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PREVALENCE OF *BACILLUS CEREUS* AND DETECTION OF SOME ENTEROTOXIGENIC GENES WITH QUALITY EVALUATION IN SOME FAST-FOODS

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ABSTRACT

Sixty samples of fast-food sandwiches were collected randomly from various fast-food outlets in Kafrelshiekh governorate including beef patties (Egyptian Hawawshi), beef burgers and chicken shawarma sandwiches (20, each). *Bacillus cereus* and quality parameters were assessed in the collected samples. The incidence rates of *B. cereus* were 30%, 15% and 15%, with mean values 3.02 ± 2.83, 2.01 ± 1.68 and 2.52 ± 2.08 log10 cfu/g in beef patties, beef burgers and chicken shawarma sandwiches, respectively. PCR findings confirmed 12 isolates, which were positive for the *groEl B. cereus* gene. Additionally, 33.33% of sandwiches that initially tested negative for bacterial isolation carried the *groEl B. cereus* gene. The enterotoxin genes *hbl* and *cytK* were present in 41.66% and 91.66% of the identified *B. cereus* isolates, respectively. Ginger essential oil (GEO) exhibited substantial antibacterial activity against *B. cereus* isolate at Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), about 25% and 30%, respectively. Quality criteria including Aerobic Plate Count (APC) and chemical analysis (pH, TVBN, and TBARS) were performed on the samples. The results revealed that 30% and 10% of the examined beef patties and beef burger sandwiches, respectively, reached the unsatisfactory limit of APC stated by the Center for Food Safety ($\geq 10^5$/g). In addition, a substantial positive correlation was observed between pH, TVBN, TBARS and APC values in the examined samples. Therefore, sanitary procedures should be followed when preparing ready-to-eat (RTE) meals to reduce microbial contamination and public health hazards. Moreover, incorporating spices like ginger into RTE foods could assist in minimizing the presence of *Bacillus cereus* and improve food quality.

Keywords: Aerobic plate count (APC), *Bacillus cereus* enterotoxins, Chemical parameters, Ginger essential oil, meat products sandwiches.

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INTRODUCTION

The fast-food industry has the potential to develop internationally because of people's busy lifestyles and the fact that many individuals, especially young people, spend a lot of time away from home. Fast food is defined as meals that can be prepared and served rapidly in packaged form from restaurants as takeaways (Ajaja *et al.*, 2020). Sandwiches are the most popular fast-food type due to their tasty, simple, and easy preparation. They frequently have a range of components, such as eggs, raw vegetables, and salad dressings like mayonnaise and ketchup. Nevertheless, some factors greatly affect the quality of these sandwiches, including raw vegetables and meat's initial levels of microbial contamination, preparation technique, and the length of time and temperature at which they are stored before consumption (Malak and Soliman, 2021).

Although ready-to-eat (RTE) sandwiches have their benefits as mentioned before, numerous investigations have shown that consuming more of these products raises the risk of foodborne intoxication, since minimal heat treatment is used in such food production (Hussein *et al.*, 2018). It has been discovered that pathogenic bacteria, like *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogene* commonly contaminate RTE sandwiches (Wu *et al.*, 2016).

Gram-positive *Bacillus cereus* is a common foodborne pathogen found in meat products. Its ability to withstand several treatments during food production is attributed to its resistant endospores. Food that may pose a risk has *B. cereus* levels higher than 10^5–10^6/g (Griffiths and Schraft, 2017).

Since meat fast food now constitutes a significant portion of the diet, it is alarming to consider that *B. cereus* may contaminate and grow in RTE sandwiches, threatening public health. *Bacillus cereus* can enter the gastrointestinal tract via ingestion and cause diarrheal and vomiting syndrome, as it can produce a variety of virulence factors (Song *et al.*, 2019).The three enterotoxins hemolysin BL (*Hbl*), cytolysin K (*CytK*) and non-hemolytic enterotoxin (*Nhe*) produced in the small intestinal tract by vegetative *Bacillus cereus* cells result in watery diarrhea and abdominal pain that emerge 8–16 hours following consuming food containing *Bacillus cereus* vegetative spores. These are the main symptoms of diarrheal syndrome associated with food poisoning. On the contrary, the initial signs of emetic food poisoning, including nausea, vomiting, and headaches, appear 1-5 hours after consuming food containing cereulide (a peptide resistant to heat and gastric acid), and the consumption of cereal products, particularly rice is most frequently linked to this kind of food poisoning (Berthold-Pluta *et al.*,2019).

Reducing *B. cereus* contamination in RTE meals is vital since it poses a serious threat to food safety. Essential oils have recently been utilized to increase food's shelf life as natural additives (Tundis *et al.*, 2023). Essential oils comprise complicated combinations of volatile plus aromatic secondary compounds that originate from aromatic plants, typically extracted from fruits, leaves and flowers (Spadaccino *et al.*, 2021). Certain essential oils (EOs) possess antibacterial effects, like ginger essential oil (GEO), derived from the ginger plant (*Zingiber officinale*), has a distinct, pungent scent and is edible when added to food as a food spice (Alsherbiny *et al.*, 2019). In addition, it contains various bioactive compounds, including 6-gingerol, zingiberene and α-curcumin (Kieliszek *et al.*, 2020). GEO has possible applications in the food, cosmetics and pharmaceutical industries (Mesomo *et al.*, 2013). Furthermore, the Food and Drug Administration (FDA) in 2018 designated ginger essential oil as Generally Recognized As Safe (GRAS) (Al-Harrasi *et al.*, 2022). GEO demonstrated significant efficacy, as it

inhibited the normal growth of *Bacillus cereus* FNCC 0057(Ashari *et al.*, 2019).

