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Papain *(Carica papaya)* and Bromelain (*Ananas comosus)* Enzymes*:* Potential Roles on Gene Expression of *Bacillus cereus* Isolated from Minced Meat and Meat Products

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Abstract

Bacillus cereus is considered a significant foodborne pathogen. Because of the disease's effects on human health and the economy, the regulatory agencies, food safety researchers, and the food industry have paid attention to *B. cereus.* Nonetheless, the need to investigate the efficacy of natural enzyme that act as antimicrobials has arisen due to the growing consumer desire for natural components. Investigating the prevalence of *B. cereus* in different beef products and assessing the effectiveness of papain (Pa) and bromelain (Br) enzymes against *B. cereus* toxigenic genes were the goals of this study. The analysis revealed an incidence of *B. cereus*, especially in rice kofta (44%), followed by sausage and minced meat (34% and 20%), respectively. The antimicrobial agent resistance of the *B. cereus* isolates was ampicillin, oxacillin, amoxicillin-clavulanic acid, ciprofloxacin, ceftriaxone trimethoprim-sulfamethoxazole and gentamicin with percent 100, 81.6, 61.2, 59.3, 57.2, 51 and 44.9, respectively. While, all isolates were sensitive to vancomycin (100%). Enterotoxigenic genes *nhe* and *cyt*K were present in all studied samples (100%), whereas the *hbl* gene was found in 73.3%. Additionally, there was a considerable difference between control and treated groups with papain and bromelain enzymes on physicochemical properties, *B. cereus* count and sensory attributes of minced meat as well as delayed spoilage, highlighting their potential as natural antimicrobial agents. Also, reduction in *B. cereus* enterotoxin genes (*nhe*, *cytK* and *hbl*) expression in tested isolates was observed after exposure to bromelain and papain. The findings underscore the importance of monitoring food safety and the potential role these natural enzymes in mitigating the virulence genes associated with *B. cereus*. The results also suggest a viable strategy for creating antibacterial systems for meat products.

Keywords: *Bacillus Cereus*, Bromelain, Papain, Sausage, Toxigenic genes.

Introduction

Bacillus cereus (*B. cereus*) considered a widespread, highly resilient bacterium responsible for food poisoning, posing a significant concern for food safety. It has the potential to contaminate a diverse range of foods globally. Furthermore, its prevalence in developed countries was considerably less than in the developing countries [1]. The estimated annual incidence of foodborne illnesses attributed B. cereus in the United States is approximately 63,623 case, with a 0.4% readmission rate [2]. The incidence of B. cereus in beef burger was 15% [3] while, it was 29% in minced meat [4]. *Bacillus cereus* (*B. cereus*) is a novel emerging pathogen contaminated extensively in animal feed and food chains, posing a huge economic loss for animal industry and high risk for human health [5]. Food contamination by B. cereus can be exacerbated by several factors, like alterations in eating patterns, the need to feed large populations, Use of complex food ingredients and prolonged preservation, increased global food trade, and insufficient hygiene standards [6]. A member of the Bacillaceae family, this rod-shaped, aerobic, motile, β-hemolytic bacterium is gram-positive. It can

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produce paracentral or centrally located, ellipsoid-shaped spores without causing the sporangium to expand. Furthermore, this bacterium grows across a wide pH range of 4.9 to 9.3, with optimal growth requiring a minimum pH of 4.35 [7].

The pathophysiology of food poisoning caused by B. cereus is still unclear. The microbe transports a wide range of potentially hazardous substances, like proteases, phospholipases, and haemolysins [8-9]. Both diarrheal and emetic illnesses have been connected to B. cereus food poisoning [10]. Cytoxin K, a strong enterotoxin, is assumed to be the major virulence factor responsible for severe diarrhea. In addition, heat-labile enterotoxins cause diarrheal illness. Moreover, the cereulide toxin is the primary cause of the emetic sickness [11]. Among the most frequent virulence factors linked to B. cereus infections are the enterotoxins that are heat-labile: The cytK gene regulates cytotoxin K, the nheABC gene complex encodes non-hemolytic enterotoxin, the hblA BCD gene complex encodes haemolysin BL, the ces gene regulates the cereulide toxin, and the pc-plc gene regulates phospholipase C [12].

The bacteria produce diarrheal toxins as they multiply in the small intestine. In contrast to the diarrheal type, emetic syndrome is brought on by emetic toxin, which is created in the food while the bacteria is growing [13]. Because of their numerous therapeutic advantages and safety, medicinal plants are increasingly being used [14]. Papaya (Carica papaya) is the source of the significant peptidase known as papain (Pa). It may hydrolyse proteins into smaller units because of its high proteolytic capability. The antibacterial activity of papain and other papaya extracts has been documented in many studies against Salmonella typhimurium, B. cereus, Enterobacter cloacae, E. coli, L. monocytogene and Proteus vulgaris [15-17]. Ananas comosus, the pineapple, is the source of the proteolytic enzyme bromelain (Br), which belongs to the Bromeliaceae family. According to certain research, bromelain has both helminthic activity against gastrointestinal worms and an antibacterial impact [18]. Even so, it is unclear exactly how bromelain works to combat bacteria [19], it is believed that by hydrolysing certain peptide bonds found in the cell wall of bacteria, Br may prevent bacterial development [20].

The public can purchase Br, which is regarded as a food supplement, at pharmacies and health food stores across the US and Europe. It can pass through the human gut without breaking down or losing any of its biological function. Bromelain has a minimal potential for toxicity and mutagenicity, according to numerous assessments [21]. Thus, the primary aim of this investigation is to determine the prevalence of B. cereus genes in meat products including hbl, nhe, and cytK genes. As well as to assess the effects of Pa and Br on B. cereus count inoculated in minced meat and its virulence genes.

Material and Methods

Sampling

One hundred fifty samples of minced meat, sausage, and rice kofta (50 each) were randomly gathered from various supermarkets in Menoufia Province, Egypt in the period From February to June 2023, then, samples were transferred to the laboratory for examination.

