral Patterns of the Isolated *Bacillus cereus* Strains from Milk and Some Milk Products in Yoghurt and Damietta Cheese

Salwa S. Thabet*, Lamiaa M.T. Al Shrief

Food Hygiene Department, Animal Health Research Institute, Agriculture Research Center (ARC), Egypt.

***Correspondence**
Corresponding author: Salwa S. Thabet
E-mail address: salwas.t.73@gmail.com

Abstract

The Present study was undertaken to assess the prevalence of *B. cereus* in milk and some milk Products and its survival in yoghurt and Damietta cheese. A total of 100 samples of raw milk, Pasteurized milk, Damietta cheese and yoghurt (25 for each) were collected from supermarkets in Assiut city and were examined for isolation of *B. cereus*. It was isolated from 24, 32, 68 and 20% of the tested samples, respectively. All the isolated strains were confirmed positive for *B. cereus* except one strain for each raw milk and Damietta cheese. 14.7%, 85.3, 50.0 and 76.5% of the confirmed strains were carried *Ces*, *Nhe*, *Hbl* and *cytK* genes. Concerning its survival in yoghurt, there was a significant difference between the effect of yoghurt and acidophilus yoghurt on *B. cereus* growth, where the organism remained viable to the 9th day in yoghurt and failed to be isolated at the 5th day in yoghurt with probiotics. In case of Damietta cheese, 2 and 5% salt were more favorable for the pathogen growth, while 10% salt was inconvenient for its growth. In addition, the inhibitory effect of thyme essential oil was more obvious than the effect of rosemary EO, where the bacterial population was decreased to a count of 2.04 and 2.46 log cfu/ g. at the 2nd week in case of addition of 1.65% thyme EO and could not be detected at the 4th week, whereas the organism could survive to the 6th week in case of rosemary. In conclusion, strict hygienic measures must be implemented during the manufacture of dairy products and addition of biopreservatives to control *B. cereus* growth is substantial.

KEYWORDS
B. cereus, Milk, Proteolytic, Lipolytic, Virulent genes

INTRODUCTION

B. cereus is a Gram-positive rod, motile, facultative anaerobe, and spore formers in unsuitable circumstances, while the spores germinate and the vegetative cells multiply under improper storage (Logan, 2011; Fiedoruk *et al*., 2017). *Bacillus cereus* is widely distributed in the environment, including soil, water, and decaying materials, and is regularly isolated as a food contaminant (Tirloni *et al*., 2023). It is a psychotropic bacterium that has the ability to grow at refrigeration temperature and yields proteases and lipases enzymes which are important in milk processing. Proteases cause unpleasant tastes and gelation. While lipases deteriorate milk fatty acids and cause off-flavor in UHT, pasteurized milk, cheese, and milk powder (Chen *et al*., 2004; Furtado, 2005).

Bacillus cereus food poisoning depends on the presence of many toxins – encoding genes that play a crucial role in its pathogenicity (Tirloni *et al*., 2020). The toxins produced by this organism play a sustainable role in food safety as it can cause public health hazards (Owusu- Kwarteng *et al*., 2017). The major toxins of *B. cereus* that have been detected include non- haemotylic enterotoxin (*Nhe*), haemolysin bL (*Hbl*) and cytotoxin K (*cytK*) which are the cause of the diarrheal syndrome as well as cereulide that induce the emetic syndrome. *Nhe* is the most virulent enterotoxin of *B. cereus* (Moravek *et al*., 2006; Zhu *et al*., 2013; JeBberger *et al*., 2014).

Much attention has focused to control spread of pathogenic bacteria and to increase the shelf life of food, so new methods for inhibiting growth of pathogenic bacteria by using nonchemical compounds become of major interest and that makes essential oils commonly used in food industries (Ibanez and Blazquez, 2021).

The spices and herbs have been used for centuries all over the world in food preservation to make it more palatable and to improve its flavor and its essential oils (EOs) are natural and ecofriendly compounds with antioxidant and antimicrobial activities which rendered them the ability to be used in food manufacture (Bhat *et al*., 2014; Shahidi, 2015; Peter and Hardin, 2021). Thyme is an aromatic plant and is broadly distributed in Europe, Asia and North Africa and several researchers reported it as a rich source of bioactive compounds as a consequence of the existence of the phenolic components. Among these components are thymol and carvacrol and the second is an isomer of the first. Depending on the place of origin, thymol serves as 40-80% of the essential oil constituent and up to 55% of carvacrol (Nieto, 2020). Also, the essential oils of rosemary have antibacterial and antifungal effect and the component that is responsible of these activities are rosmanol, carnosol, carnosic acid, ursolic acid, rosmariquinone, caffeic acid and rosmaridiphenol (Kauer *et al*., 2021).

In addition, the antimicrobial effect of Lactic acid bacteria against other pathogenic bacteria and the ability of lactobacilli to compete with these bacteria when they are found together are the key of their ability to increase the keeping quality of milk products (Tallent *et al*., 2012). Consequently, the objective of the present study was to detect the incidence of *B. cereus* in milk and some milk products and the assessment of *B. cereus* viability in yoghurt fortified with Lactobacilli and in cheese that containing

ISSN: 2090-6277/2090-6269/

different concentration of salt and supplemented with EOs.

MATERIALS AND METHODS

Collection of samples

A total of one hundred random samples of milk and milk products including twenty-five samples for raw milk, pasteurized milk, Damietta cheese and yoghurt were collected from dairy shops and supermarkets in Assiut City, Egypt. Milk samples were tested for heat treatment by using storch's test (Lampert, 1975).

Isolation of B. cereus (Tallent et al., 2012)

Twenty five ml from each sample of raw milk was added to 225 ml buffered peptone water and 25 g from each sample of Damietta cheese and yogurt was homogenized in a stomacher bag with 225 ml buffered peptone water, then the prepared samples were incubated at 37±1°C for 18±2 h. Loopful from each tube of buffered peptone water was streaked on mannitol egg-yolk polymixin (MYP) agar plate and the inoculated plates were incubated at 37±1°C for 18±2 h and were examined for the suspected red colonies surrounded by a precipitation zone. The colonies were examined macroscopically and biochemically according to Tallent *et al*. (2012).

Detection of the Proteolytic and lipolytic activity of the isolated strains

Proteolytic activity of *B. cereus* (Beerens and Luquet, 1990)

All the isolated and identified strains of *B. cereus* were tested for their proteolytic activity at different temperatures. The identified strains were subcultured on Milk Agar plates (Plate Count Agar supplemented with 10% milk powder). The plates were incubated at 7°C for 10 days, 10°C for 7 days and 30°C for 48 hours. The cultures were considered positive when presented a precipitation halo zone around the colonies indicating the release of proteolytic enzymes into the growth medium.

Lipolytic activity (Nabrdalik and Grata, 2011)

Tributyrin Agar plates (Plate Count Agar supplemented with 1% tributyrin) were used to detect the lipolytic activity of the isolated *B. cereus*. In this respect, the inoculated plates were incubated at 7°C for 10 days, 10°C for 7 days and 30°C for 48 h. Production of the lipase enzyme splits tributyrin resulting in lipolytic colonies surrounded by a clear zone in an opaque medium indicating positive result.

Molecular confirmation of the isolated strains (Das et al., 2013)

The previously isolated and identified 36 strains of *B. cereus* were submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health Research Institute, Dokki, Giza, Egypt for confirmation by using PCR technique.

