



Evaluation of Lysozyme and Propolis as Natural Antimicrobials Against Foodborne Pathogens Isolated from Dairy-Based Desserts

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Abstract

THE increasing demand for natural food preservatives has prompted a shift toward bio-based antimicrobials in the food industry. This study investigated the antimicrobial activity of lysozyme and propolis against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* isolated from dairy-based desserts. A total of 100 samples (rice pudding, ice cream, crème caramel, mahalabiya, and cheesecake) were analyzed for the presence of these pathogens. Isolation and identification were conducted using selective media, biochemical profiling, and PCR confirmation, including detection of virulence genes such as *phoA*, *stx1*, *eaeA*, *sea*, *sed*, *groEL*, *bceT*, and *cytK*. Lysozyme (0.1%) and propolis (1.5%) were selected based on well-diffusion assay and further tested in rice pudding. Microbial enumeration during 5-day storage demonstrated that lysozyme significantly reduced viable counts across all pathogens, while propolis showed moderate antimicrobial effects. Sensory evaluation revealed that lysozyme maintained acceptable product quality, whereas propolis negatively affected sensory attributes. These findings support the application of lysozyme as a safe and effective natural preservative in dairy desserts.

Keywords: Antimicrobials, Virulence genes, PCR, Lysozyme, Propolis.

Introduction

Dairy-based desserts such as rice pudding, mahalabiya, crème caramel, cheesecake, and ice cream are widely consumed across Egypt due to their nutritional value, flavor, and convenience. However, these ready-to-eat products are considered microbiologically sensitive, especially when handled improperly during production, distribution, or storage [1]. Milk and its derivatives provide an ideal environment for microbial growth due to their rich content of proteins, carbohydrates, and water [2]. Foodborne pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* have been frequently isolated from dairy desserts and represent a major public health concern [3]. Contamination may occur due to poor hygiene, improper refrigeration, or post-processing handling,

especially in ready-to-eat products that are not subjected to further heat treatment before consumption. Although most *E. coli* strains are non-pathogenic, specific pathotypes such as enteropathogenic (EPEC), enterotoxigenic (ETEC), and especially enterohemorrhagic *E. coli* (EHEC), are significant foodborne pathogens. These pathogenic strains often harbor virulence genes such as *stx1*, *eaeA*, and *phoA*, which encode shiga toxins, adhesion factors, and alkaline phosphatase, respectively [4]. These toxins can cause a wide range of gastrointestinal symptoms, it is a well-known foodborne pathogen capable of producing a wide range of extracellular toxins. *Staphylococcus aureus* is a well-known foodborne pathogen capable of producing a wide range of extracellular toxins, particularly staphylococcal enterotoxins (SEs), which are responsible for staphylococcal food poisoning

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[5]. Among these, enterotoxin A (*sea*) and enterotoxin D (*sed*) are frequently implicated in dairy product contamination and foodborne outbreaks [6]. Contamination typically occurs due to poor hygiene during food handling and improper refrigeration, allowing the organism to proliferate and produce toxins [7]. *Bacillus cereus* frequently associated with foodborne illnesses and is capable of producing two distinct types of toxins: the diarrheal enterotoxins (such as *bceT*) and cytotoxin K (*cytK*), which contributes to emetic syndrome [8]. The diarrheal syndrome typically results from ingestion of contaminated foods such as milk-based desserts that have been improperly stored, allowing the bacteria to grow and release enterotoxins. In contrast, the emetic syndrome, caused by the heat-stable cereulide toxin, is more often associated with starchy foods such as rice and pasta [9]. Therefore, the need for preserving such products using different techniques became a mandatory addition during processing in order to safeguard the human health against such pathogens, and to improve the final product quality with additional option of increasing its shelf life [10]. Due to increasing consumer demand for clean label products and growing concerns over the potential health risks of synthetic preservatives, the food industry has shifted its focus toward natural alternatives for microbial control. Natural antimicrobials derived from plants, animals, and microorganisms offer promising efficacy against a broad spectrum of foodborne pathogens while maintaining food quality and sensory attributes [11]. Lysozyme is a naturally occurring antimicrobial enzyme and a bio-preservative in dairy products, which act by hydrolyzing the β -1,4 glycosidic bonds between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of bacterial cell walls, leading to osmotic imbalance and cell lysis especially in gram-positive bacteria like *Staphylococcus aureus* and *Bacillus cereus* [12,13]. Propolis is a natural resinous substance collected by honeybees from plant buds and exudates, mixed with beeswax and bee enzymes. It has been traditionally used for its antimicrobial, anti-inflammatory, and antioxidant properties, and has gained increasing interest as a food preservative due to its broad-spectrum activity against bacteria, fungi, and viruses [14]. In dairy and dessert products, propolis has been investigated for its potential to inhibit the growth of foodborne pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*; although its strong flavor and dark color can affect sensory quality at higher concentrations [15]. While several studies have investigated the antimicrobial potential of natural compounds in raw milk and fermented dairy products, limited research has focused on their application in cooked, ready-to-eat dairy-based desserts. Products such as rice pudding, crème caramel, and ice cream are often consumed

without further heat treatment and may pose a risk if contaminated post-processing. Moreover, few investigations have evaluated the individual efficacy of lysozyme and propolis in such food matrices, particularly against multiple foodborne pathogens under the same experimental conditions. Therefore, the present study aimed to evaluate the individual antimicrobial efficacy of lysozyme and propolis against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* in various milk-based desserts.