Preparation of the samples

The samples were moved right away to a fully aseptic condition in order to isolate and identify *B. cereus.* Briefly, 225 ml of 0.1% sterile buffered peptone water (Oxoid, UK) were mixed with 25 gm. of each product, and the mixture was stomached for two minutes to produce a homogenate **[22]**. As a primary enrichment, 1 ml of the original dilution was transferred to a sterile tube together with 9 ml of sterile buffered peptone, and then incubated at 34°C for 24 hours.

Isolation and characterization of B. cereus

A bent glass rod was used to streak the bottles, which displayed turbidity over the top of *B. cereus* selected agar medium (Oxoid, United Kingdom), According to [25] the disc diffusion method was used to test for antibiotic susceptibility using ten commercial antibiotic discs (Oxoid, UK): ampicillin (10 µg) , amoxicilin (10 µg), vancomycin (30 µg), clindamycin (2.0 mg/ml), erythromycin (15 mg/ml), gentamicin (10 µg), sulfamethoxazole/trimethoprim (25 µg), and ceftriaxone (30 µg), erythromycin (15 µg) chloramphenicol (30 µg).The plates were then incubated at 37°C for 18-24 hours. The inhibition zone is interpreted by comparing it to the established CLSI breakpoints to categorize the antimicrobial susceptibility as Susceptible, Intermediate or Resistant [26]. Molecular detection of *B. cereus* enterotoxins genes *nhe*, *cyt*K and *hbl* according to Ehling-Schulz [27]

Preparation of plant enzyme concentrations

In this investigation, plant enzymes, papain (Pa) and bromelain (Br), were purchased from Sigma-Aldrich Chemie GmbH (Munich, Germany) supplied papain (EC 3.4.22.2) from papaya (Carica papaya) and bromelain (EC 3.4.22.32) from pineapple (Ananas comosus) stems (enzymatic activity 3000 U/g). A 0.45 µm syringe filter membrane was used to sterilize both enzymes.

Determination of MIC of Pa and Br against B. cereus

Then the plates incubated at 30°C for 48 hours. To investigate hemolysis, putative typical colonies were inoculated onto sheep blood agar (Oxoid, UK) and incubated at 37°C for 24 hours [23]. Moreover, according to [23, 24] biochemical reactions such as lecithinase, oxidase, motility, and sugar fermentation

(mannitol, xylose, and arabinose) were employed for additional validation

Antimicrobial Susceptibility test

Double fold serial dilution of Br (bromelain) and Pa (papain) enzyme (100, 50, 25, 12.5, 6.25 and 3.125 mg/mL) were tested against *B. cereus* using disc diffusion method according to Doughari [28].

Inoculum preparation

The bacterial concentration (10^7cfu/ml) of *B. cereus*, was first determined using the McFarland standard (0.5 McFarland, or ~1.5×10^8 CFU/mL). This was followed by serial dilution in sterile saline. , the final concentration was verified by plating suitable dilutions on aerobic plate count and counting colony-forming units (CFU) following incubation.

Application of Br and Pa fresh minced meat

Thin sheets of minced meat [4kg (1000g for each group)] sterilized with UV-B (Medium wave 280nm for 20 minutes) according to Hinds [29]. After complete sterilization all group inoculated with *B .cereus* (10^7cfu/ml) using spraying method and mixing well to ensure even distribution. Then four groups were prepared, the first group, Control, while the second (Br 50 mg/ml), the third (Pa 50 mg/ml) the forth (Br:Pa 25 mg/ml) the groups treated with Br and Pa using spraying method and mixing well to ensure even distribution of the enzymes then refrigerated for one hour ($4 \pm 1^{\circ}$C). Then assessed for the physicochemical quality, *B. cereus* count and sensory qualities, After that, the groups were kept at $4 \pm 1^{\circ}$C in sterile polyethylene plastic bags for further examination until spoilage occur (Fig. 1).

Physicochemical examination

The Jenway 3510 Standard Digital pH Meter was used to measure the pH value (230 VAC/UK) (ES 63-11/2006). The TBARs as a parameter used to determine the oxidative status was measured according to Choe [30]. According to Cai et al. (2014), the Kjeldahl method was used to determine TVB-N content.

B. cereus count

For preparation of initial dilution, 225 ml of peptone water (HIMEDIA, M028) were mixed with 25 gm of the sample, and the mixture was stomached for two minutes to produce a homogenate, tenfold serial dilution on peptone water then 0.1 ml distributed to the *B. cereus* selective agar medium surface (Oxoid, UK), then the plates incubated at 30°C for 24 to 48 hours

Sensory attributes

Nine trained male and female panelists, ages 40 to 45, assessed sensory qualities in a controlled setting with 22°C, 55% humidity, and light. They were chosen according to ISO [31]. The attributes (odor, color, and texture) served as the foundation for the samples were evaluated using a continuous nine-hedonic scale and the organoleptic descriptive assessment. The panel was given a set of descriptors to rate on continuous and numerical scales ranging from 0 (very bad) to 10 (very good) according to Cullere [32].

RT-PCR analysis of B. cereus enterotoxin gene expression in different meat products

Minced meat, sausage, rice kofta were weighed as 80 gm, then added to 50 ml distilled water in a flask (3 flasks for each) for sterilization by autoclaving for 25 min. Briefly, *B. cereus* isolates were enriched in BHI broth media at 37°C overnight, and then 2 colonies were inoculated into 10 ml sterile saline. One ml is added to the sterilized flasks and vigorously shaken, then incubated for 16 to 18 hr. at 37°C according to Wei [33] with some modifications.