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, G. bH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

PCR amplification

Primers (Table 1) were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, G.bH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Generuler100 bp ladder (Fermentas, Germany) was used to determine the fragent sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Detection of the virulence genes (Ehling- Schulz et al., 2006)

The formerly confirmed strains of *B. cereus* were sent to The Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health Research Institute, Dokki, Giza, Egypt for detection of the virulence genes and Uniplex PCR was used for detection of *Ces* gene, while Multiplex PCR was applied for detection of the diarrheal type encoded genes (*Hbl*, *cytK* and *Nhe*).

Oligonucleotide Primers

Primers used for confirmation of *B. cereus* and detection of the virulence genes were supplied from Metabion (Germany) are listed in Table 1.

Viability of B. cereus in Damietta cheese and yoghurt

Preparation of *B. cereus* strains (Tallent *et al*., 2012)

Strains of *B. cereus* used in this study were the earlier isolated and confirmed pathogenic strains. One emetic strain (ES) and one diarrheagenic strain (DS) were propagated in BPW for 48h at 37±1° C and ten-fold serial dilution was made, and the tubes were matched to 0.5 McFarland tubes to detect the count of the organism in the broth.

Preparation of *L. acidophilus* strain

L. acidophilus 20079 strain was the used reference strain in the experiment. The strain was cultured on De Man, Rogosa and Sharpe broth (MRS) and was incubated anaerobically at 37ºC for 48 h, then ten – fold serial dilution was performed for detection of the bacterial count/ ml of broth by the aid of 0.5 McFarland tube.

Preparation of Oils (NCCLS, 2002)

Thyme and rosemary essential oils were obtained from oil extraction unit of National Research Center, Egypt and two fold

Table 1. Oligonucleotide Primers used in the study.

Target gene	Primers sequences	Amplified segment (bp)	Primary Denaturation	Amplification (35 cycles)			Final extension
				Secondary denaturation	Annealing	Extension	
B. cereus gro-EL	TGCAACTGTATTAGCACAAGC T TACCACGAAGTTTGTTCACTACT	533	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.
Ces	GGTGACACATTATCATATAAGGTG GTAAGCGAACCTGTCTGTAACAACA	1271			49˚C 1 min.	72˚C 1.2 min.	
Hbl	GTA AAT TAI GAT GAI CAA TTTC AGA ATA GGC ATT CAT AGA TT	1091					
Nhe	AAG CIG CTC TTC GIA TTC ITI GTT GAA ATA AGC TGT GG	766			49˚C 1 min.	72˚C 1 min.	
cytK	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421					

serial dilution of Thyme and rosemary was carried out, where, the first dilution was made by mixing 5 ml of oil with 0.1 ml tween 80 as an emulsifier and 4.9 ml sterilized deionized water, at that time, two fold serial dilution was made by using only deionized water up to 0.39% and these dilutions was used in detecting the inhibitory concentration.

Detection of minimum inhibitory concentration (NCCLS, 2002)

Detection of the minimum inhibitory concentration (MIC) of the used essential oils was performed by using agar well diffusion method.0.1ml from the previously matched dilution of the target organism to 0.5 McFarland tube was surface plated on Mueller Hinton agar dishes with wells of 4mm diameters. Then, 0.1 ml from the prepared two-fold serial dilution of the used essential oils were put in the wells. In addition, one control well in each Muller Hinton agar plate was filled by 0.1 ml of sterile deionized water. Finally, the plates was kept at room temperature for 45 min before incubation of the plates for 24 h at 37°C to detect the diameter of the inhibition zones.

Experimental Technique

Survival of *B. cereus* in yoghurt (Abo –Donia, 2008)

One Liter of pasteurized milk was obtained from Animal Production Research Institute and were examined before used to make sure it was *B. cereus* free, then, it was warmed to 42ºC, and 2% yoghurt culture was added (L. bulgaricus and streptococcus thermophiles). The inoculated milk was divided into five portions in a bottle of 200 ml milk. The first portion was not inoculated with *B. cereus* as control negative and the other four portions were divided to two groups of two bottles and one group was inoculated with *L. acidophilus* in a count 5×10^9 cfu/ml. One bottle from each group was inoculated with an emetic strain of *B. cereus* and the other tube with the diarrheagenic strain in a count of 5×10^6 cfu/ ml. Lastly, yoghurt was kept at 4-8ºC in the refrigerator. The count of *B. cereus* was taken at the time of coagulation and at the 1st, 2nd, 3rd, 7th and 9th days of storage.

Survival of *B. cereus* in Damietta cheese

Effect of salt and storage temperature (El-Kholy et al, 2016)

Three liters of pasteurized condensed milk were obtained from Animal Production Research Institute and were examined before used to make sure they were *B. cereus* free, and they were warmed at 37ºC. Then, rennet and calcium chloride were added in percentage of 0.05 and 0.02% and 600 ml of milk was put in two bottles of 300 ml as negative control one of them was kept at room temperature and the other in the refrigerator. The remaining part was divided to two groups each group of 6 bottles contained 200 ml of milk and the first group was inoculated with the emetic strain and the second group was inoculated with the diarrhaegnic strain to obtain a count of 5×10^6 cfu/ ml, then Sodium chloride was added in a percentage of 2%, 5% and 10% in two tubes for each salt concentration in these groups. Finally, one tube of each concentration of salt was kept at room temperature (30- 37ºC) and the other one was kept in the refrigerator (4- 8°C).

Effect of Essential oils (El-Kholy et al, 2016)

Two liters of pasteurized condensed milk were obtained from Animal Production Research Institute and were examined before used to make sure they were *B. cereus* free. They were warmed to 37ºC and rented to make cheese of 2% salt concentration and 400 ml of milk were removed in a bottle as control negative. The remaining part was divided into two groups of 4 bottles of 200 ml milk the first group was inoculated with the emetic strain and the second with the diarrheagenic strain. Moreover, thyme essential oil (EO) was added to obtain 0.78 and 1.56 % in two tubes of each group and rose marry was also added in the same concentration in the other two tubes of each group (0.78 is the minimum inhibitory concentration and 1.56 was the previous dilution).

Statistical analysis

The variables were analyzed by using MedCalc Statistical Software Version 20. Shapiro- Wilk normality test was used to assess whether the data met the assumption of the statistical approach, while Levine's test and F- test were used to assess the equality of variance. Unpaired T- Test, Welch- test, Mann- Whitney- test and Kruskal- Wallis's test ANOVA with Conover Multiple Comparison Test for Post hoc were used to detect the significance between the groups and P- value less than 0.05 were considered significant and less than 0.01 were considered high significant.

RESULTS

As observed in Table 2, *B. cereus* could be isolated from 6 out of the examined raw milk samples in a percentage of 24%. While the incidence of *B. cereus* in the examined pasteurized milk samples was 32%. On the other hand, 68% and 20% of the tested Damietta cheese and yoghurt samples were contaminated with *B. cereus*.

The data illustrated in Table 3 revealed that 83.3 and 66.7% of the isolated strains of *B. cereus* from raw milk samples had proteolytic and lipolytic activity, respectively. While 62.5% of the

isolated strains from pasteurized milk were proteolytic and lipolytic. On the other hand, 82.4 and 80% of the isolated *B. cereus* strains from Damietta cheese and yoghurt were proteolytic and 59.6 and 60.0% were lipolytic, respectively.