The most accurate measure of meat quality, hygienic processing, and meat product storage life is the aerobic plate count (APC) (ICMSF, 1980), which measures the degree of bacterial contamination during the production of food (Kim *et al.*, 2018). Meat's pH value is correlated with its chemical characteristics, and hence measuring the meat's pH directly allows for the early diagnosis of spoiling. Total volatile base nitrogen (TVBN) is a useful indicator of protein degradation by tissue enzymes and microorganisms throughout food preservation (Greer and Murray, 1991).

Thus, the objectives of this study were to determine the incidence of *Bacillus cereus* and its virulence genes in some fast-food sandwiches, and to investigate the antibacterial efficacy of ginger essential oil (GEO) against the *Bacillus cereus* strain previously identified in fast-food sandwiches. Additionally, assess the bacteriological quality (Aerobic plate count) and chemical analysis (pH, TVBN, and TBARS) of fast-food sandwiches.

MATERIAL AND METHODS

1. Samples collection

Sixty random samples of fast-food meat sandwiches were obtained from various restaurants in Kafrelsheikh governorate, Egypt. The samples included beef patties (Egyptian Hawawshi), beef burgers and chicken shawarma sandwiches (20 samples, each). All samples were promptly delivered in a cooler under hygienic conditions to the Food Hygiene section lab for the bacteriological examination of their core contents.

2. Preparation of samples

The procedures were followed per ISO 6887-2: (2003)'s method. From every sandwich core, 10 g was weighed and aseptically placed in a sterile mortar with 90 ml of 0.1% sterilized peptone water inside. Then, it homogenized for 2.5 minutes before being serially diluted 10 times using the same diluents.

3. Counting and isolation of *Bacillus cereus*

The *Bacillus cereus* count was carried out in compliance with ISO 7932: (2004). A 0.1 ml of every produced serial dilution of the samples was equally distributed over a dry surface of *B. cereus* selective agar base (CM617, Oxoid, UK) containing egg yolk and polymyxin supplement (SR99, Oxoid). After 24 hours of incubation at 37 °C, the plates were checked for *B. cereus* typical colonies, which are 5 mm in diameter and have a turquoise blue color with the same color hallow zone surrounding them. The plates had a 24-hour re-incubation period prior to performing another count to monitor the additional microbial growth. Suspected colonies were collected on slopes of nutrient agar, then incubated at 37 °C for 24 hours, followed by chilling to 4 °C for morphological and biochemical identification, according to Cowan (1974).

3.1. Detection of *Bacillus cereus groEL* gene and enterotoxigenic genes (*hbl* and *cytK*) using PCR

3.1.1 Extraction of DNA

DNA was extracted from samples using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH), with modifications performed in compliance with the manufacturer's guidelines. In brief, 200 µl of sample suspension with 200 µl lysis buffer and 10 µl of proteinase K were incubated at 56 °C for 10 minutes. Following incubation, the lysate was mixed with 200 µl ethanol (100%). After that, the samples were washed and centrifuged according to the producer's instructions. The nucleic acid was eluted using the elution buffer (100 µl) that was included with the kit.

3.1.2. Oligonucleotide Primer

The primers used were given by Metabion (Germany) and are listed in Table (A).

3.1.3. PCR amplification

A 25 µl reaction including 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol concentration), 5 µl of DNA template, and 5.5 µl of water was utilized to employ the primers. The procedure was carried out using an Applied Biosystem 2720 thermal cycler.

3.1.4. PCR product analysis

Electrophoresis was done on agarose gel 1.5% (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature, utilizing gradients of 5V/cm for separation of PCR products. For gel analysis, 15 µl of every product was placed into a gel slot. The Generuler 100 bp ladder (Fermentas, Germany) and Gelpilot 100 bp plus ladder (Qiagen, Gmbh, Germany) were used to measure the fragment sizes. Pictures of the gel were taken using a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed using computer software.

Table A: Target genes, Primers sequences, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
hbl	GTA AAT TAI GAT GAI CAA TTTC AGA ATA GGC ATT CAT AGA TT	1091	5 min. 94˚C	30 sec. 94˚C	40 sec. 49˚C	1 min. 72˚C	10 min. 72˚C	**Ehling-Schulz *et al.*, 2006**
cytK	ACA GAT ATC GGI CAA AAT GC CAA GTI ACT TGA CCI GTT GC	421	5 min. 94˚C	30 sec. 94˚C	40 sec. 49˚C	45 sec. 72˚C	10 min. 72˚C	
Bacillus cereus groEL	TGCAACTGTATT AGCACAAGC T TACCACGAAGTT TGTTCACTACT	533	5 min. 94˚C	30 sec. 94˚C	40 sec. 55˚C	45 sec. 72˚C	10 min. 72˚C	**Das *et al.*, 2013**

4. Antibacterial effect of ginger essential oil (GEO) against *Bacillus cereus* isolates

4.1. Inoculum preparation

Mueller Hinton agar was used to cultivate the bacterial isolate overnight at 37°C. Following the 24-hour incubation period, colonies were gathered, and the bacterial culture was diluted in sterile nutrient broth, before being incubated for another 24 hours at 37 °C. Once the cells were incubated, the turbidity of the cell suspension was regulated using McFarland 0.5 (1.5×10^8 cfu/ml approximately) (CLSI, 2012).

