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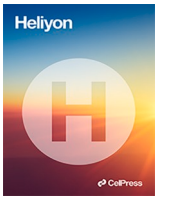

Research article

The potential of pulsed electromagnetic field-generated shock waves for reducing microbial load and improving homogenization in raw milk

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ABSTRACT

Milk is a highly nutritious food essential for human consumption. However, traditional thermal processing methods can reduce its nutritional value and cause unwanted changes. The use of shock waves produced by pulsed electromagnetic fields (PEMFs) has been explored as a means to reduce pathogenic microorganisms. The effect of shock wave treatment on microbial load and particle distribution in packaged fresh cow's milk was investigated. Additionally, the impact of shock wave treatment on *Salmonella enterica* counts in a bacterial suspension of phosphate-buffered saline (PBS) was evaluated, as this bacterium is a significant milkborne pathogen. Treatment with 1000 impulses from an electromagnetic shock wave generator resulted in a 0.7-log reduction in the total bacterial count of milk. In a separate experiment, a 300-impulse shock wave treatment applied to a *Salmonella enterica* suspension achieved a 3-log reduction in bacterial counts. Furthermore, shock wave treatment resulted in a decrease in milk particle size compared to untreated milk. Notably, the volume of milk used in this study aligns with commercially available packaged products, enhancing the experiment's industrial relevance. The use of PEMF to generate shock waves could provide a novel approach for future studies focused on reducing the microbial load of milk and improving its homogenization.

1. Introduction

Milk is a nutrient-rich liquid, providing essential proteins, vitamins, and minerals [1], and has evolved to optimize the survival chances of infants [2]. It contains phenolic compounds with potential antioxidant and antitumor properties [3,4]. To ensure safety and extend shelf life, milk undergoes pasteurization to destroy pathogens and homogenization to reduce fat globule size [5]. However, pasteurization can alter the nutritional and sensory profile of milk, affecting bioactive compounds and leading to the formation of undesirable substances [6]. Additionally, the thermal pasteurization process can be energy-intensive, potentially impacting the taste, color, and nutritional value of milk [7,8].

Raw milk contains a diverse microbiota, particularly lactic acid bacteria (LAB) such as *Leuconostoc*, *Streptococcus*, and *Enterococcus*. Notably, *Lactobacillus* and *Lactococcus* species are well-recognized for their potential health benefits. These microbes play a crucial role

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in natural fermentation processes within raw milk. During this process, they produce a variety of antimicrobial compounds, including bacteriocins, antifungal agents, organic acids, and hydrogen peroxide. These compounds act as natural preservatives in the milk. Among these, LAB-derived bacteriocins, particularly nisin produced by *Lactococcus* lactis strains commonly found in raw milk, exhibit broad-spectrum antimicrobial activity against various pathogenic bacteria, including *Listeria monocytogenes*, *Escherichia coli*, and several *Staphylococcus species* [9,10]. Unfortunately, these health-promoting and useful bacteria are significantly destroyed during thermal pasteurization processes. In the other side, a 13-year study (1993–2006) in the United States associated raw milk consumption with 1571 reported cases of foodborne illness. This resulted in 202 hospitalizations and 2 fatalities. The primary cause of these illnesses was attributed to contamination of raw milk products with pathogenic bacteria, including *Salmonella* spp., *Listeria* spp., *Escherichia coli (E. coli)*, *Campylobacter* spp., *Brucella* spp., and *Shigella* spp [10]. Therefore, it would be ideal to have a non-thermal process that can eliminate pathogenic bacteria while preserving the useful bacteria in the milk.

To address these issues, there has been a growing interest in alternative non-thermal processes to maintain microbial safety and bioactive compounds [11,12]. The increasing need for research on new non-thermal processing methods is also driven by the emergence of bacterial mutations and the rise of heat, drug, and disinfectant-resistant species in the food processing industry [4]. Several non-thermal processes have been explored in the processing of milk and dairy products, including Ultraviolet processing (UV–C), High-Pressure Homogenization (HPH), High-Pressure Processing (HPP), cold plasma, ultrasound, Pulsed electric fields (PEF), and Membrane Filtration (MF) [4,13–18].

One promising non-thermal mechanism that has the potential to replace pasteurization and homogenization is the use of shock waves [19]. Shock waves are produced by the sudden release of mechanical, electrical, chemical, or nuclear energy in a limited space, causing a sudden change in pressure, density, temperature, entropy, and speed of particles in the fluid [20,21]. Shock waves have been widely studied for their destructive effects on bacterial cells, and the use of shock waves as a non-thermal food processing method has been explored in recent years [11]. During the last two decades, shock waves have been used to improve the quality and nutritional aspects of food, such as increasing the shelf life [11], softening the meat texture, and increasing the yield and nutritional value of water and oil extracted through the softening of the plant tissues [12,22].