It is worthwhile to state that, the incidence of the organism in raw milk, pasteurized milk, Damietta cheese and yoghurt were 20, 32, 64 and 20%, where 83, 100, 94 and 100% of the isolated strains were confirmed positive for *B. cereus*, respectively, based on molecular confirmation of the isolated strains of *B. cereus* as recorded in Table 4, and Figs. 1 and 2.

It is clear from the findings in Table 5 and Figures 3, 4 and 5 that 40, 12.5 and 20% of the confirmed strains were emetic in raw milk, Damietta cheese and yoghurt, respectively, where *Ces* gene could be detected in 5 strains out of the confirmed 34 strains. While 60,100, 87.5 and 80% of the confirmed strains were diarrheagenic, respectively, based on detection of the virulent *Hbl*, *Nhe* and *cytK* genes, which can be detected in 17, 29 and 26 strains out of the confirmed 34 strains of *B. cereus* and in percentage of 50.0, 85.3 and 76.5% of the confirmed *B. cereus* strains.

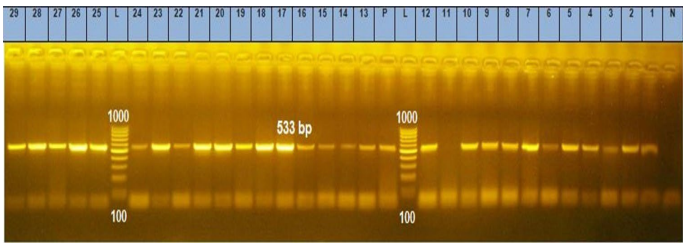

Fig. 1. Molecular confirmation of the isolated strains (1- 29).

The recorded results in Table 6 showed low rate of decline in the count of *B. cereus* strains in yoghurt not supplemented with *L. acidophilus* was reported along the experimental time, where the bacterial densities of the ES and DS strains were decreased to

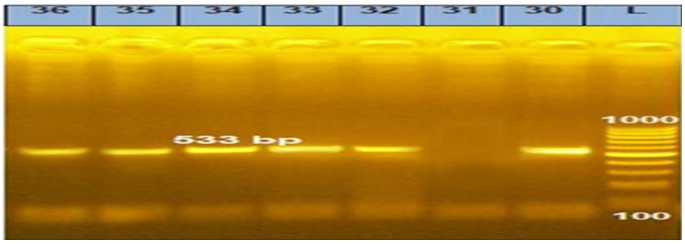

Fig. 2. Molecular confirmation of the isolated strains (30- 36).

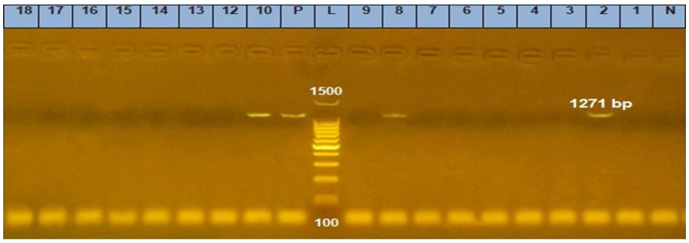

Fig. 3. Detection of the virulent *Ces* gene in the cnofirmed *B. cereus* strains (1- 18).

5.69 and 5.57 log cfu/g. at the time of fermentation, respectively, and the count was decreased gradually from 5.38 and 5.14 log cfu/g. On the first day to 4.32 and 4.9 log cfu/g. at the 7th day and yoghurt get spoiled at the 9th day. In the case of yoghurt with probiotic, rapid reduction in the count of the ES from 5.11 at zero time to 4.90, 4.00 and 2.30 log cfu/g. On the 1st, 2nd, and 3rd days. Also, the initial count of the DS was 5.18 log cfu/g, and its microbial load was continuously declined at the 1st, 2nd, and 3rd days with a count of 4.93, 4.89 and 2.54 log cfu/g, and finally, the organism could not be isolated at the 5th day and at the 14th day the tested yoghurt samples were putrefied.

The data illustrated in Table 7 showed that, the initial count of *B. cereus* in cheese samples containing 2% Salt was 6.60 and 6.48 log cfu/g in the refrigerated samples and 6.50 and 6.46 log cfu/g in the samples kept at room temperature for the ES and DS,

Table 2. Incidence of *B. cereus* in the examined samples.

Product	No. of examined samples	Positive Samples	
		No.	%
Raw milk	25	6	24
Pasteurized milk	25	8	32
Damietta Cheese	25	17	68
Yoghurt	25	5	20

Table 3. Lipolytic and proteolytic activity of the isolated strains.

Product	Proteolytic activity		Lipolytic strains	
	No. of stains	%	No. of strains	%
Raw milk	5/6	83.3	4/6	66.7
Pasteurized milk	5/8	62.5	5/8	62.5
Damietta Cheese	14/17	82.4	10/17	59.6
Yoghurt	4/5	80.0	3/5	60.0

Table 4. Results of molecular confirmation of the isolated strains.

Product	Prevalence of the confirmed strains		Incidence of *B. cereus*	
	No.	%	No.	%
Raw milk	5/6	83	5/25	20
Pasteurized milk	8/8	100	8/25	32
Damietta Cheese	16/17	94	16/25	64
Yoghurt	5/5	100	5/25	20

respectively. While the ES count was 5.02 and 5.52 log cfu/g. On the 1st day and 5.46 and 5.66 cfu/g for the DS at refrigeration and room temperature, respectively. On the 2nd day the strains return to increase gradually where the count of the ES was 6.82, 6.94, 6.95, 7.78, and 8.95 log cfu/g. On the 2nd, 7th days and 2nd, 3rd, and the 4th week for the refrigerated sample. At room temperature the count of the ES was 6.72, 6.30 and 9.91 log cfu/g. On the 2nd, 7th days and the 2nd week. The behavioral pattern of the DS was similar to the ES and its count was 6.76, 6.99, 6.99, 7.49 and 8.93 log cfu/g. in the refrigerated sample and 6.77, 6.58 and 9.95 log cfu/g at room temperature, respectively. The samples that were preserved in the refrigerator were spoiled at the 6th week and at the 3rd week for the samples that were kept at room temperature. In the case of cheese of 5% Salt, the count of the ES was decreased to 4.90 and 3.32 log cfu/g on the 1st day and 4.97 and 3.58 log cfu/g for the diarrheagenic strain, respectively. Moreover, the bacterial population of the strains gradually increased from the 2nd day to the 6th week with a count from 5.55 to 8.43 log cfu/g. for the ES and from 5.77 to 8.90 log cfu/g. For the DS in the refrigerator and the cheese gets spoiled on the 8th week. On the other hand, the microbial density of the ES and the DS was raised from 5.00 and 5.88 log cfu/g on the 2nd day to 9.47 and 9.69 log cfu/g at the 3rd week and the cheese became invalid for human consumption at the 8th and 4th weeks, respectively. On the other hand, the ES and DS were decreased to 4.90 and 4.53 log cfu/g in the refrigerated cheese that containing 10% salt and the count was progressively decreased until the count become 2.47 and 2.31 log cfu/g at the 4th week and could not be detected at the 6th week. The growth patterns of the organism at room temperature was nearly similar, where the count was declined to 4.95 and 4.91 log cfu/g at the 1st day and the reduction in the count continued till the 4th week with a count of 2.34 and 2.30 log cfu/g At last, the organism can not be detected in the cheese at the 6th week and it remained fit for consumption till the 16th and 12th week in the refrigerator and at room temperature, respectively.