Material and Methods

Sample Collection

A total of 100 dairy-based dessert samples including rice pudding, mahalabiya, crème caramel, cheesecake, and ice cream were randomly collected from retail outlets and street vendors in Cairo, Egypt. Samples were collected in sterile containers, placed in an icebox, and transported to the laboratory under chilled conditions for immediate analysis.

Isolation of Target Bacteria

Each sample was homogenized in buffered peptone water (BPW) and prepared as a 1:10 dilution by mixing 10 mL of the sample with 90 mL of BPW. A 1 mL aliquot from this dilution was streaked onto selective media: For *E. coli*, both eosin methylene blue (EMB) agar and TBX agar (ISO 16649-2:2001/Amd 1:2017) were used without prior enrichment. *S. aureus* was isolated using Baird-Parker agar (ISO 6888-1:2021), while *B. cereus* was isolated using MYP agar (ISO 7932:2004/Amd 1:2020) [16,17,18].

Biochemical Identification

Presumptive *E. coli* colonies were confirmed using Microbact™ GNB 12E [19] biochemical identification kits (Oxoid, UK), according to the manufacturer's instructions. Presumptive *Staphylococcus aureus* colonies were verified by Gram staining (Gram-positive cocci in clusters), catalase test (positive bubbling with hydrogen peroxide), and coagulase test (clot formation in plasma), in accordance with ISO 6888-1:2021 [17]. Presumptive *Bacillus cereus* colonies were examined microscopically (Gram-positive rods, often in chains) and confirmed by observing hemolytic activity on blood agar, motility in semi-solid media, and lecithinase production on MYP agar, following ISO 7932:2004/Amd 1:2020 [18].

Molecular Identification by PCR

Genomic DNA was extracted from presumptive colonies using the QIAamp DNA Mini Kit (Qiagen, Cat. No. 51304), following the manufacturer's instructions and general molecular biology protocols [20]. PCR amplification was conducted using Emerald Amp GT PCR Master Mix (Takara, Code

No. RR310A) in a final volume of 25 μ L, containing 12.5 μ L of master mix, 1 μ L of each primer (20 pmol), 5.5 μ L of DNA template, and 5 μ L of nuclease-free water. Amplification was carried out using a Biometra T3 thermal cycler. PCR products were separated on 1.5% agarose gels prepared in TBE buffer and stained with ethidium bromide (0.5 μ g/mL). Electrophoresis was run at 5 V/cm for 45 minutes, and bands were visualized using a gel documentation system. A 100 bp DNA ladder was used to estimate amplicon sizes [21]. Primers targeting identification and virulence genes were selected based on published studies. For *Escherichia coli*, genes such as *phoA*, *eaeA*, and *stx1* were amplified [22, 23]. For *Staphylococcus aureus*, primers targeting 16S rRNA, *nuc*, *sea*, and *sed* genes were used following previous studies [24-26]. For *Bacillus cereus*, primers targeting *groEL*, *bceT*, and *cytK* were adopted from published protocols [27, 28].

Screening of Antibacterial Activity Lysozyme and Propolis

To determine the effective concentrations for application, different concentrations of lysozyme and propolis as shown in table (2) were tested using the well diffusion assay (well diameter: 0.7 cm). Mueller-Hinton agar plates were inoculated with each isolate, and wells were filled with 100 μ L of each concentration. The plates were incubated at 37°C for 24 hours, and inhibition zones were measured [29]. This step was repeated three times and mean results were recorded to select the lowest concentration that showed consistent antibacterial activity for further application.

Evaluation of the antimicrobial activity of selected Lysozyme and Propolis concentrations in Rice Pudding

Rice pudding was freshly prepared in the laboratory using fresh full fat buffalo raw milk obtained from Faculty of Agriculture, Cairo university farm to ensure its quality and purity (about 3 liters of milk were needed for each trial). The whole raw milk bulk was then laboratory pasteurized at 85°C for 20 minutes followed by rapid cooling to 6°C to be ready for use. Firstly, the milk was reheated to 85°C and the rice was first cooked in milk until became soft, then sugar and corn starch were added with continuous stirring until the mixture thickened. The final product was poured into sterile containers (500 g capacity) in 4 portions for inoculation with the bacterial suspensions (4 groups). Each group was divided then into 3 parts to be fortified with selected concentrations from natural additives (Lysozyme 0.1% and Propolis 1.5%) and a control part without fortification, to obtain finally 12 treatments as described in table 2. After good mixing of each treatment, the mixtures were poured in small sterile containers to be used for examination during

storage, and then it was left to cool at room temperature and stored at refrigerator (6°C) for 5 days. This experiment was performed in triplicate to ensure the final results. Viable microbial counts were performed using ISO-recommended selective media: *Escherichia coli*: TBX agar (ISO 16649-2:2001/Amd 1:2017 [16]), *Staphylococcus aureus*: Baird-Parker agar (ISO 6888-1:2021 [17]) and *Bacillus cereus*: MYP agar (ISO 7932:2004/Amd 1:2020 [18]). Duplicate plates for each treatment were incubated aerobically at 37°C for 24 hours and mean counts were expressed as CFU/g. Samples from the fourth portion with treatments number 10 to 12 were assessed daily for five days by well-trained panelists from the Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University staff members. Samples were evaluated for different sensory parameters (color, odor, taste, and texture) using a 9-point hedonic scale, following the protocol described by Meilgaard et al. [30]. The sensory evaluation was also performed for the triplicates of the experiment and average data were calculated.

Statistical Analysis

The determination of p-values ($p \leq 0.05$) between mean values of the investigated parameters in the examined samples was achieved by one-way ANOVA using post hoc Tukey HSD through SPSS v.25.