Analysis of the SYBR green rt-PCR results

Performed according to the method stated by Yuan[34]

Statistical analysis

Data management and its analysis statistically conducted with GraphPad Prism 8.0.2 (263). The data normality was evaluated through the Shapiro-Wilk test. The quantitative data were expressed as means and standard deviations. The significance of *B. cereus* incidence was performed using Chi-square test. The mean values of disc diffusion diameters of the inhibition zones of Pa and Br against *B. cereus* were analyzed using paired t-test. The multiple comparisons between the studied groups in the trial were performed using one -way ANOVA. Also, Pearson's correlation was utilized to assess relationships between control and treated groups. Independent t- test was performed between control and treated groups to evaluate the significance of fold change result on genes regulation. P values less than 0.05 were considered statistically significant for all two-sided statistical tests. The trials were conducted in triplicate [35].

Results and Discussion

Incidence of B. cereus isolates and their phenotypic characteristics

Incidence of *B. cereus* varied among the meat products under investigation indicating a significant difference between minced meat, sausage and rice kofta ($p < 0.05$) as it was 20, 34 and 44%, respectively (Fig.2). One of the underreported foodborne illnesses in the globe is meat product contamination with toxic *B. cereus* [36]. Although accurate monitoring data on the frequency of *B. cereus*-caused food poisoning cases in Egypt is

lacking, but higher results were attained by Tharwat [37] as the authors isolated *B. cereus* from minced meat 76% while, nearly similar incidence in sausage with 32%. The symptoms may be similar to those of other foodborne infections, which could account for the paucity of reliable data [38] also, This result is in line with the findings published by Mohamed and Ghanyem [39] and is higher than the findings of Heikal [40]. The higher incidence rate of *B. cereus* in rice kofta and sausage as opposed to minced meat can be ascribed to the use of seasonings, spices, and additives, on the other hand, it is lower than the findings of [41]. Foodborne illness may become more common as a result of these additives, which are a possible risk factor that could increase Bacillus spore proliferation. Overall, 32.66% of the tested meat and meat products in the collected samples contained *B. cereus*. Lower results were obtained in turkey in which prevalence of *B. cereus* in meat was 22.4 % [42]. A nearly similar prevalence was obtained by Tewari [43] (27.8 %) and Naas [44] who stated a prevalence of 29 % .

All the bacterial isolates were Gram-positive, short bacilli, motile, with non-bulging endospores, and they all displayed the characteristic morphological features of *B. cereus*. Colonies on *B. cereus* media had a distinctive precipitation zone and were pink (lecithinase positive) fig.3 (A,B). Additionally, tests for catalase, citrate utilization, Voges-Proskauer, glucose fermentation and nitrate reduction, were positive for the isolates. Similarly, oxidase, H_2S generation, methyl red, indole, and mannitol fermentation tests were negative for the isolates.

Antimicrobial susceptibility

The susceptibility of 49 *B. cereus* isolates to antibiotics was further examined. Table 1 shows the antimicrobial agent resistance of the B. cereus isolates. It was identified as follows: ampicillin (100%, *n* = 49), oxacillin (81.6%, *n* = 40) amoxicillin-clavulanic acid (61.2%, *n* = 30), ciprofloxacin (57.2%, *n* = 28), ceftriaxone (59.3%, *n* = 19), trimethoprim-sulfamethoxazole (51%, *n* = 25), gentamicin (44.9%, *n* = 22). Moreover, all isolates were sensitive to vancomycin (100%, *n* = 49) and erythromycin (91.8%, *n* = 45) and clindamycin (87.8% = 43). The findings were differed from that obtained by Shawish and Tarabees [45] as A total of 51 B. cereus isolates were further tested for their antimicrobial susceptibility. All the tested isolates were resistant to penicillin G, whereas sensitive to oxacillin, clindamycin, vancomycin, erythromycin,) 41 gentamicin, ciprofloxacin, and ceftriaxone. While, the data obtained here in nearly is agreed with Organji [46], Jawad [47] as their results demonstrated that Bacillus cereus exhibits a wide range of antibiotic susceptibility patterns and confirmed its resistance to penicillin G, as evidenced by contrasting susceptibility to clindamycin, vancomycin, and erythromycin.

Presence of B. cereus entertoxigenic genes

The multi-drug resistant isolates of *B. cereus* (n=15) were examined for their virulence-related genes and results showed that *nhe* genes (100%, *n* = 15), *cyt*K (100%, *n* = 15), followed by *hbl* (73.3%, *n* = 11) as shown in table (1) and figure (4A, 4B, 4C). PCR has been recognized as the fastest and most reliable approach for confirming enterotoxigenic strains [48, 49]. This was consistent with the results achieved previously by Awny [50].

Bacillus cereus isolates have some virulence factors that possess its significant pathogenicity and virulence such as lipases, hemolysins, proteases and enterotoxins [51]. These virulence factors were expressed potentially by several genes as *nhe*, gene of nonhemolytic enterotoxin, and *hbl*, gene of hemolysin BL [52, 53]. Besides cytotoxin K *(cyt*K*)* gene and improved the *B. cereus* ability to cause diarrheal syndrome [36]. The role of various enterotoxins in increasing B. cereus's pathogenicity principally *nhe* gene is supported by Many investigations as in Gao [54] who reported that *B. cereus* had enterotoxigenic genes in about 45% of cases and the incidence of *nhe ABC* gene complex in the majority of isolates in addition the presence of other genes as *cyt*K, *ces*b, *ent*FM, *hly*II and *bce*T). As well as, Hendriksen[55] mentioned that *nhe*, *hbl* and *cyt*K are dominant genes responsible for enterotoxins production in *B. cereus* isolates. Results that were almost identical to those Shawish and Tarabees [45] who discovered that the *hblC* and *cytK* genes were detected in 18 isolates (90%) and 20 isolates (100%) respectively.

The result is similar to the results of Shawish and Tarabees [45] who demonstrated that *cytK* gene was the key virulence gene in isolates of *B. cereus* with 100% and followed by *hblC* gene with 90%. Furthermore, the enterotoxigenic profile of the isolates reported by Abdeen [51] who stated that, *nhe* gene was the most predominant gene among the isolates. The prevalence of *cyt*K and *hbl* genes was 55.5% and 33.3% respectively. Also, [56], found the *nhe* virulace gene in more than 97% of the isolates, whereas *hbl* and *cyt*K were 66% and 50%, respectively. Interestingly, the wide variety in incidence of *hbl* genes of the *B. cereus* is controlled by many factors such as food matrix, nutritional availability and suitable temperature for growth [57] or probably due to the influence of *hbl*, hemolytic enterotoxin gene that encoded chiefly by the *hbl*CDA cluster [58].