The mechanical phenomena that occur simultaneously with the passage of shock waves in water include high-pressure compression, tensile stress, and cavitation, which create areas of shear stress and high point temperature that facilitate microbial inactivation [12,19]. Although the effectiveness of shock wave treatment on different microbial species has been studied, its effectiveness on milk and dairy products has not been investigated [12]. In addition to reducing the microbial load, shock waves produced by pulsed electromagnetic fields (PEMF) may also affect the size of milk particles, including casein micelles, which play a crucial role in the stability, texture, and processing properties of milk and dairy products [23–25]. However, the effect of mechanical effects of shock waves on milk particles and their potential for milk processing has not yet been explored.

This research aims to investigate the effectiveness of shock wave treatment applied to milk after packaging, with a focus on reducing the total microbial load and enhancing milk homogeneity. Additionally, a separate experiment was conducted to assess the treatment's effect on *Salmonella enterica*, a bacterium associated with notable foodborne illnesses [26]. Moreover, the effect of shock waves on the structural and size distribution of milk particles, including casein micelles, was assessed using dynamic light scattering techniques. The objective of this study is to investigate the mechanical effects produced by shock waves. To augment this analysis, both simulations and experimental measurements of the electromagnetic field were conducted. These efforts aimed to quantify the electromagnetic pulse strength and evaluate its potential impact on the experimental results. The aim of this study is to enhance the development of a non-thermal method that has the potential to replace traditional thermal pasteurization processes in future applications.

To the best of our knowledge, this is the first study to investigate the use of shock waves created by PEMF as an alternative to conventional thermal processes for raw milk. The results of this study can provide valuable insights into the use of non-thermal methods for food processing, particularly for milk and dairy products.

2. Materials and methods

2.1. Milk samples

Fresh cow's milk sample (3.6 % fat, 3.1 % protein, 1039 kg/m^3 density) was prepared from the livestock breeding farm of Bawan Kalhor Keshto Sanat and Animal Husbandry Company (Gwawer District, Gilan-e Gharb County, Kermanshah, Iran). Also, commercially available pasteurized and homogenized milk (3.5 % fat, 3.3 % protein, density 1036 kg/m^3) was obtained from Manizan brand and was transferred to the laboratory under cold conditions (under 4 °*C*). The chemicals and culture media used were obtained from Merck (Germany).

2.2. Preparation of bacterial samples for shock wave treatment

In this study, the *Salmonella enterica* subsp. *enterica serovar Anatum* ATCC 9270 was used. To prepare the bacterial samples, a sterile nutrient broth (5 mL) provided by Merck, Germany, was inoculated with a single bacterial colony selected from a nutrient agar plate. The culture was incubated at 37 °C overnight to achieve an approximate concentration of 6×10^8 CFU/mL. The inoculum concentration was standardized by adjusting the turbidity to match the McFarland standard No. 2, which corresponds to an optical density of 0.2 at 600 nm, as measured by a spectrophotometer [27]. After incubation, the bacterial suspension was stored at 4 °C. For the experiments, 1.67 mL of this suspension was added to 1000 mL of the PBS (Phosphate-Buffered Saline) to achieve a final bacterial

concentration of approximately 10^6 CFU/mL.

2.3. Shock waves generated by pulsed electromagnetic field

To treat the samples, Dornier MedTech, Germany, Compact Delta II electromagnetic shock wave generator was used (Fig. 1).

The device used in this study (Fig. 1A) generates shock waves by moving a metal diaphragm (Fig. 1B) with a diameter of approximately 120 mm, which is located at the base of a shock tube filled with water. When a very short electrical pulse (16–22 kV/cm) is sent through the coil, it produces an increasing magnetic field that induces eddy currents in the metal membrane. These eddy currents create a magnetic field that is opposite to the magnetic field of the coil, causing the membrane to repel and transfer mechanical energy to the water. The sudden movement of the membrane creates a pressure wave that propagates through the water [20].

As shown in Fig. 2, the raw milk sample was treated with shock waves in a 1000 mL polypropylene container. Polypropylene was chosen as the packaging material because its acoustic impedance (1.9 MRayl) [28] is similar to that of water (1.56 MRayl) [29], resulting in minimal shock wave absorption by the container. This allows for the efficient transfer of shock wave energy into the food fluid, inducing cavitation [12]. The device was set to operate at a frequency of 70 pulses per minute. The corresponding treatment times for the various pulse settings were: 300 pulses (4 min 17 s), 1000 pulses (14 min 17 s), 2000 pulses (28 min 34 s), and 3000 pulses (42 min 52 s). The shock wave energy flux density was set to 0.28 mJ/mm^2. Typically, shock waves with a high peak pressure (100 MPa) have an energy flux density in the range of 0.003–0.89 mJ/mm^2 [30]. For this study, an energy flux density value of 0.28 mJ/mm^2 was chosen based on the limitations of the instrument to avoid potential damage to its electrical circuits. Three 1000 mL samples of raw milk at 4 °C were subjected to 1000, 2000, and 3000 pulses. While control samples of raw and pasteurized milk were kept at the same temperature and environmental conditions as treated samples, but without exposure to shock wave pulses. Furthermore, in a separate experiment, 1000 mL of *Salmonella enterica* subsp. at 4 °C was treated with 300 shock pulses at a constant energy flux density of 0.28 mJ/mm^2 and compared to untreated bacterial suspension as a control to study the effectiveness of shock wave treatment against *Salmonella enterica*.