Results

The microbiological analysis of dairy dessert samples (Table 4) revealed varying prevalence rates among the targeted foodborne pathogens. *Escherichia coli* was presumptively isolated from 9% of the samples, with full biochemical and PCR confirmation for all detected strains. Importantly, 100% of the PCR-confirmed *E. coli* isolates carried virulence genes, indicating their toxigenic potential. Similarly, *Staphylococcus aureus* was isolated in 15% of the samples and confirmed biochemically and molecularly at the same rate. However, only 20% of the PCR-confirmed *S. aureus* isolates were found to harbor enterotoxin genes, suggesting a relatively lower risk of enterotoxigenicity among these strains. *Bacillus cereus* showed the highest isolation rate, being presumptively and biochemically identified in 22% of the samples. PCR confirmation was achieved in 16% of the cases, and all confirmed isolates were positive for toxin genes, indicating that 100% of PCR-confirmed *B. cereus* strains were toxigenic. The antimicrobial potential of lysozyme and propolis against *E. coli* isolates was evaluated using the well diffusion method, and the results are presented in Table 5. Lysozyme exhibited a concentration-dependent inhibitory effect, with the highest inhibition zone diameter (2.1 cm) observed at 1% concentration (L1), categorized as extremely sensitive (ES). Decreasing the concentration to 0.1%

(L2) and 0.01% (L3) still maintained measurable zones of 1.8 cm and 1.5 cm, respectively, classified as very sensitive (VS). At the lowest tested concentration (0.001%, L4), the zone was reduced to 0.9 cm, corresponding to a sensitive (S) reaction. In contrast, propolis required higher concentrations to exhibit activity. No inhibition zone was detected at 0.01% (P1), while mild inhibition was observed at 0.1% (P2) and 0.5% (P3) with diameters of 0.8 and 1.4 cm, respectively, both considered sensitive (S). At 1.5% concentration (P4), the inhibition zone slightly increased to 1.6 cm and was categorized as very sensitive (VS). These findings indicate that lysozyme was more effective than propolis at lower concentrations against *E. coli*, suggesting a higher antimicrobial potency under the tested conditions. Table 6 illustrates the antimicrobial effectiveness of lysozyme (L2) and propolis (P3) on the viability of *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* inoculated into rice pudding and stored under refrigeration for five days. The initial microbial loads at day zero showed high counts across all tested strains, with no significant differences among the treatment groups. However, marked reductions were observed in lysozyme-treated samples beginning from day one. For *E. coli*, a significant decline was evident by day 1 in the L2 group (2.6×10^2 CFU/g), and by day 4, the organism became undetectable (<3 CFU/g), indicating complete inhibition. In contrast, *E. coli* remained detectable in P3 (1.0×10^3 CFU/g) and control samples (1.0×10^4 CFU/g), with significant differences persisting throughout the storage period. Similarly, *S. aureus* counts declined more effectively in L2 samples compared to P3 and control groups, with counts reaching 3.3×10 CFU/g by day 5 versus 1.2×10^3 CFU/g in P3 and 2.7×10^4 CFU/g in control. Although both treatments contributed to bacterial suppression, lysozyme demonstrated superior performance in reducing pathogen levels more rapidly and consistently. For *B. cereus*, a relatively higher resistance was noted, as the bacterial counts remained above 10^3 CFU/g in all groups throughout the storage period. Nonetheless, the L2 and P3 treatments significantly suppressed *B. cereus* growth compared to the control, which maintained a notably high count of 2.2×10^5 CFU/g by day 5. The statistical analysis confirmed significant differences ($p < 0.05$) among treatment groups and time points, particularly in *E. coli* and *S. aureus* responses, whereas *B. cereus* exhibited more tolerance to both agents.

Discussion

Natural antimicrobials such as lysozyme and propolis are gaining increasing attention as alternatives to synthetic preservatives in food preservation. These compounds have demonstrated effective microbial inhibition while maintaining

consumer-friendly 'clean-label' status. Lysozyme and propolis have been particularly studied in dairy products due to their natural origin and antimicrobial efficacy [11]. Propolis has shown potent effects against both Gram-positive and Gram-negative bacteria [14], while lysozyme has been successfully incorporated into dairy matrices like yogurt and cheese without altering sensory qualities [13]. The microbiological examination of dairy-based desserts revealed varying prevalence levels of key foodborne pathogens. Table 4 summarizes the outcomes of bacterial isolation, molecular confirmation, and toxigenic gene detection for *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*.

Escherichia coli is widely recognized as an indicator of fecal contamination and a significant foodborne pathogen, particularly when carrying virulence factors such as *stx1* and *eaeA*. In the current study, *E. coli* was presumptively isolated from 9% of the examined dairy-based dessert samples, including rice pudding, mahalabiya, ice cream, crème caramel, and cheesecake, using EMB and TBX agars without pre-enrichment, according to ISO 16649-2:2001/Amd 1:2017 [16]. The use of dual selective media enhanced the reliability of detection, with TBX agar showing better selectivity and colony differentiation. The isolation rate observed in this study (9%) is comparable to findings from Egypt. Abushaala et al. (2022) reported a prevalence rate of approximately 8% of verotoxigenic *Escherichia coli* in locally manufactured dairy products [31]. These relatively moderate isolation rates might be attributed to the absence of an enrichment step, which could have reduced the sensitivity of detection, especially in samples with low bacterial loads. Biochemical confirmation using the Microbact™ GNB 12E identification system showed full agreement with molecular identification by PCR targeting the *phoA* gene, highlighting the accuracy of the preliminary biochemical tests [19]. Among the PCR-confirmed isolates, 67% harbored the *eaeA* gene, and 33% carried the *stx1* gene, indicating a significant presence of potentially pathogenic strains. The high detection rate of the *eaeA* gene, encoding intimin, suggests a predominance of enteropathogenic *E. coli* (EPEC) strains, which are known to cause attaching and effacing lesions in the intestinal mucosa. On the other hand, the presence of the *stx1* gene, a hallmark of shiga-toxin producing *E. coli* (STEC), underlines the public health threat posed by consumption of contaminated dairy desserts without sufficient heat treatment post-processing. The findings of this study are in line with previous reports, such as that by Sobeih et al., who documented a high prevalence of *eaeA* and *stx1* virulence genes in *Escherichia coli* isolated from dairy products in Egypt [32]. *Staphylococcus aureus* is a significant foodborne pathogen responsible for various food poisoning