Effect of bromelain (Br) and papain (Pa) on B. cereus

As seen in table (3) the diameter of inhibition zone varied according to Pa and Br enzyme concentrations as it was 21 ± 0.1 mm for Pa with conc. 100 mg/ml while the inhibition zone disappeared at 6.25 mg/ML. More pronounced

antibacterial effect was seen at 100 mg/ML of Br (24 ± 0.2 mm). Sani [59] revealed that papaya extract has antibacterial effect against *B. cereus* with MIC of 11.25 mg/ml. Bromelain (Br) preferentially cleaving at amino acid sites involving lysine, alanine, tyrosine and glycine. Whereas, papain prefers hydrophobic sites that include Valine and also Lysine. The enzyme breaks bonds at selected locations dividing the protein chain into fragments.

Gram-negative bacterial cell walls are more complex than Gram-positive cell walls containing an outer membrane comprised of protein, lipoprotein and lipopolysaccharides, a peptidoglycan layer then a plasma membrane that also contains proteins. Gram-positive bacteria have a thick peptidoglycan layer and an inner plasma membrane. The surface layer of both Gram-positive and Gram-negative bacteria contains protein components that can be targeted by proteases to compromise cell wall structure to varying degrees. For example, the peptidoglycan layer (outer layer of Gram-positive bacteria) consists of subunits that are joined by crosslinks between the amino group of one amino acid and the carboxyl group of alanine [60], a preferred scission site for bromelain. In contrast, Gram-negative bacteria have an outer lipopolysaccharide layer that contains porin proteins that a lined with exclusively charged amino acids to facilitate passage of molecules through the membrane [61], papain prefers uncharged (hydrophobic) amino acid sites so the antibacterial activity of Br is more prominent against gram positive bacteria (*B. cereus*) than Pa [62].

Effect of bromelain (Br) and papain (Pa) on the quality examination of minced meat

One crucial factor that affects the quality, safety, and shelf life of minced meat is its pH. As seen in fig. (5) The control group's pH levels steadily elevated from 5.75 to 6.29 during the cold storage period at 4^{th} day then exceed the permissible limit at 6^{th} day (6.55) [63]. The difference between the control and treatment groups was significant ($p<0.05$). Also, papain treated group increased during storage but in lower rate than control one. While group treated with bromelain and mixture group decreased from 5.71 and 5.70 to 5.52 and 5.55, respectively at 4^{th} day of storage then start to rise till the storage term is over (16^{th} day) with no significance difference ($p > 0.05$). The correlation coefficient (r) was 0.97 with $p < 0.0001$ between Control and Pa 50 mg/ml, 0.81 with $p = 0.01$ between Control and Br 50 mg/ml and 0.79 with $p = 0.01$ Control and Br: Pa 25 mg/ml with indicated that the least correlation in pH between different groups was observed between control and mixture group. This increase in pH values during storage due to *B. cereus* growth and protein degradation resulting in production ammonia which deprotonates H_2O and generate ammonium ions and hydroxide OH- [64]. Studies have shown that the application of bromelain and papain can lead to different trends in pH changes during storage. For example, while untreated minced meat often exhibits an increase in pH due to microbial activity and the breakdown of amino acids into basic compounds, the addition of these enzymes can modulate this effect [65, 63]. Br and Pa may initially lower the pH due to their proteolytic activity, which can lead to the release of organic acids from protein degradation. This is particularly crucial when microbial populations are still low in the early phases of storage [66]. In contrast, enzyme-treated samples may show more stable or even decreasing pH levels due to reduced microbial loads facilitated by the antimicrobial properties of Br and Pa or their ability to enhance meat tenderness without promoting spoilage [67].

The effects of Pa and Br on TBARs in minced meat are significant in evaluating their potential as meat preservative agents as TBARs are commonly used as indicators of lipid oxidation in food products, which can lead to off-flavors, rancidity, and reduced shelf life [68]. As presented in fig.5 the value of TBARs increased gradually in treated groups with Pa and Br but in lower rate than control one without exceed the permissible limit as there were significance differences in TBARs values of control group and groups treated with Pa and Br. This lower rate of increasing TBARs values indicated a lower extent of lipid oxidation, which is crucial for maintaining meat quality during storage. These enzymes' protective properties could be a factor in this outcome. by scavenging free radicals or by modifying the lipid composition in a manner that makes them less prone to oxidation [69].

Data in fig. (5) Revealed that the mean of value TVB-N in control group ranging from 2.4 to 41.2 mg/100g at the end of refrigerated period (16^{th} day). Minced meat samples of control group begins to spoil as the TVB-N levels increase exceeding 20 mg/100g [63] and in advanced spoilage, it reaches above 30 mg/100g with significant difference compared to treated groups. The rate of alteration decreases in group treated mixture of Br and Pa followed by Br and Pa respectively. Similar results obtained by [70] as the authors found that the level of TVB-N increases in lower rate in samples treated with Pa and Br. Increasing TVB-N during storage occurs due to the quick of *B. cereus* bacteria growth which caused breakdown of protein and the production of free amines such trimethylamine and dimethylamine as well as ammonia as it produces protease enzyme [71].