2.4. Milk particle size and distribution

The size distribution of fat globules and other particles in milk was analyzed by measuring particle distribution in samples using a particle size analyzer and dynamic light scattering (DLS) at a temperature of 25 °C (Zetasizer; Mal 1033452, Malvern Instruments, UK) [31,32]. The refractive indices of the dispersed phase and the scattering medium were 1.59 and 1.33, respectively. Additionally, the milk absorption rate was 0.01 [31,33].

The dispersion index (PI) was used to compare the heterogeneity of the samples. The dispersion index is a measure of the heterogeneity of a sample based on size and can occur due to size distribution in a sample or sample aggregation during separation or analysis [34].

2.5. Microbiological tests

Consecutive dilutions of milk samples ranging from 10^{-1} to 10^{-5} were prepared in normal saline solution (0.9 %) and the total bacteria count was determined using PCA (Plate Count Agar) culture medium after incubating at 37 °C for 48 h [35].

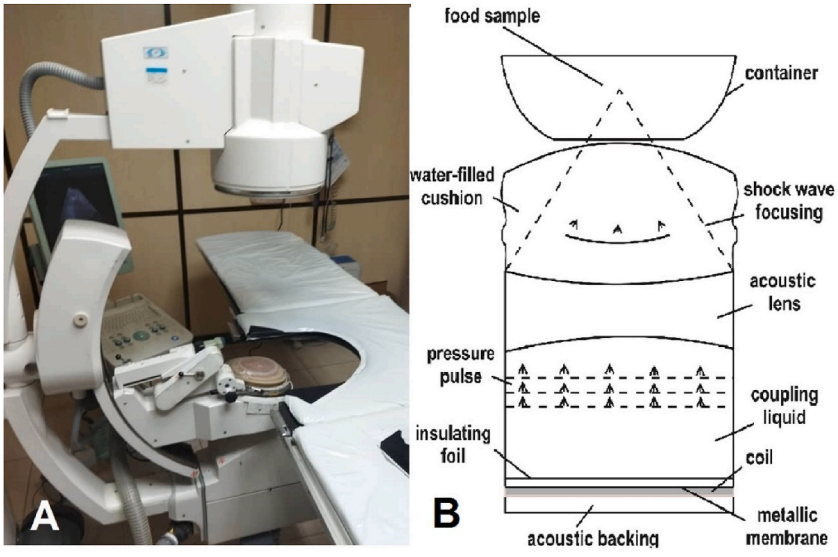

Fig. 1. A) Electromagnetic shock wave generator device B) Electromagnetic shock wave generator schematic [20].

Fig. 2. Placement of the raw milk sample package on the electromagnetic shock wave generator.

To quantify the amount of living *Salmonella enterica* before and after extracorporeal shock wave treatment, the samples were divided into treated and untreated groups and diluted them into different concentrations using PBS. The bacteria were then cultured at 37 °C on SSA (*Salmonella* Shigella Agar) for 24 h, and their concentration was determined by colony counting. For quantifying microbial concentration, plates with 30–300 colonies were selected for counting. The number of colonies was then multiplied by the reciprocal of the dilution factor to determine the microbial concentration [36].

2.6. Simulation and measurement of electromagnetic field

In this study, COMSOL Multiphysics 5.6 Software (COMSOL, Stockholm, Sweden) was utilized to simulate the electromagnetic field generated by an electric pulse within the electromagnetic shock wave generator device. The dimensions of the electromagnetic shock wave generator (Dornier MedTech, Germany, Compact Delta II) were taken into account to develop a 2-D axisymmetric model of the inductors using the magnetic fields physics module. The simplified geometry of the electromagnetic shock wave generator is illustrated in Fig. 3. An axisymmetric two-dimensional representation was adopted for the model. The generator comprises an array of planar spiral-type coils and a copper metal diaphragm, both positioned within the air section. The physical properties of the materials employed in the simulation are provided in Table 1, with the metal diaphragm having a radius and thickness of 60 mm and 0.1 mm, respectively. The planar spiral-type coil consists of 60 turns, each with a diameter of 1 mm. In the simulation model, Dirichlet boundary conditions have been incorporated, with the infinite element domain delineated at the external boundaries, as depicted by the blue color in Fig. 3. Boundary along the r = 0 axis functions as the axis of symmetry. The simulation resolves around this axis, employing Maxwell's equations [37]. The equations solved in a time-dependent analysis are as follows [38]:

$$E = -\frac{\partial A}{\partial t} \tag{1}$$

where, E is electric field intensity, t is time and A is the magnetic vector potential. The total electric current J defined as:

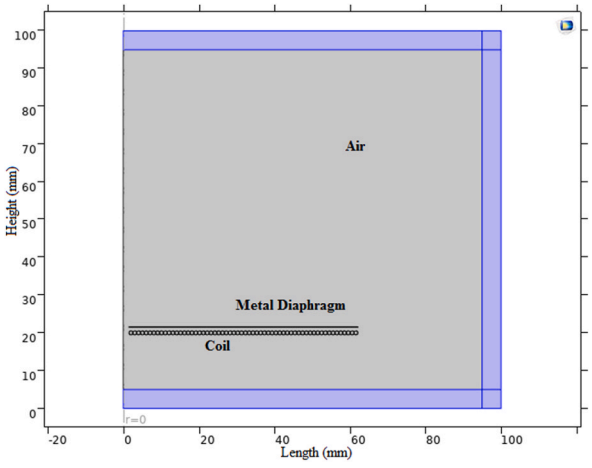

Fig. 3. The simplified geometry of the electromagnetic shock wave generator.

Table 1
The properties of materials.

Materials	Relative permeability	Electrical conductivity (S/m)	Relative permittivity
Copper/Cu	1	5.998e7	1
Air	1	0	1

$$J = \nabla \times H \tag{2}$$

where , H is magnetic field intensity. B magnetic flux density is defined as:

$$B = \nabla \times A \tag{3}$$

Constitutive realtion between J and E defined as:

$$J = \sigma E \tag{4}$$

where, σ is electric conductivity of material. Constitutive realtion between B and H defined as:

$$B = \mu_0 \mu_r H \tag{5}$$

where, μ_0 and μ_r are free space and material magnetic permeability respctivly. Constitutive relation between electric displacement field, D and electric field intensity E:

$$D = \epsilon_0 \epsilon_r E \tag{6}$$

where, ϵ_0 and ϵ_r are free space and material permittivity, respctivly.

The simulation utilized a typical measured current pulse with a peak intensity of 1.92 kA (as depicted in Fig. 4) applied to the coil during the simulation process [39]. In addition, an automated physics-driven meshing approach was used.

2.7. Statistical analysis

A one-way analysis of variance was utilized to examine the significant impact of the independent variable (the number of shock waves in different treatments) on the mean values of the dependent variable (the logarithm of the number of microorganism colonies). A p-value less than 0.05 in Duncan's test was used to indicate a significant difference. The bacterial count test was performed in triplicate.

3. Results and discussion

3.1. Impact of pulsed electromagnetic field-generated shock waves on milk microorganisms and Salmonella enterica

Fig. 5 shows the total bacteria count for raw milk, commercially available pasteurized and homogenized milk, and milk subjected to varying numbers of shock waves generated by the pulsed electromagnetic field. The results indicate that the shock wave treatment led

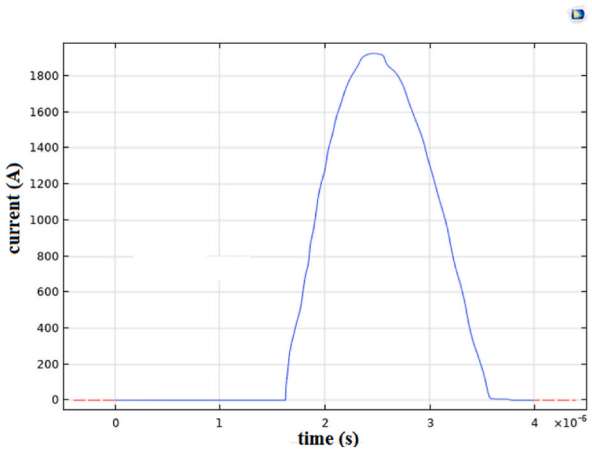

Fig. 4. Current pulse utilized in magnetic field simulation.
The magnetic field was measured using the Gauss Meter Android smartphone application developed by Keuwlsoft, which employs the magnetic sensors embedded in smartphones.

to a maximum logarithmic reduction of 0.7 of total microbial load of raw milk ($P < 0.05$), which is not a significant reduction compared to commercially available pasteurized milk. In addition to the direct impact of the shock wave, other phenomena associated with the treatment, such as high pressure, tensile stress, and cavitation, may also contribute to the inactivation of bacteria [19].