outbreaks worldwide, primarily due to its ability to produce heat-stable enterotoxins. In the present study, *S. aureus* was isolated presumptively from 15% of dairy-based dessert samples using Baird-Parker agar, showing characteristic black or gray colonies with clear halos, in accordance with ISO 6888-1:2021 [17]. This isolation rate (15%) is lower than that reported by Saad et al., who documented *Staphylococcus aureus* in up to 68% of dairy-based sweets (milk and ice cream) collected from retail vendors in Qena governorate, Egypt [33]. The moderate prevalence observed may reflect both the intrinsic susceptibility of dairy-based products to contamination and the relatively favorable growth conditions for *S. aureus* due to high protein and moisture content. Regarding enterotoxin gene detection, 20% of PCR-confirmed isolates were found to harbor enterotoxin genes, with 13% positive for the *sea* gene and 7% positive for the *sed* gene. The predominance of the *sea* gene in this study is in agreement with global reports. For example, Zhang et al., found that *sea* was the most frequently detected classical enterotoxin gene among *Staphylococcus aureus* isolates from milk and dairy products worldwide [34]. The *sea* gene encodes enterotoxin A, the most commonly implicated toxin in staphylococcal food poisoning cases, while *sed* encodes enterotoxin D, often associated with contaminated dairy and meat products. The detection of these genes highlights the potential risk of foodborne illness from consumption of improperly handled dairy desserts. In this study, *B. cereus* was presumptively isolated from 22% of the examined dairy-based dessert samples using MYP agar, characterized by pink, dry colonies with lecithinase activity, following ISO 7932:2004/Amd 1:2020 [18]. The observed isolation rate (22%) is consistent with Elgushi et al., who documented an overall *B. cereus* prevalence of 10.5% in dairy samples in Egypt, with the highest rate in milk powder (24.99%) [35]. The high prevalence detected reflects the capacity of *B. cereus* spores to survive harsh environmental conditions, including pasteurization processes, and to germinate under favorable storage conditions, particularly in high-protein, high-moisture foods such as dairy-based desserts. Biochemical identification methods were further supported by molecular confirmation through PCR targeting the *groEL* gene. Approximately 73% of the biochemically presumptive isolates were confirmed by PCR, demonstrating the reliability of the molecular approach in reducing false positives typically encountered with traditional methods. Importantly, 100% of the PCR-confirmed isolates were found to carry enterotoxin genes, specifically *bceT* and *cytK*. The *bceT* gene encodes for a component of the enterotoxin complex associated with diarrheal syndrome, while the *cytK* gene encodes cytotoxin K, a pore-forming toxin that

contributes to severe cytotoxic effects. These findings are consistent with previous studies in Egypt, such as those by El-Haw et al. (2024), who reported a high prevalence of toxigenic *B. cereus* strains in dairy products and detected multiple virulence genes, including *cytK* and *bceT* [36]. Based on the well diffusion test (Table 5), lysozyme exhibited noticeable antibacterial activity against all tested pathogens, particularly *Escherichia coli* and *Bacillus cereus* at a concentration of 1% (L1). Even at lower concentrations like 0.01% (L3), inhibition zones were observed, and the intermediate concentration (L2) was selected as the minimum inhibitory concentration. These findings are in agreement with Khorshidian et al. (2022), who reported that lysozyme possesses strong antimicrobial activity, especially against Gram-positive bacteria, and under certain conditions, can be effective against Gram-negative bacteria such as *E. coli*, particularly when used in food matrices like dairy products [37], while Benkerroum (2008) highlighted its effectiveness in milk matrices [38]. Similarly, Arslan et al., confirmed strong inhibition of lysozyme against *S. aureus* and *B. cereus*, especially in dairy systems [12]. Propolis also exhibited antimicrobial activity, although to a lesser extent than lysozyme. At a concentration of 1.5% (P3), propolis showed measurable inhibition zones against *E. coli*, *S. aureus*, and *B. cereus*, but required higher concentrations compared to lysozyme. This is consistent with the findings of Przybyłek & Karpiński, and Silici & Kutluca, who reported effective but concentration-dependent activity of propolis [15, 14]. Moreover, Ugur & Arslan, stated that the antimicrobial potential of propolis can vary based on its botanical origin, which may explain the moderate effects observed in our study [39]. In general, both lysozyme and propolis had higher antimicrobial activity against *E. coli*, followed by *B. cereus*, while *S. aureus* was more resistant. Finally, the well diffusion results validated the selection of L2 (0.1%) and P3 (1.5%) as practical concentrations for in-product trials, combining antimicrobial effectiveness with reasonable feasibility for food application. The antibacterial effect of lysozyme and propolis against *E. coli*, *S. aureus* and *B. cereus* was then evaluated in produced rice pudding to evaluate their effect within the food matrix in the presence of other factors. The in-product evaluation results tabulated in Table 6, revealed a pronounced antimicrobial effect of lysozyme (L2 – 0.1%) against all three tested pathogens. In *E. coli*-inoculated samples, lysozyme reduced the bacterial count from 4×10^6 CFU/g at the production day to undetectable levels by day 4. This significant reduction confirms the ability of lysozyme to retain activity in complex food matrices such as rice pudding. Khorshidian et al., reviewed several applications of lysozyme in dairy matrices and confirmed its effectiveness in