4.2. Antibacterial activity of ginger essential oil (GEO)

The technique of agar well diffusion was used for screening the antibacterial efficacy of ginger essential oil (GEO) (Harraz, Planta Medical Group, Egypt) against the isolated *Bacillus cereus*. Mueller-Hinton agar (CM337, Oxoid, UK) plates were prepared with 6 mm diameter wells using a sterilized borer and 100 µl of standardized inoculum (1.5×10^8cfu/ml) of the tested fresh bacterial cells was swabbed over the plates's surface.

Thereafter, for each well, 100 µl of GEO was added. The plates were incubated for 24 hours at 37°C, after being left to stand for diffusion to take place for at least an hour. The transparent inhibition zones's diameter surrounding the wells was measured and documented (Okeke and Lamikanra, 2001), and three duplicates of the antimicrobial assay were

performed. The inhibitory zone diameter (mm) was used to express the results: no activity: <9 mm; partial activity: 9–12 mm; activity: 13–18 mm and very activity: >18 mm (Alves *et al.*, 2000).

4.3. Detection of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ginger essential oil (GEO)

The minimum inhibitory concentration (MIC) of GEO was measured by using the broth microdilution method on Mueller Hinton broth (Abou-Dobara *et al.*, 2013), with adding 0.05% tween 80 to improve the oil's solubility in the broth to reach the desired concentrations, which range from 50 µg/ml to 1.75 µg/ml.100 µl from each dilution was blended with the inoculum suspension of *B. cereus* isolate (1.5×10^8cfu/ml). After preparing the positive and negative growth controls, all dilutions were incubated 24 hours at 37°C. Following that, the last tube that showed no evidence of bacterial growth (turbidity) was selected to act as the minimum inhibitory concentration (MIC). Following that, Mueller Hinton agar plates were coated with 100 µl of the tubes that did not exhibit any visible bacterial growth (no turbidity). Then, the plates were incubated at 37°C for 24 hours to determine Minimal Bactericidal Concentration (MBC)(µg/ml), which is known as the minimal concentration of GEO that inhibits 99.9% of bacterial growth.

5. Quality evaluation of fast-food meat sandwiches

5.1. Aerobic plate count (APC)

APC was done in compliance with ISO (4833-1:2013). Two distinct sterile Petri dishes were filled with approximately 1 ml of each dilution previously prepared for each sample. Each dish received around 15 ml of melted, sterilized and tempered standard plate count agar (45°C). Once the inoculation plates were properly mixed, and given time to solidify, they incubated at 37°C for 48 hours. The plates with between 30 and 300 colonies were counted, and after recording each count separately, the colony forming unit (CFU)/gram was computed.

5.2. Chemical analysis

5.2.1. Measurement of pH:

According to Pearson (2006), an electrical pH meter (Bye model 6020, USA) was used to record the pH rate.

5.2.2. Measurement of total volatile basic nitrogen (TVBN): TVBN was assessed in compliance with ES: 63-9/ (2006).

5.2.3. Measurement of thiobarbituric acid reactive substances (TBARS) was carried out following ES: 63-10/ (2006), and is based on malondialdehyde (MDA) measurement, which is considered a lipid peroxidation byproduct.

6. Statistical analysis

Each measurement was analyzed using SPSS 22.0 (IBM Corp., Armonk, NY, USA), (Statistical Package for the Social Sciences). Each parametric data was tested for significance using ANOVA and LSD tests. The outcomes were provided as mean ± Standard Errors (SE). The statistical significance of the samples' mean differences was assessed at $P<0.05$ (a significance level).

RESULTS

Table 1: Statistical analysis of *Bacillus cereus* count in the examined fast-food samples.

Sample type	No. examined samples	positive samples		Min.	Max.	Mean ± SE (log 10)	*Bacillus cereus* count/g*	Unaccepted samples
		No.	%					
Beef patties (Hawawshi)	20	6	30	1.30	3.60	3.02 ± 2.83[a]	> 10^5	0
Beef burger sandwiches	20	3	15	1.69	2.30	2.01 ± 1.68[a]	> 10^5	0
Chicken shawarma sandwiches	20	3	15	2	2.69	2.52 ± 2.08[a]	> 10^5	0

*Center for Food Safety (2014)**,** unsatisfactory limit.
Means of various superscript letters in the same column significantly differ (P< 0.05)

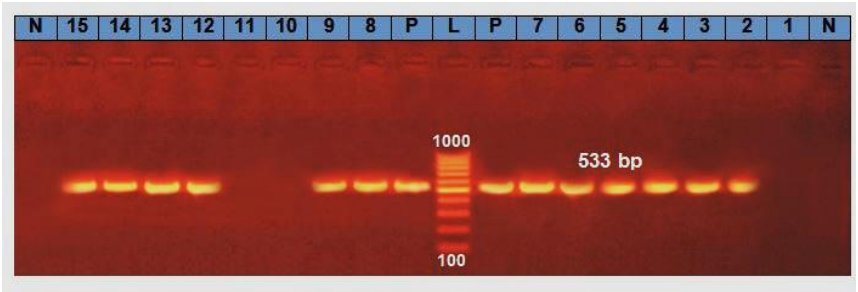

Fig. (1): PCR amplification products of *Bacillus cereus groEL* on agarose gel electrophoresis (No. of isolates = 15)
Lane L: 1000 bp molecular size marker.
Lane P: Positive control, **Lane N:** Negative control.
Lanes: **1: 7** isolates from beef patties (Hawawshi), **8:12** from beef burgers and 13:15 from chicken shawarma sandwiches.
Lanes 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14 and 15: Positive *Bacillus cereus groEL* (533 bp), (80%).
Lanes 1, 10 and 11: Negative *Bacillus cereus groEL* (533 bp).