Fig. 6 depicts the variation in colony-forming units (CFU) following extracorporeal shock wave treatment on a *Salmonella* suspension, as part of an independent experiment.The initial concentration of *Salmonella* in the untreated control suspension was 6 log CFU/mL. Following the application of 300 impulses (with an energy flux density of 0.28 mJ/mm^2), a significant reduction of up to 3 logarithms in the untreated control's CFU was observed ($P < 0.05$). Alvarez et al. [40] reported that shock wave treatment was highly effective in inactivating *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* bacteria, due to the occurrence of cavitation. Cavitation, a process in which the formation and collapse of microbubbles create shock waves and high energy levels, leads to the destruction of the bacterial cell wall [19].

The study conducted by Pandur et al. (2023) showed that fluid jet and mechanical pressure are the main causes of bacterial wall destruction and the antimicrobial effects of cavitation [41]. The results of the study by Zevnik et al. (2022) suggest that local stresses caused by the mechanical loads of the cavitation bubble can exceed the cell membrane perforation threshold and that bacterial cell damage can be explained only by mechanical effects in the absence of thermal and chemical effects, such as the production of free radicals [42]. Conversely, the study by Pandur et al. (2022) suggests that the effectiveness of cavitation-related processes in the fluid is influenced by both the physiological state of the bacteria and the architecture of the bacterial cell wall. The peptidoglycan structure of the bacterial cell wall is considered the most crucial factor in determining the effectiveness of shock wave treatment [43]. Given the significant structural variation in the peptidoglycan of different bacterial species, it can be inferred that the effectiveness of shock wave treatment may vary depending on the type of bacteria being targeted [44].

Despite the application of 1000 shock waves resulting in a relatively minor decrease in microbial load (0.7 logarithmic decrease) compared to raw milk, no significant change was observed after the application of 2000 and 3000 shock waves ($P > 0.05$). It is plausible that bacteria sensitive to shock waves were eradicated after 1000 shocks, while other more resilient bacteria persisted despite the subsequent shocks up to 3000. The varying response of bacteria to extracorporeal shock wave treatment may be attributed to the presence of different microbial strains in the milk, each exhibiting a distinct sensitivity to the stress induced by the shock waves, including the effects of cavitation. However, it is important to note that different conditions, including varying impulse numbers and bacterial medium, were utilized, making direct comparisons challenging. Therefore, although the reduction of *Salmonella enterica*, a dangerous pathogenic bacterium [26], showed a significant decrease of up to 3 log, correlating this directly with the overall bacterial inactivation observed under various shock wave conditions proves challenging. Further investigations considering these limitations are warranted to provide a more comprehensive understanding of the microbial response to shock wave treatment.

Previous research has confirmed that shock wave processing can have different effects on various types of bacteria, and even different strains of a single bacterial species can respond differently to this treatment [19]. Bacterial cells are classified based on their shape (spherical, rod-shaped, curved, and spiral), spore formation (sporous and non-sporous), and oxygen requirement (aerobic, microaerophilic, and anaerobic). These classifications can help explain the diverse results observed in shock wave processing studies due to the different sensitivities of bacteria to stress induced by the shock wave, including the effects of cavitation [42]. Raw milk can contain a wide range of bacterial species, including Gram-positive species such as *Lactococcus, Streptococcus, Lactobacillus, Enterococcus, Leuconostoc, Bacillus, Propionibacterium, and Mycobacterium*, as well as Gram-negative species such as coliforms, *Pseudomonas,* and *Acinetobacter* [4]. However, studies indicate a predominance of Gram-positive species in the microbiota of raw milk [10]. *Salmonella enterica*, a Gram-negative, rod-shaped, aerobic bacterium [45], possesses a thinner peptidoglycan layer in its cell wall compared to Gram-positive bacteria commonly found in raw milk [10]. Following shock wave application, *Salmonella enterica*

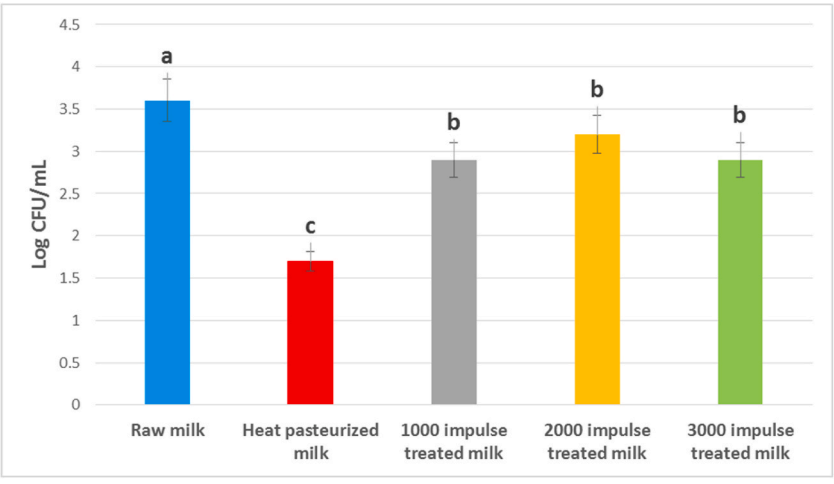

Fig. 5. The effect of different variables on the total count of bacteria in milk samples (Error bars represent standard deviation from three replicates. Different lowercase letters above the error bars indicate a significate difference between mean treatment groups at 95 % confidence intervals.).