Up to the end of the storage period, *B. cereus* count revealed considerably lower levels in all treated groups than control one (Fig. 6). At 16^{th} day of cold storage, the mean count of control group increased from 7.47(log cfu/gm) to 9.10 (log cfu/gm). The highest reduction in *B. cereus* count became evident in group treated with mixture of Pa

and Br followed by Br treated group while, the lowest reduction observed in group treated with Pa only with significance difference between the treated group. Emeruwa [72] found that the extract of papaya fruit had antibacterial activity against *B. cereus*. The cell wall is ruptured when Br breaks down the surface proteins, allowing the cell contents to leak, enlarge, and collapse [73]. Therefore, the way that Pa and Br inhibit bacterial species supports the earlier finding Br is a cysteine peptidase with endopeptidase activity that is classed as a subfamily of Pa because of their close similarities in mechanism of action. Although, Br accelerates the breakdown of muscle proteins into smaller peptides and amino acids which can potentially lead to an increase in TVB-N, however, studies have shown that Br, when used in for meat, can actually reduce the rate of increase of TVB-N during storage. This is attributed to bromelain's antimicrobial properties, which inhibit the growth of spoilage bacteria responsible for converting amino acids into volatile nitrogenous compounds [70]. They are different from Pa in minor areas in their amino acid sequence, such as the absence of asparagine-175 and the transition from serine-176 to lysine [74].

The results sensory attributes scores are presented in Fig. (7).The scores of sensory attributes exhibited a significant decrease in the color, odor and texture during refrigerated storage period but the rate of decrease was higher in control group than treated ones with Pa and Br. Pa and Br enzymes, which are plant proteases, are frequently employed to tenderise meat [75] also, they recognized as safe materials in meat industry [76] increasing meat-eating quality [77]

Enterotoxins gene expression after exposure to bromelain and papain

The RT-PCR investigations demonstrated reduction in *B. cereus* enterotoxin genes (*nhe*, *cyt*K and *hbl*) expression in tested isolates after exposure to bromelain and papain as presented in table (4) as the fold change was less than one which means downregulation also, there were significance difference between control and treated groups ($p < 0.0001$). Furthermore, the results showed that the effect of Br was more pronounced than Pa which may reflect variation in protease activity or penetration ability. Up till now, no obtainable information has been reported about effectiveness of exposure to Br and Pa on *B. cereus* enterotoxin gene expression in different meat products. Eshamah, Han [16], dos Anjos, da Silva [78] described the antibacterial activity of Pa against different of pathogens. Also, several studies suggested that the Pa antibacterial activity can be connected to some enzymatic actions, such as esterase and amidase and extend this interpretation also to Br due to its structure which is very similar to that of Pa [78, 79]. It is well-documented that Pa and Br have a great proteolytic action causing cell walls rupture and death of the bacterial cells through hindering of protein or bacterial cell walls synthesis of pathogens as *S. aureus, E. coli, P. vulgaris, Klebsiella pneumonia, S. typhimurium* and *B. subtilis* [20, 66].

Importantly, the possible explanations behind rendering the antibacterial potency of *Pa* and Br, their content of organic acids and a phenolic compounds [80, 81]. Moreover, Varilla [82] revealed that Br manifests significant apply as a prophylaxis against *E. coli* enterotoxigenic bacterial (ETEC) infection by temporarily inactivation of ETEC receptors in vivo through interacting with intestinal secretory signaling pathways.

Conclusion

The antibacterial effect of papain and bromelain against B. cereus and its entero-toxigenic genes highlights their potential as natural antimicrobial agents. Given the rise in antibiotic resistance, further research into the specific mechanisms and applications of these enzymes could pave the way for new strategies in food safety and public health as their incorporation into food preservation methods or dietary supplements may offer a promising avenue for combating foodborne pathogens effectively.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Authorship contribution statement

Asmaa Elgendy: Searching about the suitable methodology, performing the microbiological examination and writing this part. Mohebat Abd El-Aziz: Searching about the suitable methodology dealing with the trail on minced meat quality, analysis of data statistically and writing this part. Hanem Khalifa: Review the RT-PCR technique and writing its results and explain the effect of Pa and Br on toxigenic genes of *B. cereus*.

Ethical of approval

The panellists informed consent via the statement “I am aware that my responses are confidential, and I agree to participate in the sensory evaluation”. They were able to withdraw at any time without giving a reason. The products tested were safe for consumption.

TABLE 1. Antibiogram of the obtained *B. cereus* strains (n = 49)

Antimicrobial	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Ampicillin	0	0	0	0	49	100
Oxacillin	9	18.4	0	0	40	81.6
amoxicillin-clavulanic acid	10	20.4	9	18.4	30	61.2
Ceftriaxone	14	28.6	5	10.2	30	61.2
Ciprofloxacin	15	30.6	6	12.2	28	57.2
trimethoprim-sulfamethoxazole	17	34.7	7	14.3	25	51
Gentamicin	19	38.8	8	16.3	22	44.9
Vancomycin	49	100	0	0	0	0
Erythromycin	45	91.8	0	0	4	8.2
Clindamycin	43	87.8	0	0	6	12.2

TABLE 2. Molecular detection of enterotoxigenic genes of *B. cereus* isolated from examined samples.

Target gene	NO of examined isolates	Positive isolates	%
Nhe	15	15	100
***Cyt*K**	15	15	100
Hbl	15	11	73.3

TABLE 3. MIC of varying concentrations of Papain (Pa) and Bromelain (Br)

Concentrations	Papain (Pa)	Bromelain (Br)	p value
100 mg/Ml	21 ± 0.1 mm	24 ± 0.2 mm	
50 mg/Ml	14 ± 0.2 mm	18 ± 0.3 mm	
25 mg/mL	10 ± 0.1 mm	14 ± 0.1 mm	0.008
12.5 mg/mL	7 ± 0.1mm	10 ± 0.2 mm	
6.25 mg/mL	ND*	6 ± 0.1 mm	
3.125 mg/mL	ND*	ND*	

The values are expressed as the mean of triplicate ± SD, the difference between values are significant at $p < 0.05$. ND* means that the inhibition zone not detected.

TABLE 4. RT-PCR expression of *cyt*K, *nhe* and *hbl* genes in *B. cereus isolated* from different studied groups of minced meat, sausage and rice kofta treated with bromelain (Br) and papain (Pa).