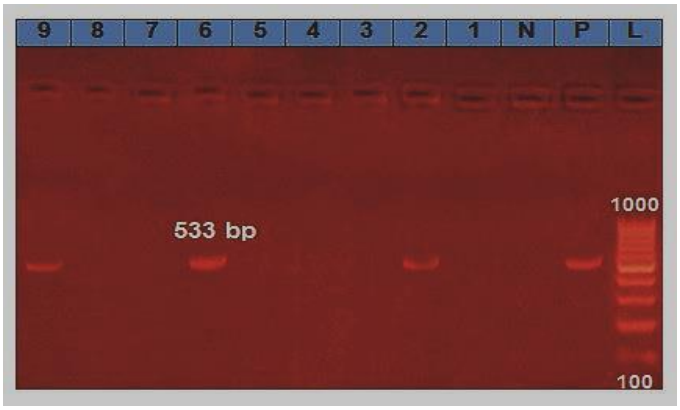

Fig. (2): PCR amplification products of *Bacillus cereus groEL* directly in some negative isolation samples (No. of samples = 9) on agarose gel electrophoresis
Lane L: 1000 bp molecular size marker.
Lane P: Positive control, **Lane N:** Negative control.
Lanes1:3 samples from beef patties (Hawawshi), **4:6** from beef burgers and **7:9** from chicken shawarma sandwiches.
Lanes 2, 6and 9: Positive *Bacillus cereus groEL* (533 bp),(33.33%).
Lanes 1, 3, 4, 5, 7 and 8: Negative *Bacillus cereus groEL* (533 bp).

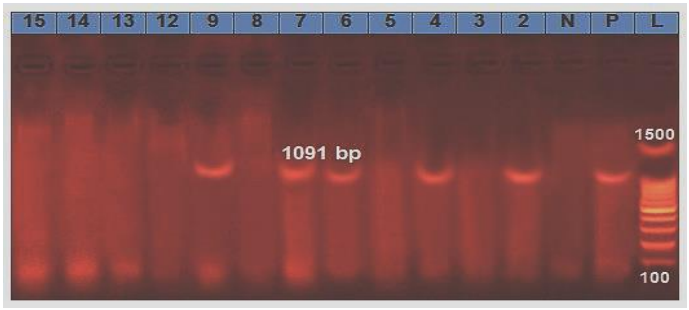

Fig. (3): PCR amplification products of enterotoxic gene (*hbl*) in positive isolates for *Bacillus cereus groEL* on agarose gel electrophoresis (No. of isolates = 12)
Lane L: 1500 bp molecular size marker.
Lane P: Positive control, **Lane N:** Negative control.
Lanes 2, 4, 6, 7 and 9: Positive *Bacillus cereus hbl* (1091bp), (41.66%).
Lanes 3, 5, 8, 12, 13, 14 and 15: Negative *Bacillus cereus hbl* (1091bp).

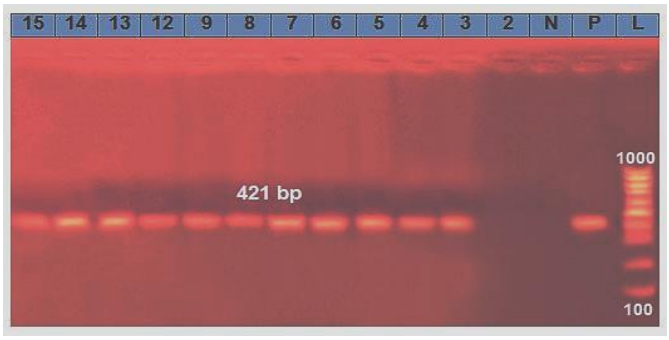

Fig. (4): PCR amplification products of enterotoxic gene (*cytK*) in positive isolates for *Bacillus cereus groEL* on agarose gel electrophoresis (No. of isolates = 12)
Lane L: 1000 bp molecular size marker.
Lane P: Positive control, **Lane N:** Negative control.
Lanes 3, 4, 5, 6,7, 8, 9, 12, 13, 14 and 15: Positive *Bacillus cereus cytK* (421bp), (91.66%).
Lanes 2: Negative *Bacillus cereus cytK* (421bp).

Table 2: Inhibition zone (mm), MIC (Minimum Inhibitory Concentrations) and MBC (Minimum Bactericidal Concentration) of ginger essential oil against *Bacillus cereus* strain isolated from the examined fast-food samples.

Inhibition zone(mm)	Ginger essential oil %	
19 ± 0.58	**MIC**	25.0
	MBC	30.0

Table 3: Statistical analysis of Aerobic plate count (APC) in the examined fast-food samples.