reducing microbial load, including *E. coli*, in cheese curds and similar products. The authors highlighted that even native lysozyme at levels around 400 µg/mL was able to significantly reduce bacterial counts during refrigerated storage, supporting its role as a natural preservative in dairy-based systems [37]. Similar inhibitory effects were observed for *S. aureus* but without complete elimination, with bacterial counts dropping drastically over the 5-days storage period. It was noticeable that *S. aureus* was sensitive to lysozyme as it was significantly decreased after one day of storage (3.3×10^3 CFU/g) in their samples than propolis fortified and control samples (7.0×10^3 and 1.1×10^4 CFU/g, respectively). Lysozyme is well known for its strong action against *S. aureus* due to its thick peptidoglycan layer. Arslan *et al.*, observed significant reductions in *S. aureus* counts in dairy systems supplemented with lysozyme [12]. For *B. cereus*, lysozyme fortified rice pudding samples showed lower counts with significant difference than the control group for first day of storage (3.6×10^3 and 2.0×10^4 CFU/g, respectively) till the end of storage period (6.6×10^3 and 2.2×10^5 CFU/g, respectively). These results are consistent with Benkerroum, who reported that lysozyme is effective against vegetative cells of *B. cereus*, supporting its role in the control of spore-forming pathogens in food [38]. Propolis is a natural bee-derived product with potent antimicrobial properties due to its high phenolic and flavonoid content. Przybyłek & Karpiński, and Silici & Kutluca, documented its effectiveness against several food pathogens including *E. coli*, *S. aureus*, and *B. cereus* in various studies [15, 14]. In this study, propolis (P3 – 1.5%) showed moderate inhibition of microbial growth; it reduced the initial counts of *E. coli*, *S. aureus*, and *B. cereus*, but with antibacterial effect weaker than that of lysozyme. For example, *E. coli* counts were still detectable ($\sim 10^3$ CFU/g) by day 5 in propolis-treated samples, unlike the lysozyme-treated ones. Also, for *S. aureus*, the counts in propolis samples were significantly lower than control group but higher than those for lysozyme samples by the end of storage period (1.2×10^3 , 2.7×10^4 and 3.3×10^4 CFU/g, respectively) (Table 6). This suggests that although propolis has documented antimicrobial properties, its efficacy may be limited in rich food matrices due to interactions with proteins, sugars, and fats that may bind or neutralize its active components [14]. Interestingly, *B. cereus* showed a relatively higher resistance to both treatments compared to *E. coli*, especially in later stages of storage. This may be attributed to the spore-forming nature of *B. cereus*, which allows it to survive and potentially recover under chilled conditions, Benkerroum, [38]. Overall, the microbial enumeration data strongly support the use of lysozyme as a natural preservative in dairy-based desserts. Its rapid and sustained antimicrobial effect across multiple

pathogens makes it suitable for extending shelf life while maintaining product safety. The impact of lysozyme and propolis addition to rice pudding on sensory parameters was evaluated as shown in Table 7. Results of sensory evaluation revealed a clear distinction between lysozyme and propolis fortified samples. Rice pudding samples fortified with lysozyme (L2 – 0.1%) consistently maintained high sensory scores throughout the 5 days storage period, with no significant deviations from the control in terms of color, odor, taste, or texture. These results confirm the compatibility of lysozyme with dairy-based desserts from a sensory standpoint; therefore, it could be easily added to dairy desserts as a preservative without any obvious changes in all sensory parameters. This makes lysozyme a promising candidate for use in products requiring both microbial safety and consumer acceptability. Conversely, propolis fortified rice pudding samples (P3 – 1.5%) exhibited a marked decline in sensory acceptability, especially in color (3.7 ± 0.5) and odor (5.6 ± 0.5) attributes since the day of production. During storage, the scores for color dropped below 4 on a 9-point scale, While taste and texture became less favorable as storage progressed, the propolis-fortified rice pudding samples were deemed completely unacceptable by the panelists after three days of storage across all sensory parameters. These findings align with those reported by El-Sakhawy *et al.*, who highlighted that despite the antimicrobial efficacy of propolis, its practical application in dairy-based foods is often constrained due to its strong bitter taste and intense aroma, especially at higher concentrations [40]. Przybyłek & Karpiński, also emphasized that the phenolic compounds in propolis, while effective against pathogens, can adversely affect organoleptic qualities [15]. Otherwise, it may be added to dairy desserts as a new additive known to the consumer as a novel product, which could be accepted as a fresh product by some consumers, especially those concerned with healthy nutrients. Therefore, while both compounds possess antimicrobial properties, only lysozyme demonstrated a balance between microbial control and sensory acceptability, highlighting its superior suitability for preserving dairy desserts like rice pudding. The sensory attributes of rice pudding samples fortified with lysozyme (L2) and propolis (P3) were evaluated over a 5-day refrigerated storage period and are summarized in Table 7. On the day of production, both L2 and control samples received the highest possible scores across all parameters (color, odor, taste, texture), reflecting excellent initial acceptability. In contrast, the P3 samples exhibited significantly lower scores, particularly for color (3.7 ± 0.5) and odor (5.6 ± 0.5), though taste and texture remained relatively acceptable (7.3 ± 0.5 and 5.3 ± 0.5 , respectively). Throughout the storage period, L2 samples consistently maintained high