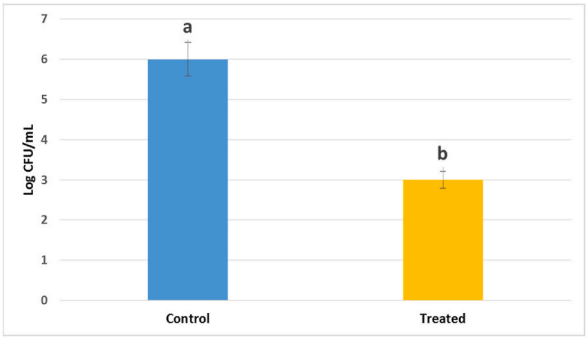

Fig. 6. Decrease in *Salmonella enterica* in a suspension after treatment with 300 impulses of extracorporeal shock waves (Error bars represent standard deviation from three replicates. Different lowercase letters above the error bars indicate a significate difference between mean treatment groups at 95 % confidence intervals.).

exhibited a significant reduction in population (3 log reduction) compared to the minimal decrease observed in the total raw milk bacterial count (0.7 log reduction). This enhanced susceptibility of *Salmonella* aligns with established research highlighting the role of mechanical stress from shock waves in targeting the bacterial cell wall. The resulting disruption of the peptidoglycan layer likely compromises the integrity of the *Salmonella* cell membrane, leading to cytoplasmic content release and ultimately, cell death.

In a study conducted by Takemoto et al. (2006) [46] on the antimicrobial effects of shock waves produced by explosive fuse, the effectiveness of the treatment varied depending on the bacterial species. Specifically, *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* were found to be significantly affected, with effectiveness rates of 57 % and 49–100 %, respectively. However, no significant effects were observed for bacteria such as *E. coli*, *Bacillus cereus*, and *Staphylococcus aureus*. The study treated samples with three shock waves at an intensity of 300 MPa.

Horn et al. (2009) conducted a study in which standard suspensions of *Staphylococcus aureus* bacteria were subjected to shock wave treatment at varying energy flux densities (0.38–0.96 mJ/mm^2) and impulse numbers (2000–12000) under both static and dynamic growth conditions. The results of the study showed that shock wave treatment was effective in reducing *Staphylococcus aureus* bacteria by 56–100 % [47].

In another study, the effect of shock waves generated by an electrical discharge of 18.8 kV–31.6 kV with a frequency of 1 Hz on *Vibrio* spp. bacteria was investigated. The results showed a five logarithmic decrease in the bacterial population after applying 160 impulses [48]. Therefore, based on the results of this study and those of previous research, it can be concluded that the use of shock wave treatment is an effective means of reducing bacterial populations.

The results of this study provide insights into the potential effects of shock wave treatment on milk microorganisms, particularly when compared to conventional thermal pasteurization methods. While exposing milk to 1000 shock wave impulses resulted in a modest 0.7-log reduction in microbial load, it is important to note that this reduction is not as significant as that typically achieved through thermal pasteurization. However, a separate experiment targeting the pathogenic bacterium *Salmonella enterica* demonstrated a notable 3-log reduction in bacterial counts within a suspension medium. This substantial reduction highlights the potential of shock wave treatment to selectively target harmful pathogens like *Salmonella* while preserving the beneficial microflora present in raw milk. Given that lactic acid bacteria (LAB) dominate the microbiota of raw milk, it is essential to acknowledge the complex microbial composition of raw milk, which includes a diverse array of both pathogenic and beneficial bacteria [10]. These findings suggest a promising direction for future research aimed at optimizing shock wave treatment to effectively control pathogenic microorganisms while minimizing disruption to beneficial microbial populations. Further investigations employing advanced techniques are warranted to elucidate the varying effects of shock wave treatment on the different bacterial species present in milk before/after packaging, thereby contributing to a more comprehensive understanding of its microbiological impact.