Sample type	No. examined samples	positive samples		Min.	Max.	Mean ± SE (log 10)	APC/g *	Unaccepted samples	
		No.	%					No.	%
Beef patties (Hawawshi)	20	20	100	3.09	5.74	4.87 ± 4.48[a]	$\geq 10^5$	6	30
Beef burger sandwiches	20	20	100	3.03	5.49	4.69 ± 4.21[a]	$\geq 10^5$	2	10
Chicken shawarma sandwiches	20	20	100	3.23	4.88	4.33 ± 3.61[a]	$\geq 10^5$	0	0

*Center for Food Safety (2014), unsatisfactory limit.
Means of various superscript letters in the same column significantly differ (P< 0.05).

Table 4: Statistical analytical results of pH, TVBN and TBARS values in the examined fast-food samples

Sample type	No. samples	PH			TVBN			TBARS		
		Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min.	Max.	Mean ± SE
Beef patties (Hawawshi)	20	6.15	6.59	6.27±0.03[a]	14.67	19.88	16.81±0.49[a]	0.59	0.87	0.71±0.02[a]
Beef burger sandwiches	20	5.85	6.97	6.75±0.06[b]	10.92	17.80	15.71±0.39[b]	0.37	0.84	0.71±0.03[a]
Chicken shawarma sandwiches	20	5.79	6.18	5.97±0.03[c]	15.54	18.20	16.86±0.19[a]	0.30	0.67	0.49±0.03[b]

Means of various superscript letters in the same column significantly differ (P< 0.05)

Table 5: The correlation between Aerobic plate count (APC) and pH, TVBN and TBARS values in the examined fast-food samples

Correlations					
		APC	**pH**	**TVBN**	**TBARS**
APC	Pearson Correlation	1	.118	.440**	.467**
	Sig. (2-tailed)		.370	.000	.000
	N	60	60	60	60
pH	Pearson Correlation	.118	1	.062	.615**
	Sig. (2-tailed)	.370		.636	.000
	N	60	60	60	60
TVBN	Pearson Correlation	.440**	.062	1	.394**
	Sig. (2-tailed)	.000	.636		.002
	N	60	60	60	60
TBARS	Pearson Correlation	.467**	.615**	.394**	1
	Sig. (2-tailed)	.000	.000	.002	
	N	60	60	60	60
** Correlation is significant at the 0.01 level (2-tailed).					

DISCUSSION

One of the possible spoiling bacteria linked to meat products is *Bacillus cereus*, and its high level suggests a potential hazard of producing toxins and occurrence of food poisoning outbreaks globally (Ceuppens *et al.*, 2013). The findings presented in Table (1) clarified *B. cereus* incidence was 30%, 15%, and 15% in beef patties (Egyptian hawawshi), beef burgers and chicken shawarma sandwiches, respectively.

Nearly comparable results were reported by Hussein *et al.* (2018) that detected *B. cereus* in 33.33% of hawawshi sandwiches, as well as 13.3% in chicken shawarma sandwiches by Abou Zeid *et al.* (2023), and 12% in beef burgers sandwiches by Abd El-Rahman *et al.* (2018). Conversely, Hussein *et al.* (2021) observed a higher occurrence of *B. cereus* (60% and 70%) in beef burgers and chicken shawarma sandwiches, respectively, additionally, a higher incidence (40%) in hawawshi sandwiches was observed by El-Shenawy *et al.* (2016). The goodness of the raw ingredients and the sanitary conditions

through the preparation and manufacturing of the product are two potential sources of these variances.

The samples under examination yielded mean *B. cereus* values of 3.02 ± 2.83, 2.01 ± 1.68and 2.52 ± 2.08 log_{10}cfu/g for beef patties (Egyptian hawawshi), beef burgers and chicken shawarma sandwiches, respectively (Table, 1). All of the analyzed samples showed no discernible difference ($p > 0.05$) among them. Furthermore, in accordance with the Centre for Food Safety (2014), which detected an unsatisfactory *B. cereus* limit of $>10^5$ in ready-to-eat food, all analyzed samples were considered acceptable (counts between 10^2 to $>10^3$). Under certain circumstances, such as excessive handling, inadequate hygiene, and inappropriate time and temperature control, these samples constitute an urgent risk because they may encourage *B. cereus* growth and reach unacceptable levels. Food with *B. cereus* counts more than 10^5–10^6cfu/g indicates the organism is actively growing, proliferating and represents a risk to human health (EFSA, 2005).

The results attained were consistent with those of Buyukyoruk *et al.* (2014), who found ready-to-eat sandwiches provided close to hospitals and schools in Turkey had *B. cereus* count of 2 to 3.2 log10 cfu/g, whereas, Hussein *et al.* (2018) reported higher counts of *B. cereus* in burgers and shawarma (3.98 ± 0.25 and 3.17 ± 0.21 log10 cfu/g, respectively), while a lower count in hawawshi (2.84 ± 0.24. log10 cfu/g). Also, Ajaja *et al.* (2020) found higher average *B. cereus* counts in fast-food shawarma sandwiches (1.9×10^5cfu/g), meanwhile, *B. cereus* count was 3.34 ± 0.15 and 3.11 ± 0.13 cfu/g in beef burgers and shawarma sandwiches, respectively according to Hussein *et al.* (2021).

GroEl gene consistently serves as a diagnostic biomarker, compared to the *gyrB* gene to distinguish *B. cereus* from other pathogens found within food products (Wei *et al.,* 2018). Based on the findings of biochemical testing, 15 isolates of *B.cereus* were identified, and 12 isolates out of 15 (80%) were detected using PCR with *B. cereus groEl* gene, were positive for *B. cereus groEl* gene (fig. 1). Moreover, *groEl* gene was found in 3 out of 9 analyzed samples (33.33%) that were taken immediately from food with a negative isolated result (fig. 2). These outcomes were lower than those of Abou Zeid *et al.* (2023) who detected *groEl* gene in 50% immediately from food of a negative isolated result.