Table 5. Detection of the virulent genes in the confirmed strains of *B. cereus*

Product	*Ces*	*Hbl*	*Nhe*	*CytK*	Emetic strains		Diarrheagenic strains	
					No.	%	No.	%
Raw milk	2	2	3	3	2/5	40	3/5	60
Pasteurized milk	0	5	8	8	0/8	0	8/8	100
Damietta cheese	2	9	14	11	2/16	12.5	14/16	87.5
Yoghurt	1	1	4	4	1/5	20	4/5	80
Total	14.7%	50.0%	85.3%	76.5%	5/34	14.7%	29/34	85.3%

Table 6. Survival of *B. cereus* in yoghurt.

Time	Yoghurt				Yoghurt with *L. acidophilus*			
	ES		DS		ES		DS	
	Count	pH	Count	pH	Count	pH	Count	pH
Zero time	5.69	6.8	5.57	6.9	5.11	6.5	5.18	6.7
1st day	5.38	5.9	5.14	5.7	4.9	4.9	4.93	4.8
2nd day	4.6	5.6	4.53	5.5	4	4.8	4.89	4.6
3rd day	4.98	5.3	4.97	5.2	2.3	4.6	2.54	4.6
5th day	4.6	4.9	4.97	4.8	ND	4.3	ND	4.4
7th day	4.32	4.8	4.9	4.7	ND	4.3	ND	4.2
9th day	Spoiled	NT	Spoiled	NT	Spoiled	NT	Spoiled	NT

ES: Emetic strain of *B. cereus*; DS: Diarrheagenic strain of *B. cereus*; NT: Samples were not tested; ND: Organism was not detected.

Table 7. Behavioral patterns of *B. cereus* in cheese of different salt concentration.

Time	2% Salt				5% Salt				10% Salt			
	Refrigeration		Room Temp.		Refrigeration		Room Temp.		Refrigeration		Room Temp.	
	ES	DS	ES	DS	ES	DS	ES	DS	ES	DS	ES	DS
Zero time	6.6	6.48	6.5	6.46	6.53	6.68	6.46	6.69	6.5	6.6	6.3	6
1st day	5.02	5.46	5.52	5.66	4.9	4.97	3.32	3.58	4.9	4.53	4.95	4.91
2nd day	6.82	6.76	6.72	6.77	5.55	5.77	5	5.88	3.23	3.77	3.2	3.46
7th day	6.94	6.99	6.3	6.58	6.84	5.91	6.18	6.56	3.36	3.47	3.89	3.79
2nd week	6.95	6.99	9.91	9.95	6.92	6.34	8	8.46	3.48	3.46	3.58	3.96
3rd week	7.78	7.49	Spoiled	Spoiled	7.59	7.3	9.47	9.69	3.92	3.84	2.47	2.75
4th week	8.95	8.93	NT	NT	8	8.79	Spoiled	Spoiled	2.47	2.31	2.34	2.3
6th week	Spoiled	Spoiled	NT	NT	8.43	8.90k	NT	NT	ND	ND	ND	ND
8th week	NT	NT	NT	NT	Spoiled	Spoiled	NT	NT	ND	ND	ND	ND
12th week	NT	NT	NT	NT	NT	NT	NT	NT	ND	ND	Spoiled	Spoiled
16th week	NT	NT	NT	NT	NT	NT	NT	NT	Spoiled	Spoiled	NT	NT

ES: Emetic strain of *B. cereus*; DS: Diarrheagenic strain of *B. cereus*; NT: Samples were not tested; ND: Organism was not detected.

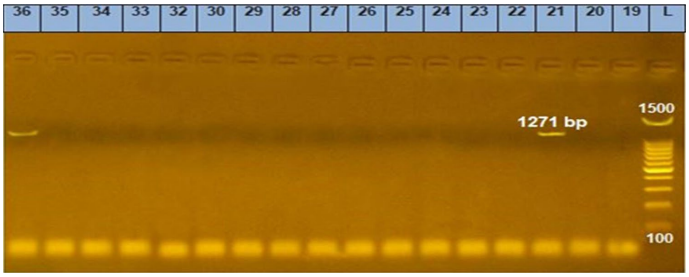

Fig. 4. Detection of the virulent *Ces* gene in the cnofirmed *B. cereus* strains (19- 34).

As represented in Table 8, there was slight increase in the count of *B. cereus* strains in cheese samples supplemented with 0.78 thyme EO at the 1st day with a count of 6.60 and 6.63 and at the 2nd day the count was 6.79 and 6.77 log cfu/g for the ES and DS, respectively. Then, the bacterial densities of the ES and the DS were reduced from the 1st week to the 6th week with a count of 5.75, 5.15, 4.08, and 2.62 log cfu/g for the ES and 5.92, 5.57, 4.59, and 2.32 log cfu/ g. for the DS. While in case of 1.65% concentration of Thyme EO there was somewhat decrease in the count of the ES and DS at the 1st day with a count of 6.36 and 6.23 log cfu/g for the ES and DS, thereafter, the count for the tested strains was rapidly reduced to 3.25 and 2.04 log cfu/g. At the 1st and 2nd weeks for the ES and 4.97 and 2.46 log cfu/g, respectively, and at the 4th week the organism could not be detected, and the cheese samples remain viable for human consumption for two months in case of 0.78% concentration of thyme EO and have become decomposed at the 8th weeks, while, in case of 1.65% the cheese was deteriorated at the 12th week. In the case of 0.78% rosemary concentration, the microbial load of the ES and DS was reduced at the 1st day to 6.30 and 6.59 log cfu/g and returned to increase at the 2nd day to 6.74, 6.77 log cfu/g, then the count was declined to 6.50, 5.34, 5. 23 and 3.25 cfu/ g. for the ES and 6.77, 6.76 and 5.91, 5.96 and 3.11 for the DS on the 1st, 2nd days and 4th and 6th weeks. In case of 1.65% concentration, the decline rate of *B. cereus* count was regular over the experimental time and the count was declined to 3.11 and 3.99 cfu/g at the 4h week and the cheese was become spoiled at the 8th week.

DISCUSSION

B. cereus food poisoning is classified as a serious public health hazard universally arising from food of milk and meat origin (Owusu- Kwarteng *et al*., 2017; Tawab *et al*., 2020). So, Detection of *B. cereus* prevalence in milk and milk product was the scope of our study to assisting the applicable food safety controls.

Regarding the results of morphological and biochemical identification, it is clearly evident that the highest incidence of *B. cereus* was reported for Damietta cheese (68%) followed by the pasteurized and raw milk. While the lower percentage was recorded for yoghurt (20%). On the other hand, molecular confirmation of the isolated strains by using PCR for detection of *B. cereus groEL* gene clarified that all the isolated strains were confirmed positive for *B. cereus* except one strain for each raw milk and Damietta cheese, so, the incidence of *B. cereus* decreased to 20% and 64% in raw milk and Damietta cheese.

Comparing the results recorded for raw milk with the recorded results of other authors we found that, nearly similar incidence (23.3%) was obtained by Liang *et al*. (2022), where they could isolate *B. cereus* from 14 out of the tested 60 raw milk samples and Lower percentage (12.86 and 6.66%) was recorded by Aouadhi *et al*. (2014) and Michel *et al*. (2018). On the other hand, 36.66, 52.0 and 36.66% were the demonstrated results by Arora *et al*. (2014); Osama *et al*. (2020) and Mostafa *et al*. (2022).