sensory scores, with no significant deterioration until day 4, where a slight decline was observed (e.g., taste: 7.6 ± 0.5). By day 5, L2 samples still retained favorable acceptability (scores of 7.0 for all parameters), indicating stability and compatibility of lysozyme with the product's sensory properties. On the other hand, propolis-fortified samples (P3) demonstrated a progressive and significant decline in all sensory parameters. By day 3, color and odor scores dropped to 3.0 ± 1 and 4.6 ± 0.5 , respectively, and further decreased by day 5 to as low as 2.0 ± 0 for color and 3.3 ± 0.5 for taste. These reductions rendered the product sensorially unacceptable to panelists, highlighting the adverse impact of propolis on the organoleptic properties of rice pudding, especially when used at higher concentrations. Control samples showed gradual but moderate declines in sensory scores over time. By day 5, their scores remained higher than P3 but lower than L2, particularly in taste and odor attributes. Overall, the results indicate that lysozyme could be effectively used as a natural preservative in dairy-based desserts without negatively impacting sensory quality, while propolis may limit consumer acceptability due to its strong aroma and flavor characteristics.

Conclusion

In conclusion, restrict application of different prerequisite programs especially those concerning

sanitation and hygiene during handling of dairy desserts is an urgent factor to protect the consumer health and extend the shelf life of the product. There is a lack of information in the Egyptian standards concerning such type of dairy products, their recipes, additives types and levels, preservatives and even the regulation about its storage conditions and shelf life. Therefore; we appeal the concerned authorities in Egypt to enforce regulations and standards for control dairy dessert production, handling and storage with strict regulations for its microbial quality and safety. Our study also recommended the use of lysozyme as natural preservative with rapid and sustained antimicrobial effect across multiple pathogens, which makes it suitable for extending shelf life of such products while maintaining product safety and without affecting its sensory attributes even during storage for up to 5 days.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Primers used for PCR detection of virulence and identification genes.

Target Gene	Organism	Primer Name	Primer Sequence (5'–3')	Product Size (bp)
<i>phoA</i>	<i>Escherichia coli</i>	F	GATTGAACGGCAGTACCGG	370
		R	CCGTTGCCAGTGATGACGAT	
<i>eaeA</i>	<i>Escherichia coli</i>	F	GACCCGGCACAAGCATAAGC	384
		R	CCACCTGCAGCAACAAGAGG	
<i>stx1</i>	<i>Escherichia coli</i>	F	ACACTGGATGATCTCAGTGG	614
		R	CTGAATCCCCCTCCATTATG	
<i>16S rRNA</i>	<i>Staphylococcus aureus</i>	F	GTA GGT GGC AAG CGT TAT CC	228
		R	CGC ACA TCA GCG TCA G	
<i>nuc</i>	<i>Staphylococcus aureus</i>	F	GCG ATT GAT GGT GAT ACG GTT	279
		R	AGC CAA GCC TTG ACG AAC TAA AGC	
<i>sea</i>	<i>Staphylococcus aureus</i>	F	TTG GAA ACG GTT AAA ACG AA	120
		R	GAA CCT TCC CAT CAA AAA CA	
<i>sed</i>	<i>Staphylococcus aureus</i>	F	CTA GTT TGG TAA TAT CTC CT	317
		R	TAA TGC TAT ATC TTA TAG GG	
<i>groEL</i>	<i>Bacillus cereus</i>	F	GTAGTAAAAGGCGGCAGTGG	441
		R	TTTCCATTGCTGTGTTCCAG	
<i>bceT</i>	<i>Bacillus cereus</i>	F	GGGATCCACATTTACAAGCC	424
		R	CGCGGATCCATTTTCTCAAG	
<i>cytK</i>	<i>Bacillus cereus</i>	F	AAAGGTATTGGTGGCAGCTT	565
		R	CTGAAGCCATCTTTAGGTGC	

TABLE 2. Different concentrations of lysozyme and propolis tested to determine the antimicrobial activity

Lysozyme (L)		Propolis (P)	
L1	1%	P1	0.5%
L2	0.1%	P2	1%
L3	0.01%	P3	1.5%
L4	0.001%	P4	2%

TABLE 3. Samples Key for the antimicrobial experiment of Lysozyme and Propolis against some pathogens in rice pudding

n=	Microbial inoculum	Fortification
1		L2 (0.1%)
2	<i>E. coli</i>	P3 (1.5%)
3	(12× 10 ⁶ CFU/mL)	Control (without fortification)
4		L2 (0.1%)
5	<i>S. aureus</i>	P3 (1.5%)
6	(9× 10 ⁶ CFU/mL)	Control (without fortification)
7		L2 (0.1%)
8	<i>B. cereus</i>	P3 (1.5%)
9	(5× 10 ⁶ CFU/mL)	Control (without fortification)
10	Without inoculation with pathogens to be used for sensory	L2 (0.1%)
11	evaluation.	P3 (1.5%)
12		Control (without fortification)

TABLE 4. Isolation and Toxigenic Confirmation Rates of *E. coli*, *S. aureus*, and *B. cereus* Recovered from Dairy Desserts

Bacteria	Presumptive (%)	Biochemical Confirmed (%)	PCR Confirmed (%)	Toxigenic (% of PCR-confirmed)
<i>Escherichia coli</i>	9%	9%	9%	100%
<i>Staphylococcus aureus</i>	15%	15%	15%	20%
<i>Bacillus cereus</i>	22%	22%	16%	100%

TABLE 5. Minimum inhibitory concentration (MIC) of Lysozyme (L) and Propolis (P) against the tested isolates.