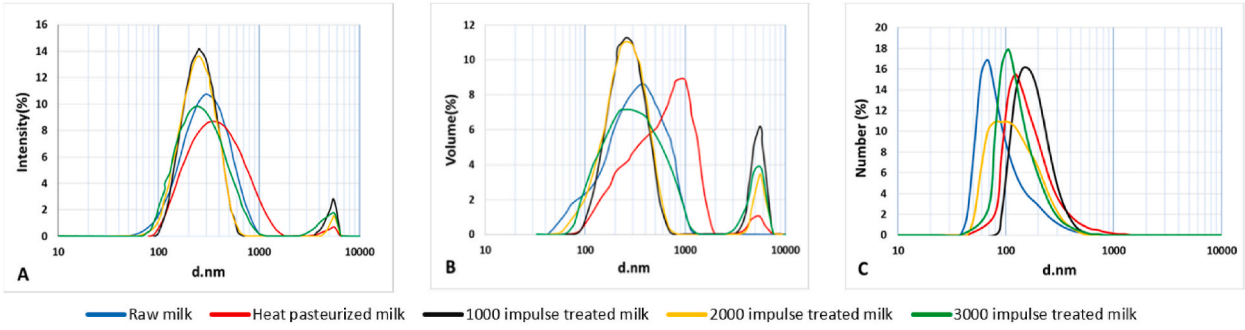

Fig. 7. Particle size distribution of milk samples according to Intensity (A), Volume (B), and Number (C).

3.2. Impact of pulsed electromagnetic field-generated shock waves on milk particle size and distribution

Fig. 7 shows the particle size distribution of milk samples: raw, commercially available pasteurized and homogenized, and shock wave treated (1000, 2000, 3000 impulses). The x-axis is in logarithmic scale. The distribution is presented according to three criteria: intensity (Fig. 7A), volume (Fig. 7B), and particle number distribution (Fig. 7C). In the particle size distribution in terms of intensity, the contribution of each particle in the distribution is determined by the intensity of light scattered by that particle. In the volume distribution, the contribution of each particle in the distribution is determined by the volume of that particle. Finally, in the particle size frequency distribution, each particle is given equal weight regardless of its size [49]. The results demonstrate a notable decrease in milk particle size following shock wave treatment compared to raw milk. However, given the absence of data regarding milk particle size prior to commercial pasteurization and homogenization, a direct comparison between the effectiveness of the commercial homogenization process and the shock wave treatment is not viable.

As shown in Fig. 7A and B, the particle size distribution diagrams of all processed milk samples (except for raw milk) exhibit two peak points. The first peak point ranges from 80 to 900 nm for shock wave processed milk, 90–1300 nm for commercially pasteurized and homogenized milk, and 70–1100 nm for raw milk. The smaller range and peak size of particles in the graphs of milk processed with shock waves generated by pulsed electromagnetic field compared to raw milk indicate greater homogeneity and smaller particle size, and thus more suitable homogenization. However, according to Table 2, the polydispersity index (PI) increased from 0.212 for raw milk to 0.32 for processed milk. The presence of a second peak in the size distribution diagram of milk particles is indicative of protein aggregation [5]. All processed milk samples exhibited a second peak within the range of 6000 nm, although intensity and volume varied. Notably, milk samples processed with shock waves displayed significantly higher values (up to sixfold) compared to those treated with conventional pasteurization. This suggests a greater degree of protein aggregation in shock wave-treated milk. The potential explanations of the mechanisms underlying this difference include: (i) the intensity and duration of the shock wave leading to protein unfolding due to rapid pressure fluctuations, (ii) localized heating caused by the shock wave further destabilizing protein structures, (iii) shear forces generated by the shock wave physically disrupting proteins, and (iv) specific interactions between shock waves and certain milk proteins. Further research is needed to elucidate the dominant factors and specific mechanisms by which shock waves induce protein aggregation in milk compared to conventional pasteurization.

The size distribution of fat globules in raw milk ranges from 1 to 10 μm, while protein micelles have a diameter of 40–300 nm [50]. Furthermore, the fat concentration in raw milk can range from 2 to 8 percent [51]. The distribution and size of emulsion particles can impact the rate of protein emulsion, clot formation, and their integration. Particle size and particle size distribution can be utilized to examine emulsion stability [52].

Mootse et al.(2014), investigated the particle size distribution of raw milk samples using the DLS technique [23]. In their study, the average size of micellar casein particles in raw milk collected from 44 cows was found to be 171.13 nm, with a range of variation of 70.1 nm. The distribution of the particles was similar to a normal distribution, which is consistent with the results obtained in the present study for the raw milk sample (Fig. 7, part A).

Tran Le et al. (2008) investigated the change in the particle size distribution of casein micellar dispersions due to heating in the presence and absence of whey proteins using the DLS technique [32]. Their results showed that heating casein micelles in the presence of whey proteins leads to the formation of a particle distribution with two peak points. These observations suggest that the average increase in particle size is primarily due to the accumulation of casein micelles, which is another consequence of whey protein precipitation on casein micelles caused by heat. In the current study, similar results were obtained in the particle size distribution of milk processed by the conventional thermal pasteurization method, as shown in Fig. 7 with two peak points and a shift towards the right side, indicating the effect of heat on whey protein deposition on casein micelles and particle accumulation. This finding is consistent with a study conducted by Ransmark et al.(2019), which also measured milk particle distribution using the DLS technique [5].