Concerning the pasteurized milk, the rate of *B. cereus* isolation reported by Singh *et al*. (2015); Gao *et al*. (2018) and Mostafa *et al*. (2022) was lower than the recorded data in this study, where they could detect the organism in the evaluated samples in a percentage of 17.64, 27 and 18.18%, respectively. Contrariwise, higher incidence was obtained by Owusu –Kwarteng (2017) and Salem *et al*. (2015) with a percentage of 47% and 40%.

In the case of cheese, lower incidence was recorded by Mo-

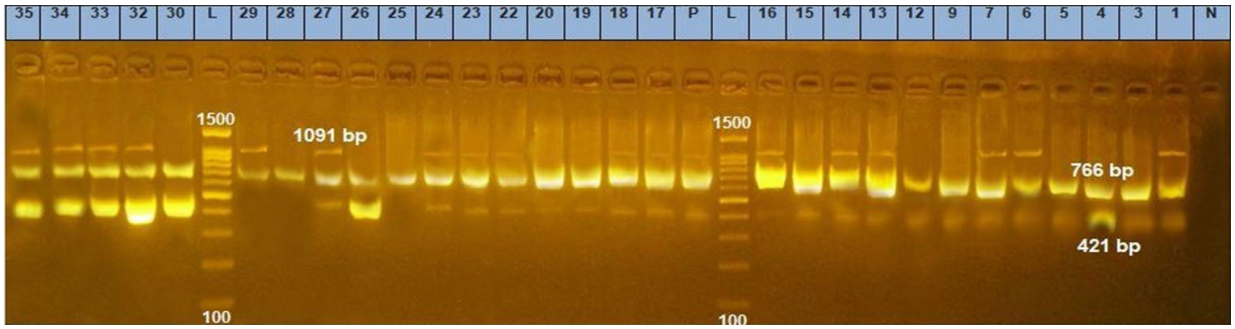

Fig. 5. Detection of the virulent Nhl, *Hbl* and *cytK* genes in the cnofirmed *B. cereus* strains

Table 8. The effect of thyme and rosemary on the viability of *B. cereus*.

Time	Thyme				Rosemary			
	0.78		1.65		0.78		1.65	
	ES	DS	ES	DS	ES	DS	ES	DS
Zero time	6.58	6.5	6.56	6.46	6.62	6.93	6.6	6.63
1st day	6.6	6.63	6.36	6.23	6.3	6.59	6.34	6.65
2nd day	6.79	6.77	5.04	5.5	6.74	6.77	6.78	6.69
1st week	5.75	5.92	3.25	4.97	6.5	6.76	6.72	6.62
2nd week	5.15	5.57	2.04	2.46	5.34	5.91	4.08	4.8
4th week	4.08	4.59	ND	ND	5.23	5.96	3.11	2.99
6th week	2.62	2.32	ND	ND	3.25	3.11	ND	ND
8th week	Spoiled	Spoiled	ND	ND	Spoiled	Spoiled	Spoiled	Spoiled
12th week	NT	NT	Spoiled	Spoiled	NT	NT	NT	NT

ES: Emetic strain of *B. cereus*; DS: Diarrheagenic strain of *B. cereus*; NT: Samples were not tested; ND: Organism was not detected.

stafa *et al*. (2022) and Osama *et al*. (2020) who found that 48.48 and 44% were contaminated. On the contrary, Salem *et al*. (2015) could isolate the organism from 80% of the estimated samples, while Ibrahim *et al*. (2015) failed to isolate *B. cereus* from examined samples.

Regarding yoghurt, the obtained result was in agreement with the result documented by Ayoub *et al*., 2003 (20%), but higher incidence was mentioned by Mostafa *et al*. (2022). Conversely, Hassan *et al*. (2010) and Salem *et al*. (2015) found that 2% and 10% of the examined yoghurt samples were contaminated with *B. cereus*.

The high incidence of *B. cereus* in milk was a result of its widespread in the environment, where it is found in the soil, food and the intestine of human and animal (Bottone, 2010). Consequently, raw milk is a vehicle for entry of *B. cereus* in the food chain in addition to, the spores present in the environment and existed in the form of biofilm which persist for a long time in the food chain (Kwon *et al*., 2017). The spores derived from the biofilm have more resistance to heat and cleaning in relation to the spores of other sources (Hayrapetyan *et al*., 2016; Ostrov *et al*., 2016).

B. cereus strains withstand boiling, pasteurization and UHT temperatures in such way this organism can be found in milk products (Vyletelova and Hanus, 2005; Ahmed *et al*., 2011). In addition, *B. cereus* is a psychrotrophic organism that has an intrinsic factor allows its proliferation at low temperature (Reyes *et al*., 2007). The aforementioned data clarified the high percentage of *B. cereus* contamination in pasteurized milk and Damietta cheese.

Most of the isolated strains have the proteolylic and lipolytic activity which has the ability to deteriorate the milk protein and fat and that is important from the economical point of view, hence, they limit the shelf life of milk products and consequently lead to unsolicited sensory changes in the texture, aroma, taste, and the nutritive value of milk (Teh *et al*., 2014). In addition, Montanhini *et al*. (2013) found that, 20°C is the optimum temperature for production of protease and the lowest proteolytic activity at 10°C and So, lots of attention from the manufacturer and researchers must be focused on milk products that maintained at room temperature.

It was obvious from the results recorded in Table 4 that, most of the confirmed strains were diarrhaegenic and its prevalence was 85.3% and all the diarrhaegenic strains carried *Nhe* virulent gene and this was non-compliant to the reported data by Dierick *et al*. (2005) who found that, the main virulent factor is *Hbl* gene. Also, most of studies showed that the pathogenicity of *B. cereus* was owed to the detection of *Hbl* diarrheal enterotoxins. On the other hand, nearly similar results were recorded by Wiwat and Thiramanas (2014) and Hefny *et al*. (2020) and higher results were reported by Ngamwongsatit *et al*. (2008) who could find 65.9% of the isolates carried *Hbl* gene. Lower percentage for detection of *Nhe* gene were demonstrated by Hefny *et al*., 2020 (53.0%) and higher finding was mentioned by Wiwat and Thiramanas (2014) who could detect the gene in 100.0% of the tested strains, moreover, Hefny *et al*. (2020) found lower results for *cytK* (33.0%). Finally, the result of *Ces* gene encding cereulid emetic toxin was nearly similar to the result of Owusu- Kwarteng (2017). Whereas Hefny *et al*. (2020) failed to detect the emetic gene. It was clear from the outcome that was observed in Fig 5 that the three detected virulent genes existed in 16 out of the confirmed strains (50.0%).

It is clearly evident that, pH is one of the most significant dealings used to maintain the bacterial growth and nowadays food of low pH are extensively produced and consumed because of its role in assuring the bacterial stability (Tirloni *et al*., 2017), so, studying the survival of *B. cereus* in yoghurt become an essential matter in this work.

The recorded data in Table 6 declared that, during the first 24 h the reduction rate of *B. cereus* was lower than that recorded at the rest of the experiment, that was a result of the lower reduction in pH at this time. Then, a gradual decrease in the count was observed in the case of yoghurt without *L. acidophilus*, where the bacterial load of *B. cereus* at the 7th day was 4.32 and 4.9 log cfu/ g. for the ES and DS. In contrarily, a high reduction rate was reported in case of yoghurt supplemented with *L. acidophilus* and the count of the ES and the DS was 2.30 and 2.54 log cfu/ g. at the 3rd day and failed to be detected at the 5th day, respectively.