Sample type	Treatment	Sample No.	*dnaJ*	*hbl*		*Nhe*		*cytK*		*P value*
			Cycle threshold (CT)	Cycle threshold (CT)	Fold change	Cycle threshold (CT)	Fold change	Cycle threshold (CT)	Fold change	
Minced meat (M)	Control	**M**	19.78 ± 0.32	21.86 ± 0.22	-	22.77 ± 0.15	-	20.55 ± 0.26	-	
	Bromelain	**M1**	19.71 ± 0.31	24.32 ± 0.18	0.1731±	26.82 ± 0.17	0.0575	22.18 ± 0.32	0.3078	
	Papain	**M2**	19.54 ± 0.25	22.50 ± 0.15	0.5434	24.10 ± 0.14	0.3368	20.97 ± 0.35	0.6329	
Rice kofta (R)	Control	**R**	20.80 ± 0.22	20.51 ± 0.33	-	21.06 ± 0.13	-	21.12 ± 0.27	-	
	Bromelain	**R1**	19.27 ± 0.16	21.22 ± 0.14	0.2117	22.71 ± 0.22	0.1103	21.70 ± 0.32	0.4633	
	Papain	**R2**	20.19 ± 0.14	20.77 ± 0.11	0.5471	21.54 ± 0.15	0.4698	21.91 ± 0.25	0.7579	*P* < 0.0001
Sausage (S)	Control	**S**	20.59 ± 0.15	21.43 ± 0.12	-	22.29 ± 0.33	-	21.34 ± 0.12	-	
	Bromelain	**S1**	20.79 ± 0.19	22.82 ± 0.15	0.4383	24.47 ± 0.21	0.2535	22.15 ± 0.12	0.6552	
	Papain	**S2**	19.85 ± 0.16	21.26 ± 0.13	0.6736	22.48 ± 0.25	0.5249	21.87 ± 0.12	0.8293	

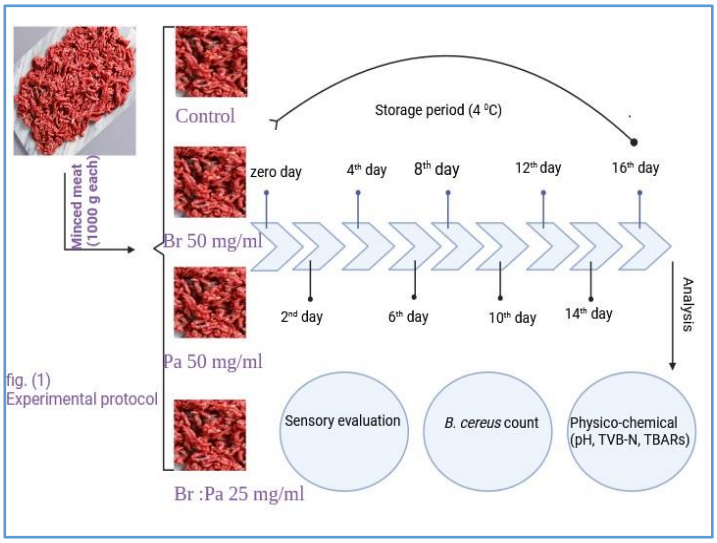

Fig. 1. Application of Papain (Pa), Bromelain (Br) on fresh minced meat

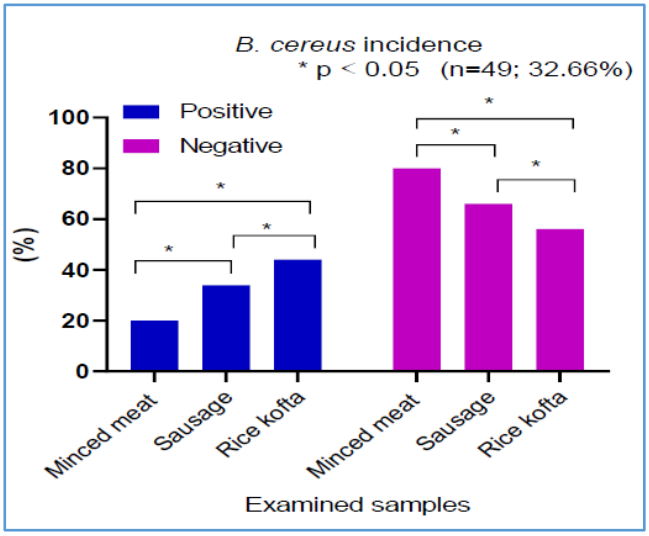

Fig. 2. The incidence of *B. cereus* isolated from minced meat and meat products (sausage and rice kofta) (n=50), the results are significantly different at p < 0.05 (*)

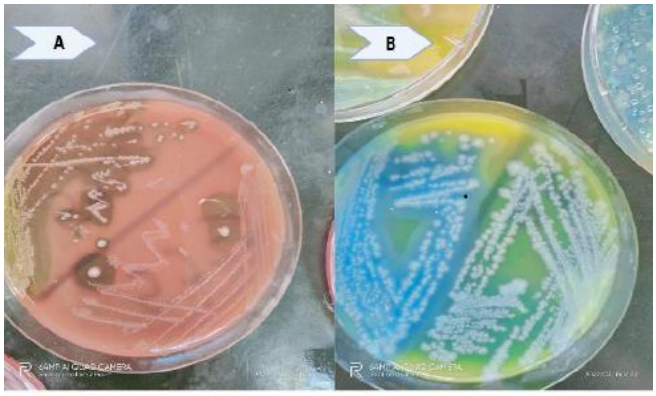

Fig. 3. *B. cereus* on blood agar media showing complete haemolysis (A) while, (B) represents typical blue colonies surrounded by an egg yolk precipitate.