Tested strains	Inhibition Zone Diameter (cm)							
	L1	L2	L3	L4	P1	P2	P3	P4
<i>E.coli</i>	2.1 (ES)	1.8 (VS)	1.5 (VS)	0.9 (S)	0.0	0.8 (S)	1.4 (S)	1.6 (VS)
<i>B.cereus</i>	2.1 (ES)	1.5 (VS)	1.2 (S)	0.3	0.0	0.6	1.1 (S)	1.3 (S)
<i>S.aureus</i>	1.1 (S)	0.9 (S)	0.9 (S)	0.0	0.0	0.5	0.9 (S)	0.9 (S)

* Values in table are averages of three triplicates.

(S) Sensitive (+) for total diameter 9–14 mm.

(VS) Very sensitive (++) for total diameter 15–19 mm.

(ES) Extremely sensitive (+++) for total diameter >20 mm.

TABLE 6. Impact of Lysozyme and Propolis on the viability of tested pathogens in inoculated rice pudding during the storage (5days).

Treatment Parameter	L2	P3	Control
Storage period (zero-day)			
<i>E.coli</i> Count	$4 \times 10^6 \pm 2.6 \times 10^{6a}$	$1.1 \times 10^7 \pm 1.1 \times 10^{6b}$	$9.3 \times 10^6 \pm 5.7 \times 10^{5b}$
<i>S. aureus</i> Count	$9.6 \times 10^6 \pm 5.7 \times 10^{5a}$	$1.0 \times 10^7 \pm 5.7 \times 10^{5a}$	$1.1 \times 10^7 \pm 1.0 \times 10^{6a}$
<i>B. cereus</i> Count	$7.3 \times 10^6 \pm 5.7 \times 10^{5a}$	$3.6 \times 10^6 \pm 1.5 \times 10^{6b}$	$7.7 \times 10^6 \pm 4.0 \times 10^{5a}$
Storage period (1 day)			
<i>E.coli</i> Count	$2.6 \times 10^2 \pm 2.0 \times 10^{2a}$	$6.6 \times 10^3 \pm 4.7 \times 10^{3a}$	$3.0 \times 10^4 \pm 9.5 \times 10^{3b}$
<i>S.aureus</i> Count	$3.3 \times 10^3 \pm 2.5 \times 10^{3a}$	$7.0 \times 10^3 \pm 2.6 \times 10^{3ab}$	$1.1 \times 10^4 \pm 3.7 \times 10^{3b}$
<i>B.cereus</i> Count	$3.6 \times 10^3 \pm 3.7 \times 10^{3a}$	$1.0 \times 10^4 \pm 2.0 \times 10^{3ab}$	$2.0 \times 10^4 \pm 9.5 \times 10^{3b}$
Storage period (2 days)			
<i>E.coli</i> Count	$2.0 \times 10^2 \pm 1.0 \times 10^{2a}$	$1.7 \times 10^4 \pm 6.6 \times 10^{3a}$	$1.4 \times 10^5 \pm 4.0 \times 10^{4b}$
<i>S.aureus</i> Count	$7.6 \times 10^3 \pm 9.8 \times 10^{3a}$	$1.7 \times 10^4 \pm 1.5 \times 10^{3a}$	$6.5 \times 10^4 \pm 2.1 \times 10^{4b}$
<i>B.cereus</i> Count	$3 \times 10^3 \pm 2.6 \times 10^{3a}$	$4.8 \times 10^3 \pm 2.8 \times 10^{3a}$	$5.5 \times 10^4 \pm 2.2 \times 10^{4b}$
Storage period (3 days)			
<i>E.coli</i> Count	$3.3 \times 10 \pm 5.7 \times 10^a$	$2.4 \times 10^3 \pm 5.0 \times 10^{2b}$	$3.4 \times 10^3 \pm 6.9 \times 10^{2b}$
<i>S.aureus</i> Count	$2.6 \times 10^2 \pm 2.0 \times 10^{2a}$	$1.6 \times 10^3 \pm 1.1 \times 10^{3ab}$	$4.3 \times 10^4 \pm 7.2 \times 10^{3b}$
<i>B.cereus</i> Count	$5 \times 10^3 \pm 4 \times 10^{3a}$	$1.6 \times 10^4 \pm 3.5 \times 10^{3a}$	$3.6 \times 10^4 \pm 5.7 \times 10^{3b}$
Storage period (4 days)			
<i>E.coli</i> Count	$< 3^a$	$1.0 \times 10^3 \pm 4.6 \times 10^{2a}$	$1.0 \times 10^4 \pm 1.1 \times 10^{3b}$
<i>S.aureus</i> Count	$1.6 \times 10^2 \pm 5.7 \times 10^a$	$1.5 \times 10^3 \pm 6.4 \times 10^{2a}$	$1.5 \times 10^4 \pm 2.7 \times 10^{3b}$
<i>B.cereus</i> Count	$5.6 \times 10^3 \pm 5.7 \times 10^{2a}$	$6.6 \times 10^3 \pm 2.3 \times 10^{3a}$	$3.5 \times 10^4 \pm 5.0 \times 10^{3b}$
Storage period (5 days)			
<i>E.coli</i> Count	$< 3^a$	$3.6 \times 10^2 \pm 1.5 \times 10^{2a}$	$6.3 \times 10^3 \pm 5.7 \times 10^{2b}$
<i>S.aureus</i> Count	$3.3 \times 10 \pm 5.7 \times 10^a$	$1.2 \times 10^3 \pm 2.3 \times 10^{2b}$	$2.7 \times 10^4 \pm 5.7 \times 10^{2c}$
<i>B.cereus</i> Count	$6.6 \times 10^3 \pm 2.0 \times 10^{3a}$	$7.3 \times 10^3 \pm 2.3 \times 10^{3a}$	$2.2 \times 10^5 \pm 1.5 \times 10^{4b}$