These observations may be as a result to the high reduction of pH in acidophilus yoghurt and the antibiogram of lactic acid bacteria which was dependent on the inhibitory substances that produced during fermentation including oxygen peroxide, aromatic compounds, reuterin, bacteriocins and bacteriolytic enzymes (Madureira *et al*., 2011; Georgalaki *et al*., 2013). Moreover, the results were compatible with the demonstrated data by Guerin *et al*. (2016) who reported that, growth of *B. cereus* can be occurred at pH < 5.4 in aerobic conditions at 10o C. Moreover, the (ICMSF) international commission for microbiological specification for foods (2005) demonstrated that, the pH equal to 5.0 as the limit for *B. cereus* growth. The previously recorded data clarified the sharp decline in the bacterial population of *B. cereus* in yoghurt supplemented with probiotics.

The previously mentioned data was statistically normal with P value > 0.05 and the difference between the behaviors of *B. cereus* strains was not significant, on the other hand, there was a significant difference between the behavior of *B. cereus* in yoghurt and the acidophilus yoghurt with P value < 0.05.

It was obvious that, the reported findings by Hassan *et al*. (2010) were higher than the result that was recorded in our study, where the count of *B. cereus* strain was decreased from 4.47 log cfu/g to > 2 log cfu/g. during the first 24 h. and from 7.95 log cfu/g to 4.27 and > 2 log cfu/g at the 1st and 2nd days, respectively.

On the other hand, Tirloni *et al*. (2017) found that, the low pH values during the whole time of yoghurt storage that was ranged from 3.85 and 4.08 not allow growth of the *B. cereus* strains and the bacterial load was somewhat stable, where there was lower than 0.5 log cfu/ g. increase in the bacterial population.

Ezeonu and Okonkwo (2009) reported that, *B. cereus* grew best and produced toxins at pH 6 to 8, while its growth and toxin production were inhibited at pH 2 to 4 and this is nearly in agreement with the result of this study, where the organism could not be isolated from yoghurt with *L. acidophilus* at pH 4.3 and 4.4 for the examined two strains at the 5th day.

The behavioral Patterns of *B. cereus* growth in Damietta cheese that containing 2% and 5% Salt were nearly similar, where there was decrease of the bacterial population for each strain at the 1st day to 5.02 and 4.46 log cfu/ g. in the refrigerator and to 5.52 and 5.66 log cfu/ g. at room temperature in case of 2% salt. Moreover, the count was 4.90 and 4.97 log cfu/g in cheese of 5% salt that was kept in the refrigerator and the count was 3.32 and 3.58 log cfu/g at room temperature, respectively. The count returned to increase along the experiment and the organism was in a count of 8.95 and 8.93 log cfu/g for cheese of 2% that was kept in the refrigerator at the 4th week and the cheese get spoiled at the 6th week, but the count of the tested strains was 9.91 and 9.95 log cfu/g at the 2nd week at room temperature and the spoilage of cheese was recorded at 3rd week. Regarding the refrigerated cheese of 5% salt, the detected count for *B. cereus* strains at the 6th week was 8.43 and 8.90 log cfu/g and spoilage of the cheese was occurred after two months, but, the cheese that was preserved at room temperature get spoiled at the 4th week.

It is clear that cheese of 10% salt is inconvenient for *B. cereus* growth and the microbial load of the organism declined gradually during the experiment where the count of the tested strains at the 4th week was 2.47 and 2. 31 log cfu/g and couldn't be isolated at the 6th week in case of the refrigeration storage of cheese. The microbial load of the ES and the DS at room temperature decreased to 2.34 and 3.3 log cfu/g at the 4th week for each strain, respectively and the cheese get spoiled at the 12th week.

Statistical analysis of the aforementioned results showed that, the data did not pass the normality test and there was no significance difference between the behaviors of the tested strains of *B. cereus* as well as between the effect of the storage temperature and the salt percentage of 2% and 5%. While there was a

very high significance difference between the patterns of *B. cereus* growth in cheese of 10% salt and the patterns of growth in cheese of 2 and 5% salt with P value < 0.001.

The high increasing rate in the count of the organism in cheese that containing 2 and 5% salted cheese that was stored at room temperature and in the refrigerator was in agreement with the recoded data of other authors which found that the optimum temperature for its growth is between 30-37°C, also, they found that, the lowest temperature for growth was 4°C and the highest was 55°C for few strains can grow at these temperatures (EFSA, 2005). On the other hand, the result of Ezeonu and Ugwu (2009) was not in consistent with our finding, where, they found no growth at 4°C, also, Ezeonu and Okonkwo (2013) recorded that, the best temperature for *B. cereus* growth was 37°C. Moreover, the intense reduction in the microbial load in cheese of 10% salt was parallel to the reported data of Rajkowski and Bennett (2003) who said that, 7.5% salt was the maximum percentage that was tolerated by *B. cereus*.

Because of the perceived safety of natural plant essential oils, they are being explored as safe alternatives to chemical preservatives in food chain (Calo *et al*. 2015).The most of the essential oils have obvious antioxidant and antimicrobial activities in addition to its ability to be used in food Manufacture as biopreservatives to prevent spoilage of food and increase the product shelf life (Burt, 2004). This was the impetus for studying the effect of essential oils on the behavior of *B. cereus* in Damietta cheese.

The data reported in Table 8 clarified that, the bacterial population of *B. cereus* in the presence of the essential oils was decreased and this was revisable to the high increasing rate in its count in control samples (cheese containing 2% salt), Where the count of *B. cereus* was increased from 6.6 and 6.48 to 8.95 and 8.93 log cfu/ g. at the 4th week for each strain, respectively. While, in case of cheese supplemented with thyme the count was decreased to 4.08 and 4.59 log cfu/ g. in case of 0.78% concentration of thyme and was failed to be detected in cheese containing 1.65% thyme at this time, As well as, in case of cheese containing rosemary the count was decreased gradually and the reduction rate was lower than that was recorded for thyme, where, the microbial load was 5.23 and 5.96 log cfu/ g. and 3.11 and 2.99 log cfu/ g. at the 4th week in both concentration of rosemary, respectively.

Based on the statistical analysis of the previously mentioned data, the recoded results was passed the normality and homogeneity test with P value > 0.05 and there was significance difference was recoded between the effect of 1.65% thyme EO and 1.65% rosemary EO on the growth patterns of *B. cereus* strains in cheese with P value < 0.05.

On the other side, the shelf-life of cheese treated with the essential oils was longer than that was recorded for cheese of 2% salt, where the cheese containing thyme in a concentration of 0.78 and 1.65% become invalid for consumption at the 8th and 12th week and at the 8th week in case of 0.78 and 1.65% concentration of rosemary. Although, the cheese get spoiled at the 12th week in both cheese of 10% salt and 1.65% thyme, but, the effect of thyme on the viability of the organism is clearly evident, where, the organism was failed to be isolated at the 4th week relative to its survival in 10% salted cheese that was kept in the refrigerator till the 6th week with account of 2.43 and 2.00 log cfu/ g. for each strain, respectively.

El-kholy *et al*. (2017) reported that, thyme essential oil was the highest effective oils against *B. cereus* and rosemary was the lowest one and this was in comply with our outcome, despite, the detected MIC (0.25% and 0.5%) which was lower than that was recorded in this investigation for thyme and rosemary EOs (0.78 and 1.65%). The difference between the recorded MIC for these oils and the result of other authors may be attributed to the extraction method of the oil (Moreira *et al*., 2005).

Pursuant to the preceding findings and the reported results of Calo *et al*. (2015), it is important to add Thyme EO as a food supplement to manage foodborne infection that is associated with *B. cereus* and to inhibit biofilm formation.