Concerning the enterotoxigenic genes, Fig. (3) showed that the *hbl* gene was present in 5 of 12 isolates (41.66%) that tested positive for *B.cereus groEl* gene. These findings are similar to those of Yu *et al.* (2020), who discovered that 39.1% of the *B. cereus* isolated from ready-to-eat meals had the *hbl* gene. In contrast, Abd El Tawab *et al.* (2020 a) reported a lower frequency of the *hbl* gene (10%), while Abd El Tawab *et al.* (2020 b) observed a higher occurrence of the *hbl* gene (70%) in the tested *B. cereus* isolates obtained from meat products. Therefore, different geographic regions and the source of origin may affect the genotype and enterotoxigenic gene incidence. Furthermore, 11 out of 12 isolates (91.66%) that tested positive for *B. cereus groEl* gene had *cytK* gene (Fig. 4). These outcomes align with those of Abd El Tawab *et al.* (2020 b), who found *cytK* gene in 90% of *B. cereus* isolates. In contrast, Abd El Tawab *et al.* (2020a) reported that the *cytK* gene was present in all *B. cereus* strains (100%) that were obtained from various meat items. However, this occurrence was higher than the findings of Abd El-Wahaab *et al.* (2018) and Tharwat *et al.* (2020) who found *cytK* gene in 25% and 31.9% of *B. cereus* strains from examined meat products, respectively.

Haemolysis, dermonecrosis, cytotoxicity and vascular permeability are caused by the *hbl* toxin. Cytotoxin K (*cytK*), which is considered the main virulent agent in

diarrhea, is extremely cytotoxic and can result in haemolysis and necrosis (Lund *et al.*, 2000).

Ginger essential oil (GEO) demonstrated a high antibacterial action against *Bacillus cereus* strain isolated from the tested fast-food samples, as demonstrated by the average diameter of the inhibition zones against the bacterium, which was 19 ± 0.58 mm, based on the agar well diffusion findings presented in Table (2). The results obtained were consistent with the findings of Albaridi and Yehia (2021), who reported that a 25 mm diameter inhibitory zone was observed when ginger extract was used against *Bacillus cereus* which was isolated from cooked rice. Furthermore, the outcomes agree with the study conducted by Harmalkar and Desai (2011), who evaluated the effectiveness of ginger extract against *P. aeruginosa*, *E. coli*, *S. typhi*, *B. cereus* and *Staph. aureus*, nevertheless, it was demonstrated to be effective only towards *B. cereus*, *P. aeruginosa* and *Staph. aureus*.

Minimum Inhibitory Concentration (MIC) refers to the lowest concentration of an antibacterial agent which prevents the microbe from growing (Coyle, 2005). The values for MIC and MBC of ginger essential oil (GEO) towards *Bacillus cereus* were shown in Table (2), which were 25% and 30%, respectively that had been used to quantify the antibacterial activity of GEO towards *Bacillus cereus*. This outcome agreed with Ashari *et al.* (2019), who revealed that GEO at a concentration of 0.25% significantly inhibited the normal growth of *Bacillus cereus* FNCC 0057.

Furthermore, certain research indicated that the antimicrobial properties of GEO originate from its bioactive constituents, which include 6-gingerol, gingerol and α-curcumin. Those essential GEO bioactive ingredients target the cell wall and cell membrane of bacteria to prevent its growth (Lei *et al.*, 2017). Since proteins and nucleic acids are necessary components found throughout the bacterial cell and are released when the bacterial cell membrane gets destroyed (Xu *et al.*, 2018), so membrane integrity is correlated with protein and nucleic acid leakage. This might happen because of the GEO treatment, which increases the cell membrane permeability, causing bacteria to lose their important structural functions and release proteins and nucleic acids from the cell.

Food safety during processing, preparation, and handling is evaluated using the aerobic plate count (APC) (ICMSF, 1978). Based on the results in Table (3), the average aerobic plate counts (APC) of beef patties (Egyptian Hawawshi) were 4.87 ±4.48 cfu/g, with a range of 3.09 to 5.74 log10 cfu/g. Ismail (2006) reported nearly identical results, where the average APC value was 5×10^4cfu/g. However, Aly (2016) found lower mean values ($9.33x10^3$cfu/g) in hawawshi samples. Conversely, Sotohy *et al.* (2019), Hussein *et al.* (2018) and Morshdy *et al.* (2018) reported higher APC values (6.30, 5.9 and 5.56 log cfu/g, respectively) in hawawshi samples. The unsatisfactory limit for APC was $\geq 10^5$, as demonstrated by the Centre for Food Safety (2014). About 6 out of 20 (30%) beef patties samples evaluated in this study were unsatisfactory for microbiological quality and safety, meaning their levels exceeded the upper limit of acceptability for human consumption. However, beef burger sandwiches had an average APC of 4.69 ± 4.21 log10 cfu/g, ranging from 3.03 to 5.49 cfu/g (Table, 3). These results were consistent with those published by Hassanin *et al.* (2015), who recorded 4.8 cfu/g APC, while higher APC mean values were reported by Morshdy *et al.* (2018) and Sotohy *et al.* (2019), since APC were 5.98±0.65 and 6.30±0.08 cfu/g, respectively in beef burger sandwiches. According to the Centre for Food Safety (2014), 2 (10%) out of 20 beef burger sandwiches examined in this study did not meet the acceptable level of microbiological quality and safety (APC), reaching the unacceptable standards for APC ($\geq 10^5$). On