* Values equal mean $\pm$ standard deviation.* For each storage period: Overall mean values with different superscript letters differ significantly ($p < 0.05$) in the same row.**TABLE 7. Grading of the produced rice pudding samples based on sensory parameters at different storage periods.**

Storage period	Sample	Color (9)	Odor (9)	Taste (9)	Texture (9)
Zero-day storage	L2	9 ± 0^a	9 ± 0^a	9 ± 0^a	9 ± 0^a
	P3	3.7 ± 0.5^b	5.6 ± 0.5^b	7.3 ± 0.5^b	5.3 ± 0.5^b
	Control	9 ± 0^a	9 ± 0^a	9 ± 0^a	9 ± 0^a
After 1 day storage	L2	9 ± 0^a	9 ± 0^a	9 ± 0^a	9 ± 0^a
	P3	3.7 ± 0.5^b	5.6 ± 0.5^b	7.3 ± 0.5^b	5.3 ± 0.5^b
	Control	9 ± 0^a	8.3 ± 0.5^a	9 ± 0^a	9 ± 0^a
After 2 days storage	L2	9 ± 0^a	9 ± 0^a	9 ± 0^a	9 ± 0^a
	P3	3.3 ± 0.5^b	5.3 ± 0.5^b	6 ± 1^b	5.3 ± 0.5^b
	Control	8.6 ± 0.5^a	8.3 ± 0.5^a	8.6 ± 0.5^a	8.3 ± 0.5^a
After 3 days storage	L2	9 ± 0^a	8.3 ± 0.5^a	8.6 ± 0.5^a	9 ± 0^a
	P3	3 ± 1^b	4.6 ± 0.5^b	5.3 ± 0.5^b	5 ± 0^b
	Control	8 ± 0^a	7.3 ± 0.5^a	8.3 ± 0.5^a	8 ± 1^a
After 4 days storage	L2	8.6 ± 0.5^a	7.6 ± 0.5^a	7.6 ± 0.5^a	8.3 ± 0.5^a
	P3	2.6 ± 0.5^b	4.6 ± 0.5^b	4 ± 0^b	4.3 ± 0.5^b
	Control	7.3 ± 0.5^a	7 ± 0^a	6.6 ± 0.5^a	7 ± 1^a
After 5 days storage	L2	8 ± 0^a	7 ± 0^a	7 ± 0^a	7 ± 0^a
	P3	2 ± 0^b	3.6 ± 0.5^b	3.3 ± 0.5^b	4 ± 0^b
	Control	6.3 ± 0.5^c	5.6 ± 0.5^c	5.6 ± 0.5^c	6.3 ± 0.5^a

* Values equal mean $\pm$ standard deviation.

* Value nine in the scale means the highest score, while value one means the lowest score.

* For each storage period: Overall mean values with different superscript letters differ significantly ($p < 0.05$) in the same column.

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تقييم اللايزوزايم والبروبوليس كمضادات ميكروبية طبيعية ضد الممرضات المنقولة بالغذاء والمعزولة من الحلويات اللبنية

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الملخص:

أدى الطلب المتزايد على المواد الحافظة الطبيعية إلى توجه متنامٍ نحو استخدام مضادات ميكروبية حيوية في صناعة الأغذية. هدفت هذه الدراسة إلى تقييم النشاط المضاد للميكروبات لكل من اللايزوزايم والبروبوليس ضد *Escherichia coli* و *Staphylococcus aureus* و *Bacillus cereus* المعزولة من الحلويات اللبنية. تم تحليل عدد 100 عينة (أرز باللبن، آيس كريم، كريم كراميل، مهلبية، وتشيز كيك) للكشف عن وجود هذه الممرضات. وقد تمت عملية العزل والتعرف باستخدام الأوساط الانتقائية، والتحليل البيوكيميائي، والتأكد بتقنية PCR ، بما في ذلك الكشف عن جينات الضراوة مثل *phoA*, *stx1*, *eaeA*, *sea*, *sed*, *groEL*, *bceT*, *cytK* : تم اختيار اللايزوزايم بتركيز 0.1% والبروبوليس بتركيز 1.5% بناءً على نتائج اختبار الانتشار في الحفر (well-diffusion assay) ، وتم اختبارهما لاحقاً في منتج الأرز باللبن. أظهرت نتائج العد الميكروبي خلال فترة تخزين لمدة 5 أيام أن اللايزوزايم قلل بشكل ملحوظ من الأعداد الحية لجميع الممرضات، في حين أظهر البروبوليس تأثيراً مضاداً متوسطاً. كما كشفت نتائج التقييم الحسي أن اللايزوزايم حافظ على جودة المنتج المقبولة، بينما أثر البروبوليس سلباً على الصفات الحسية. تدعم هذه النتائج استخدام اللايزوزايم كمادة حافظة طبيعية آمنة وفعالة في الحلويات اللبنية.

الكلمات المفتاحية: مضادات ميكروبية، جينات الضراوة، تفاعل البوليميراز المتسلسل (PCR) ، لايزوزايم، بروبوليس.