CONCLUSION

It could be concluded that, *B. cereus* is widely distributed in milk and milk products and the enteropathogenic strains are common and this may be attributed to the natural presence of the organism in the environment which led to contamination of milk and dairy products. Therefore, to optimize the quality and safety of these products, good hygienic measures, manufacturing programs and hazard analysis and critical control points must be implanted as well as keeping milk and milk products at low temperature to prevent spore germination and growth of *B. cereus*. Moreover, increasing the salt percentage and inclusion of essential oils and lactobacilli in milk products as biopreservatives are the way to control *B. cereus* growth and to extend their shelf life.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abo- Donia, S.A., 2008. Origin, history and manufacture process of Egyptian dairy products: An Overview. Alexandria J. Food Sci. Technol. 5, 51- 62.

Ahmed, M.M., Metwally, N., Dabiza, M.A., El-Kholy, W.I., Sadek, Z.I., 2011. The effect of boiling on milk microbial contents and quality. J. Am. Sci. 7, 110- 114.

Aouadhi, C., Maaroufi, A. Mejris., 2014, Incidence and characterization of Aerobic spore forming bacteria originating from dairy milk in Tunisia. Int. J. Dairy Technol. 67, 95- 102.

Arora, D., Singh, D., Kapoor, P.K., Singh, K., Jadhav, V.J., Kumar, A., 2014. Detection of *B. cereus* in pasteurized milk sold in Local market of Hisar, Haryana. Haryana Vet. 53,154-155.

Ayoub, M.A., El –Shayeb, T.M., Zaki, M.S.A., 2003. Characterization of *B. cereus* isolated from raw milk and some dairy products. S.C.V. M. J. 1, 123-133.

Beerens, H., Luquet, F., 1990. Practical guide for microbiological analyses of milk and dairy products. Editorial Acribia, S.A., Zaragoza, Spain.

Bhat, S., Kaushal, P., Kaur, M., Sharma, H.K., 2014. Coriander (*Coriandrum sativum* L.): Processing nutritional and functional aspects. African Journal of Plant Science. 8, 25- 33.

Bottone, E.J., 2010. *B. cereus*, a volatile Human Pathogen. Clinic Microbial Rev. 23, 382- 398.

Burt, S., 2004. Essential Oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol. 94, 223- 253.

Calo, J.R., Crandall, P.G., O'Bryan, C.A., Ricke, S.C., 2015. Essential oils as antimicrobials in food systems – a review. Food Control. 54, 111–119.

Chen, L. Coolbear, T., Daniel, R.M., 2004. Detection and impact of protease and lipase activities in milk and milk powder, Int. Dairy J.13, 255-275.

Das, S., Lalitha, K.V., Thampuran, N., 2013. Isolation and molecular characterization of atypical enterotoxigenic *B. cereus* with negative Voges-Proskauer reaction from Indian white shrimp Fenneropenaeusindicus (H. Milne Edwards, 1837). Indian J. Fish., 60, 113-117.

Dierick, K., Van- Collie, E., Swiecika, I., Meyfroidt, G., Delieger, H., Melemans,A., Hoedemaekers, G., Fourie, L., Hyndriks, M., Mahilon, J., 2005. Fatal family outbreak of *B. cereus* associated food poisoning. J. Clin. Microbiol. 43, 4277- 4279.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. EFSA Journal. 175, 1-48.

Ehling-Schulz, M., Guinebretiere, M., Monthán, A., Berge, O., Fricker, M. Svensson, B., 2006. Toxin gene profiling of enterotoxic and emetic *Bacillus cereus*. FEMS Microbiol. Lett. 260, 232–240.

El-kholy, W.M., Aamer, R.A., Mailam, M.A., 2017. Effect of some Essential oils on the quality of UF-Soft cheese. Alex. J. Fd. Sc. Technol. 14, 13-27.

El- Kholy, W.M., Abd El- Khalek, A.B., Mohamed, S.H.S., Fouad, M.T., Kassem, J.M., 2016. Talaga cheese as a functional dairy product. Am. J. Food Technol. 11, 182- 192.

Ezeonu, I.M., Ugwu, C.C., 2009. Isolation and characterization of strains from various food samples. Nig. J. Microbiol. 22, 1700- 1708.

Ezeonu, I.M., Okonkwo, O., 2013. Influence of incubation temperature, pH and food substrate on production of *B. cereus* extracellular proteins and toxins. Afr. J. Microbiol. Res. 7, 2050- 2056.

Fiedoruk, K., Drewnowska, J.M., Daniluk. T., Leszczynska, K., Iwaniuk, P., Swiecicka, I., 2017. Ribosomal background of the *Bacillus cereus* group thermotypes. Sci. Rep. 7, 46430.

Furtado, M.M., 2005. Main problems of cheeses: causes and prevention.