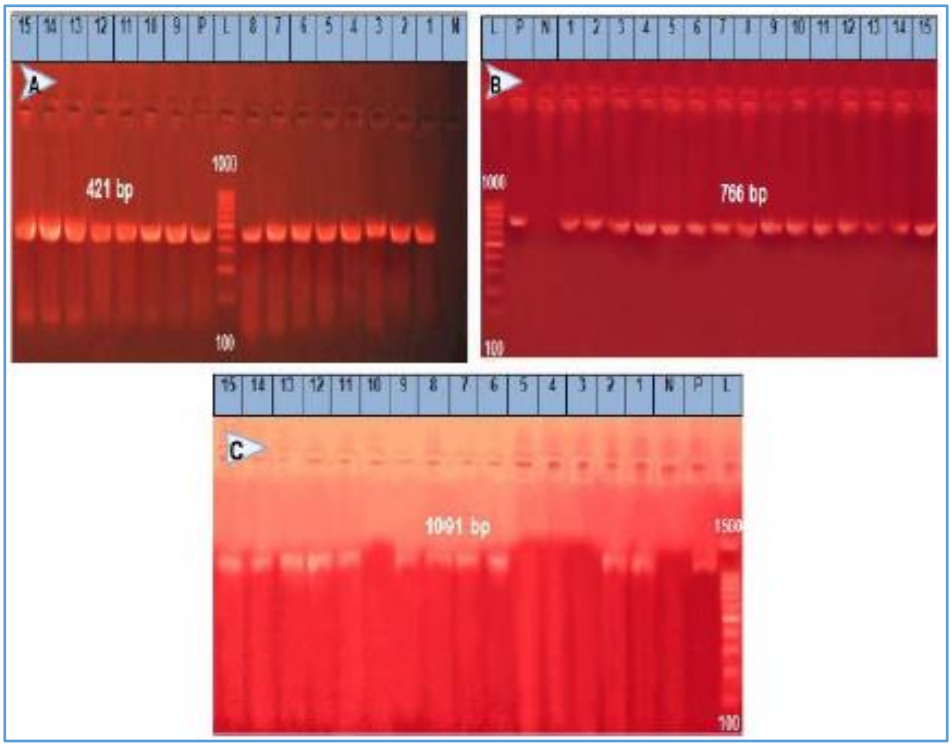

Fig. 4. Results of *B. cereus* entero-toxogenic gene encoding (*Cyt*K), (P), positive control at 421 pb, (N) negative control, Lane (1-15) represents the samples which considered positive (A), *B. cereus* entero-toxogenic gene encoding (*Nhe*), (P), positive control at 766 pb, (N) negative control, Lane (1-15) represents the samples which considered positive (B) and). *B. cereus* entero-toxogenic gene encoding (*Hbl*), (P), positive control at 1091 pb, (N) negative control, Lane (1-15) represents the samples (1, 2, 6, 7, 8, 9, 11, 12, 13, 14, 15) considered positive (B)

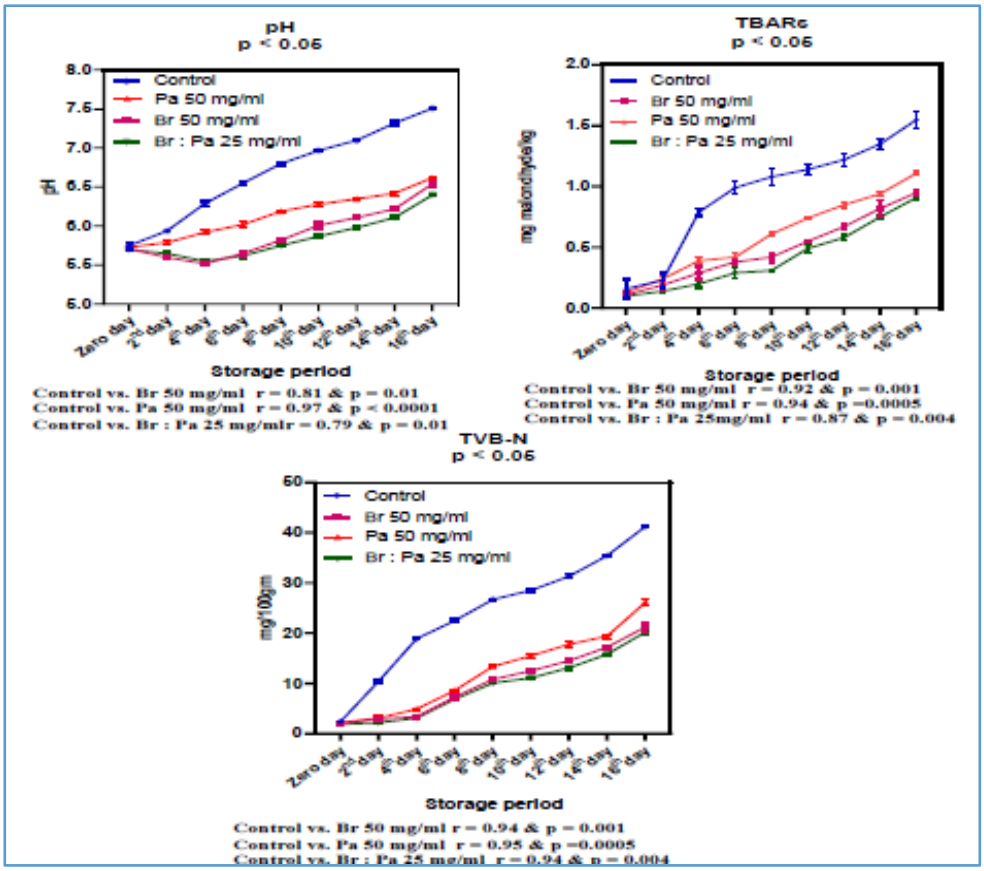

Fig. 5. Impacts of bromelain (Br) and papain (pa) and their mixture on pH, TBRs, and TVB-N in chilled minced meat and the correlation between control and treated groups (r).