the other hand, APC of chicken shawarma sandwiches varied from 3.23 to 4.88 cfu/g, with an average of 4.33 ± 3.61 log10 cfu/g (Table, 3). Hassanin *et al.* (2015) and Ahmed *et al.* (2015) reported nearly identical results for APC in chicken shawarma sandwiches, determining 4.4 log cfu/g and 4.2 x 10^4 ± 2.8 x 10^3, respectively, whereas Al-Busaidi *et al.* (2023) and El-Fakhrany *et al.* (2019 a) registered higher APC in chicken shawarma sandwiches by 5.48±1.01 and 2.36x10^{10} ± 2.14x10^9 respectively, and Farooq Ahmad *et al.* (2013) recorded lower APC of 8.1x10^2 cfu/g. In this study, all 20 samples (100%) of the microbiologically (APC) examined chicken shawarma sandwiches met the standards established by the Centre for Food Safety (2014) and were deemed suitable for human consumption.

Elevated levels of APC could be a sign of poor quality and potential issues with temperature control (Imperiale, 2017). High microbiological counts in cooked food indicate improper handling practices, a general lack of hygiene, and contamination either during or after cooking. Further research has shown that a variety of surfaces and raw vegetables are contaminated when contact with meat during preparation in kitchens. These microorganisms can survive for extended periods, which can lead to cross contamination (Gillespie *et al.*, 2000).

The outcomes presented in Table (4) indicated that pH average levels for beef patties (Egyptian Hawawshi), beef burgers and chicken shawarma sandwiches were 6.27±0.03, 6.75±0.06 and 5.97±0.03, respectively. Beef burger sandwiches had the highest pH value, followed by beef patties, while chicken shawarma sandwiches had the lowest pH. The degree of microbial spoilage of meat leads to the breakdown of proteins into free amino acids, and the subsequent production of amines and NH3, which are alkaline substances, could be the cause of the pH rises in RTE sandwiches (Karabagias *et al.*, 2011). Malak and Soliman (2021) measured a nearly identical pH value (6.94) in cheeseburger sandwiches at 25 ºC after 60 min. from preparation, while Abd Allah (2011) recorded a lower pH value (6.18) in burger sandwiches. El-Fakhrany *et al.* (2019 b) found that burger sandwiches had a lower pH value of 6.1, and chicken shawarma sandwiches had a higher pH value of 6.5.

TVB-N is often used to estimate the shelf life and rate of deterioration for different meat varieties (Morshdy *et al.*, 2021). The mean values of TVB-N for beef patties (Egyptian Hawawshi), beef burgers, and chicken shawarma sandwiches were 16.81±0.49, 15.71±0.39, and 16.86±0.19, respectively (Table 4). There was no discernible difference between beef patties and chicken shawarma sandwiches, and TVB-N value of beef burger sandwiches was found to be lower. The lower values of TVB-N were recorded by Abd Allah (2011) and Malak and Soliman (2021) in burger sandwiches (10.85 and 14.25, respectively).

The rapid growth of microorganisms' spoilage results in protein deterioration and the production of free amines like trimethyl-amine, dimethylamine and ammonia, which may be the cause of higher TVB-N (Rukchon *et al.*, 2011). Additionally, the sandwiches' shelf life may be influenced by the freezing and thawing of meat (Malak and Soliman, 2021).

TBARS test is sensitive to determine how highly unsaturated fatty acid products break down (Akoh, 2017). Meat cuts kept in a refrigerator or freezer may soon acquire rancid tastes and odors, which causes oxidative degradation and a loss of meat quality (Guillen-Sans and Guzman-Chozas, 1998). The beef patties and beef burger sandwiches had the highest mean TBARS values (0.71±0.02 and 0.71±0.03 mg MDA/Kg, respectively) (Table 4). Conversely, the TBARS value of the chicken shawarma sandwiches was the lowest (0.49±0.03 mg MDA/kg). These results were

consistent with the findings of Malak and Soliman (2021), who stated that 30 minutes after preparation at 25 ºC, the TBARS level in burger sandwiches was 0.71 mg MDA/Kg., whereas El-Fakhrany *et al.* (2019b) found that burgers and chicken shawarma sandwiches had higher TBARS levels (7.81 and 4.63 MDA/Kg, respectively). On the contrary, Abd Allah (2011) found lower TBARS values in burger sandwiches (0.09 ± 0.01). As TBARS levels rise beyond 0.9 mg MDA/kg, rancidity of fat develops, leading to alterations in the flavor, odor, color and loss of sandwiches' nutritional value (Kolakowska, 2003). The use of outdated meat or improper treatment of the meat during processing, which allows the fat to oxidize, may be the cause of the increase in TBARS readings. The given results revealed that all the examined sandwiches could not be deemed rancid, since the TBARS levels were below 0.9 mg MDA/kg.

Aerobic plate count (APC) values had a significant positive correlation with pH, TVBN, and TBARS values of the fast-food meat sandwiches under investigation (Table 5) and there was a significant correlation between aerobic plate count (APC) and TVBN and TBARS values since microbial growth and its proteolytic enzymes can cause protein breakdown, and TBARS is typically regarded as a measure of lipid oxidation, which impacts the sensory characteristics of the food products.