Sao PAulo: Fonte Press.
Gao, T., Ding, Y, m, Wu, Q., Wang, J., Zhang, J., Yu, S., Yu, P., Liu, C., Kang, L., Feng, Z., Chen, M., Wu, S., Zeng, H. and Wu, H., 2018. Prevalence virulence genes, antimicrobial susceptibility and genetic diversity of *B. cereus* isolated from pasteurized milk in China. Frontiers in Microbiology 9, 533.
Georgalaki, M., Papadimitriou, K., Anastasiou, K., Pot, B., Van. Dressche, G. Devreese, B., Tsakalidou, E., 2013. Macedovicin, the second food-grade lanlibiotic Produced by *Streptococcus macedonicus* AcA- DC 198. Food microbial. 33, 124- 130.
Guerin, A., Dargaignaratz, C., Broussolle, V., Clavel, T., Nguyen-the, C., 2016. Combined effect of anaerbiosis, low pH and cold temperatures on the growth capacities of psychrotrophic *B. cereus*. Food Microbiol. 59, 119- 123.
Hassan, G.M., Al- Ashmawy M.A.M., Meshref, A.M.S., Afify, S.I., 2010. Studies on enteroloxigenic *B. cereus* in raw milk and some dairy products. J. Food Saf. 30, 509- 583.
Hayrapetyan, H., bee, T., Groot, M.N., 2016. Sporulation dynamics and spore heat resistance in wet and dry biofilms of *B. cereus*. Food control. 60, 493-499.
Hefny, A., Mohamed, N.M.A., Eltokhy, E.I., Abd El- Azeem, M.W. 2020. Characterization of *B. cereus* isolated from raw milk and milk products. J. Vet. Ani. Res. 3, 205- 212.
Ibanez, M.D., Blazquez, Maria, A., 2021. *Crucuma longa* L. rhizome essential oil from extraction to its agri-food applications. Plants (basel). 10, 44.
Ibrahim, G.A., Sharaf, O.M., El- Khalek, A.B.A., 2015. Microbiological quality of commercial raw milk, Domiatte cheese and Kariesh cheese. Middle East J. Appl Sci. 5, 171-176.
ICMSF (International Commission for Microbiological Specification for Foods), 2005. *B. cereus*. In: Microbological Specification of Food Pathogens. Microorganisms in Foods. Vol 5. Blakie Academic and Professional, London, UK. pp. 20- 35.
JeBberger, N., Dietrich, R., Bock, S., Didier, A., Mortlbauer, E., 2014. *B. cereus* enterotoxins act as major virulence factors and exhibit distinct cytotoxicity to different human cell lines. Toxicon. 77, 49-57.
Kauer, R., Gupta, T.B., Bronlund, J., Kauer, L., 2021. The potential of Rosemary as a functional ingredient for meat products. Food Rev. Int. 16, 1- 21.
Kwon, M., Hussain, M.S., Oh, D.H., 2017. Biofilm formation of *B. cereus* under food processing related conditions. Food Sci. Biotechnal. 26, 1103-1111.
Lampert, L.M., 1975. Modern Dairy Products. 3rd ed Chemical Pub. Co., Inc. New York.
Liang, L., Wang, P., Qu, T., Zho, X., G.E, Y., Chen, Y., 2022. Detection and Quantification of *B. cereus* and its spores in raw milk by PCR and distinguish *B. cereus* from other bacteria of the genus *Bacillus* Food Quality and Safety 6, 1-10.
Logan, N.A., 2011. *Bacillus* and relatives in foodborne illness. J. Appl. Microbiol. 112, 417-429.
Madureira, A.R., Pinlado, M.E., Games, A.M., Malcata, F.X., 2011. Incorporation of probiotics bacteria in whey cheese decreasing the risk of microbial contamination. J. Food Prot. 47, 1194- 1199.
Michel, D. Amin, A., Amer, A., Abo- El-Makarem, H.S., Ashry, S., 2020. Enterotoxigenic profiles Manufacturing steps of traditional soft cheese. A.J.V.S. 66, 45- 51.
Montanhini, M.T.M., Montanhini, R.N., pinto, J.P.N., Bersot, L.S., 2013. Effect of temperature on the lipolytic and proteolytic activity of *B. cereus* isolated from dairy products. Int. Food Research J. 20, 1417- 1420.
Moravek, M., Dietrich, R., Buerk, C., Broussolle, V., 2006. Determination of the toxic potential of *B. cereus* isolates by quantitative enterotoxin analysis. FEMS Microbial. Lett. 257, 293- 298.
Moreira, M.R., Ponce, A.G., Devalle, C.E., Roura, S.I., 2005. inhibitory parameters of essential oils to reduce a foodborne pathogen. Lebens – mittel - wissenschaft und- Technologie Lwt. 38, 565-570.
Mostafa, N.F., ElKenany, R.M., Younis, G., 2022. Characterization of *B. cereus* isolated from contaminated foods with sequencing of virulence genes in Egypt. Braz. J. Biol. 84, 25.
Nabrdalik, M., Grata, K., 2011. Influence of the culture conditions on lipolytic activity of *Bacillus cereus* and *Bacillus mycoids*. Ecological Chemistry and Engineering. 18, 1727- 1735.
NCCLS, 2002. Performance standards for antimicrobial susceptibility testing, Twelfth information supplement. NCCLS document M100- S12, NCCLS, Wayne, Pa.
Ngamwongsatit, W Buasri, W., Pianariyanon, P., Pulsrikan, C., Ohbo, M., Assaving, A., Panbangred, W., 2008. Broad distribution of enterotoxin genes (*Hbl*CDA, *Nhe*ABC, *cytK* and entFM) among *B. cereus* thrringiensis and *B. cereus* as shown by novel primers. Int. J. Food Microbiol.121, 352- 356.
Nieto. G., 2020. A Review: Applications and uses of Thymus in the food industry. Plants. 9, 961
Osama, R, Ahmed, M.F.E., Abdulmawjood, A., Al-Ashmawy, M., 2020. Prevalence and antimicrobial resistance of *B. cereus* in milk and dairy products. Mansoura Vet. Med. J. 21, 11-18.
Ostrov, L., Avraham, H., Salange B., Steinberg, D., Shemesh, M., 2016. Development of a method to determine the effectiveness of cleaning agents in removal of Biofilm derived spores in milking system. Frontires in Microbiology 7, 1498.
Owusu – Kwarteng, J., Wuni, A., Akabanda, F., Tanodebrah, K., Jespersen, L., 2017. Prevalence, virulence factor genes and antibiotic resistance of *B. cereus* sensu isolated from dairy farms and traditional dairy products. BMC Bicrobial. 17, 65.k8
Peter. J.T., Hardin, D.M., 2021. Food safety and quality – based shelf life of perishable foods. In: Food Microbiology and Food Safety. This Springer imprint in published by the registered company aspringer Nature Swezerland AG, The registered company address is : Gewerbestrasse 11. 6330 Cham, Switherland.
Rajkowski, K.T. Bennett, R.W., 2003. *B. cereus* Ch 3 In: Miliotis, M.D., Bier, J.W. (eds) International Handbook of foodborne Pathogens. Marcel Dekker, New York, 27- 39.
Reyes, J.E., Bastias, J.M., Gutierrez, M.R., Rodriquez, M. de, L., 2007. Prevalence of *B. cereus* in dried milk products used by children school feeding program. Food Microbiol. 24, 1-6.
Salem, N.A., El- Jakee, J., Nasef, S.A., Badr, H., 2015. Prevalence of *B. cereus* in milk and milk products. Animal Health Research Journal, 3, 168-172.
Shahidi, F., 2015. Handbook of Antioxidants for Food Preservation. Woodhead Publishing Series in Food Science, Technology and Nutrition, UK. pp. 251- 285.
Singh, V.K., Shukla, S., Chaturvedi, A., 2015. Study the incidence of *B. cereus* isolates from dairy foods. The Pharma innovation Journal 3, 80- 85.
Tallent, S.M., Kotewicz, K.M., Strain, E.A, Bennett, R.W., 2012. Efficient isolation and identification of *B. cereus* group. J. AOSA Int. 95, 446- 451.
Tawab, A.A., Maarouf, A.A.A., Hofy, F.I.E., Mousa, D.H., 2020. Molecular characterization of enterotoxigenic *B. cereus* isolated from meat products and human in Kaluobia, Egypt. Nature and Science. 18, 71-79.
Teh, K.H., Flint, S., Palmer, J., Andrewes, P., Bremer, P., Lindsay, D., 2014. Biofilm An unrecognized source of spoilage enzymes in dairy products. Int. Dairy J. 34, 32-40.
Tirloni, E., Bernardi, C., Celandroni, F., Mazzatini, D., Massimino, M., Stella, S., Ghelardi, E., 2023. Prevalence, virulence, potential and growth in cheese of *B. cereus* strains isolated from fish and short- ripened cheese sold on the Italian market. Microorganisms 11, 521.
Tirloni, E., Ghelardi, E., Celandroni, F., Bernardi, C., Stella, S., 2017. Effect of dairy product environment on the growth of *B. cereus*. J. Dairy. Sci. 100, 7026- 7034.
Tirloni, E., Stelia, S., Bernardi, C., Mazzantini, D., Celanrani, F., Ghelardi, E., 2020. Identification and pathogenic potential of *B. cereus* strains isolated from a dairy processing plant producing PDO Taleggio cheese. Microorganism 8, 949.
Vyletelova, M., Hanus, O., 2005. Vyskyt vybranych palogenu privyrobe UHT melka, jogurtu a syru aajejich vztah K nekterym skupinam mikroorg anismu. Veterinarstvi 9, 576-572.
Wiwat, C., Thiramanas, R., 2014. Detection of haemolysin bl gene of *B. cereus* isolates. Mahindol Unv. Pharm. Sci. 41, 22- 30.
Zhu, K., Acaroz, U., Martlbauer, E.A., 2013. A cellular logic circuit for the detection of bacterial pore- Forming toxins. Chem. Commun. 49, 5198- 5200.