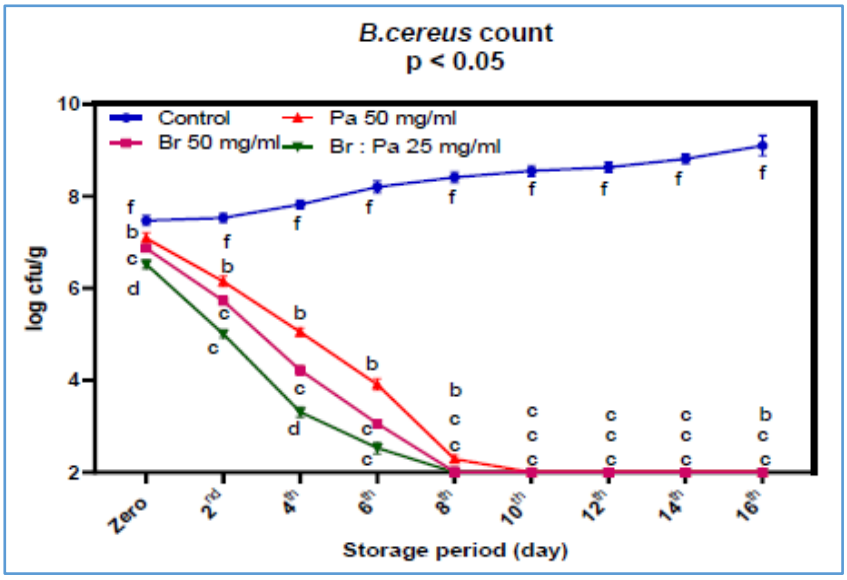

Fig. 6. Impacts of bromelain (Br) and papain (pa) and their mixture on *B. cereus* count in chilled minced meat. Values are shown as the mean ±SD. Different lowercase letters indicate significance at *P* < 0.05 between groups at the same time point.

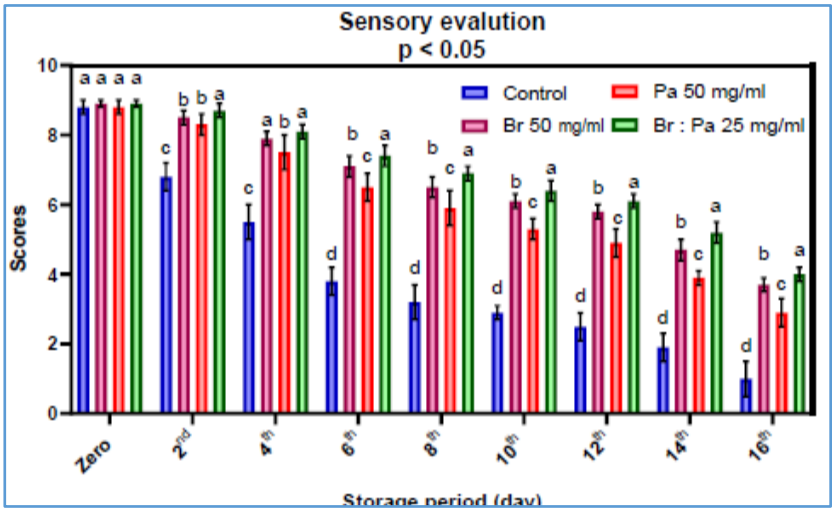

Fig. 7. Impacts of bromelain (Br) and papain (pa) and their mixture on sensory attributes in chilled minced meat. Values are shown as the mean of color, odour and over all acceptability ±SD. Different lowercase letters indicate significance at *P* < 0.05 between groups at the same time point.

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تأثير إنزيما البابين والبروميلين في التعبير الجيني لبكتيريا الباسيليس سيريوس المعزولة من اللحوم المفرومة وبعض منتجات اللحوم

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[3] قسم صحة الأغذية، مركز بحوث الصحة الحيوانية، فرع شبين الكوم، مركز البحوث الزراعية ، مصر.

الملخص

تُعتبر بكتريا البايسليس سيريوس مسبب خطيرللعدوى المنقولة بالغذاء. ونظرًا لتأثيرات البكتريا الضارة على صحة الإنسان والاقتصاد، أولت الوكالات الفيدرالية وباحثو سلامة الأغذية وصناعة الأغذية اهتمامًا خاصًا بها. لذلك نشأت الحاجه لدراسة فعالية الإنزيمات الطبيعية التي تعمل كمضادات للميكروبات نظرًا لتزايد رغبة المستهلكين في المكونات الطبيعية. كان هدف هذه الدراسة هو دراسه معدل تواجد بكتريا البايسليس سيريوس في منتجات اللحوم المختلفة وتقييم كفاءة إنزيمات البابين (Pa) والبروميلين (Br) ضد الجينات السامة لبكتريا البايسليس سيريوس. كشف التحليل عن وجود نسبة كبيرة من البايسليس سيريوس ، وخاصة في كفتة الأرز (44٪)، تليها النقانق واللحم المفروم (34٪ و20٪) على التوالي. كانت الجينات السامة nhe وcytK موجودة في جميع العينات المدروسة (100٪)، بينما وُجد جين hbl في 73.3٪. بالإضافة إلى ذلك، كان هناك فرق كبير بين المجموعة الضابطة والمعالجة بإنزيمات البابين والبروميلين في الخصائص الفيزيائية والكيميائية، وعدد بكتريا البايسليس سيريوس وكذلك الخصائص الحسية ، بالإضافة إلى تأخر التلف وزيادة مدة التخزين المبرد، مما يُبرز إمكاناتها كعوامل طبيعية مضادة للميكروبات. كما لوحظ انخفاض في التعبير عن جينات السموم المعوية (nhe، وcytK، وhbl) لبكتيريا البايسليس سيريوس في العزلات المختبرة بعد التعرض للبروميلين والبابين. تُؤكد النتائج أهمية مراقبة سلامة الغذاء والدور المُحتمل لهذه الإنزيمات الطبيعية في التخفيف من جينات الضراوة المرتبطة ببكتيريا البايسليس سيريوس. كما تُشير النتائج إلى استراتيجية فعّالة لإنشاء أنظمة مضادة للبكتيريا لمنتجات اللحوم.

الكلمات الدالة: الباسيليس سيريوس، جوده اللحوم، البابين، البروميلين، السجق، اللحم المفروم.