CONCLUSION

A higher risk of foodborne illnesses may result from the isolated strains of *B. cereus* investigated in this study, which carry different pathogenic genes, especially considering the rise in ready-to-eat (RTE) food consumption. Additionally, using the PCR approach straight from food samples would allow for quicker and more effective testing for the identification of *Bacillus cereus* and its enterotoxins, which improves our knowledge of *B. cereus*'s possible toxigenicity in food. Therefore, it is critical to increase awareness and focus on upholding appropriate hygienic measures from farm to fork. This emphasizes the necessity of better educational programs to successfully educate food vendors and consumers about the dangers of improper food preparation. Additionally, it is essential for controlling the initial contamination of raw materials used in sandwiches, since *B. cereus* vegetative cells can be destroyed by washing vegetables and cooking them at an appropriate temperature, and long-term storage of sandwiches at ambient temperature raises the microbial load and deterioration, which may lead to infectious levels to rise and onset of diseases. Subsequently, sandwiches need to be preserved at low temperatures. Furthermore, the addition of GEO (ginger essential oil) was effective in lowering the amount of *B. cereus* contamination in vitro. Therefore, the inclusion of GEO in fast-food meat sandwiches as a natural food additive resulted in a better reduction of *Bacillus cereus* and enhanced the quality of fast-food meat sandwiches.

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مدى انتشار ميكروب الباسيلس سيرس والكشف عن بعض جينات السموم مع تقييم الجودة في بعض الأطعمة السريعة

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زاد الاقبال على الأطعمة السريعة مؤخرا والذي صاحبه بعض حالات التسمم الغذائي. هدفت هذه الدراسة إلى تقييم مدي انتشار ميكروب الباسيلس سيرس والكشف عن بعض جينات السموم مع تقييم الجودة في بعض الأطعمة السريعة. كما هدفت ايضا لاختبار مدى فاعلية زيت الزنجبيل على البكتريا المعزولة. تم تجميع ٦٠ عينة عشوائية من ساندويتشات الوجبات السريعة من مختلف منافذ بيع الوجبات السريعة في محافظة كفر الشيخ والتي تضمنت فطائر اللحم البقري (الحواوشي المصري) وساندويتشات برجر اللحم البقري وشاورما الدجاج (٢٠ لكل منها). تم عزل بكتيريا الباسيلس سيرس وتقييم معايير الجودة في العينات التي تم تجميعها، حيث وجد أن معدل التلوث ببكتيريا الباسيلس سيرس كان٣٠٪ , ١٥٪ و١٥٪ بمتوسط قيم ٣,٠٢ ± ٢,٨٣ , ٢,٠١ ± ١,٦٨ و ٢,٥٢ ± ٢,٠٨ مستعمرة بكتيرية / جرام في الحواوشي المصري وساندويتشات برجر اللحم البقري وشاورما الدجاج على التوالي. وقد أكدت نتائج تفاعل PCR وجود ١٢عترة إيجابية لجين*groEl* المميز لبكتيريا الباسيلس سيرس، بالإضافة إلى ذلك، فإن ٣٣,٣٣٪ من عينات الساندويتشات التي أظهرت نتيجة سلبية لعزل بكتيريا الباسيلس سيرس بالطرق المرجعية كانت إيجابية لجين *groEl* بإستخدام PCR. كانت جينات السموم المعوية *hbl* و*cytK* موجودة في ٤١,٦٦٪ و ٩١,٦٦٪ من عزلات بكتيريا الباسيلس سيرس على التوالي. تم إجراء معايير الجودة على العينات والتى تضمنت العد الكلي للبكتيريا (APC) والتحليل الكيميائي (الأس الهيدروجيني pH، المركبات النيتروجينية الطيارةTVBN وحمض الثيوباربيتوريك TBARS)، وكشفت النتائج أن ٣٠ و ١٠٪ من فطائر اللحم البقري (الحواوشي المصري) وساندويتشات برجر اللحم البقري المفحوصة، على التوالي، وصلت إلى الحد غير المقبول للعد الكلي للبكتيريا الذي ذكره مركز سلامة الغذاء (≤ ٥١٠/جم).أيضا، كشف التحليل الإحصائي عن وجود إرتباط إيجابي كبير بين قيم الأس الهيدروجيني(pH)، المركبات النيتروجينية الطيارة (TVBN)، حمض الثيوباربيتوريك (TBARS) و العد الكلي للبكتيريا في العينات المفحوصة.و قد أظهر زيت الزنجبيل العطري نشاطًا كبيرًامضادًا للبكتيريا تجاه معزول بكتيريا الباسيلس سيرس عند الحد الأدنى للتركيز المثبط (MIC) والحد الأدنى للتركيز القاتل للبكتيريا (MBC) بنسبة ٢٥ و ٣٠٪ على التوالي.لذلك، يجب اتباع الإجراءات الصحية أثناء تحضير الوجبات الجاهزة للأكل من أجل تقليل التلوث الميكروبي الذي يقلل بدوره من المخاطر على الصحة العامة. علاوة على ذلك، فإن إضافة التوابل مثل الزنجبيل إلى الأطعمة الجاهزة للأكل يمكن أن يساعد في تقليل وجود بكتيريا الباسيلس سيرس وتحسين جودة الطعام.