3.3. Results of electromagnetic field simulation and measurement

Fig. 8 presents the simulation results of the pulsed electromagnetic field in COMSOL software, illustrating the maximum intensity of the electromagnetic pulse strength at approximately 5 T in both a two-dimensional view (Fig. 8A) and a three-dimensional view (Fig. 8B). However, in this study the sample was located approximately 14–17 cm away from the coil, resulting in an electromagnetic field intensity at the sample location of only a few microtesla (μT), as confirmed by both simulation and experimental measurements. Therefore, it can be concluded that the electromagnetic field did not directly affect the results obtained in the experiment. The findings of this study are solely attributed to the shock wave treatment.

4. Conclusion

As the number of shock waves increased up to 3000, there was a significant decrease in the size of milk particles compared to raw milk. Additionally, shock wave treatment led to a 0.7-log reduction in the total microbial load with up to 1000 impulses, which was less than the reduction observed with traditional pasteurization and homogenization processes. However, there was no further decrease in the microbial load with an increasing number of shock waves up to 2000 and 3000 shocks. In a distinct experiment aimed at assessing the impact of shock wave treatment on a suspension of *Salmonella enterica*, a significant reduction of 3 logs in the *Salmonella enterica* population was noted after administering just 300 impulses of shock wave treatment, in comparison to the untreated control sample. The study was conducted with a maintained constant shock wave energy flux density and temperature throughout the experiment, which may limit the generalizability of the findings. Future research endeavors could explore the effects of increasing the energy flux

Table 2
Polydispersity index (PI) for raw and processed milk.

Sample	Polydispersity index (PI)
Raw milk	0.212
Heat pasteurized milk	0.264
1000 impulse-treated milk	0.311
2000 impulse-treated milk	0.281
3000 impulse-treated milk	0.320

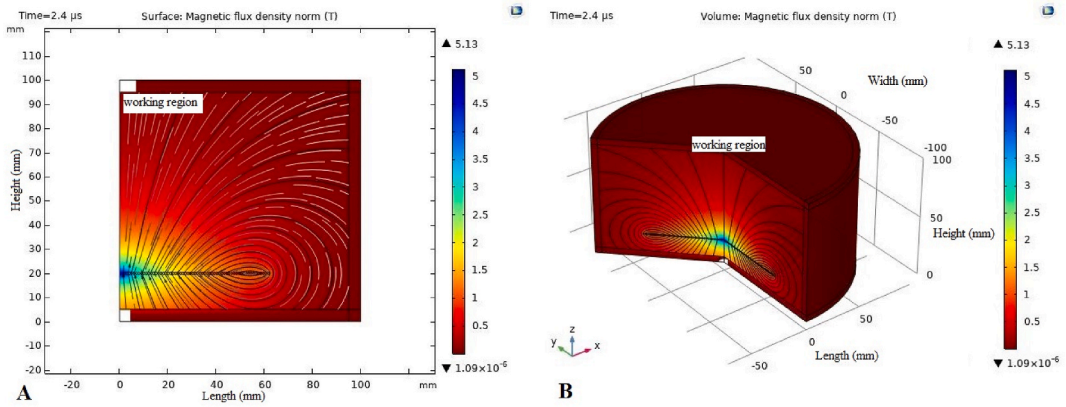

Fig. 8. Simulation results of the pulsed electromagnetic field in COMSOL software: A) Two-dimensional view, B) three-dimensional view.

density and temperature to optimize shock wave treatment parameters. Moreover, while this study provides valuable insights into the impact of shock wave treatment on milk, it represents only an initial exploration. Future research should employ more sophisticated bacterial detection and assessment methods to investigate the effects of shock wave treatment on various bacterial species present in milk. By addressing these limitations and expanding the scope of investigation, future studies can refine our understanding of shock wave treatment's efficacy in food processing. The results of this study lay the groundwork for such endeavors and may ultimately guide the development of novel food processing approaches.

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Ethics statement

This study utilized fresh milk from healthy Holstein cows that were bred at the livestock farm of Bawan Kalhor Keshto Sanat Company in Kermanshah, Iran. The cows were kept under standard conditions of feeding, housing, and welfare and did not undergo any experimental procedures or treatments throughout the study. The milk was collected by trained personnel following established animal welfare guidelines. The study did not involve any animal experiments or interventions, so ethical approval was not necessary.

Data availability statement

All data analyzed for this article is available with the corresponding author and can be provided reasonably upon request.

CRediT authorship contribution statement

Ehsan Seyfali: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohammad Hadi Khoshtaghaza:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization. **Milad Rouhi:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zahra Sarlak:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Gholamhassan Najafi:** Writing – review & editing, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

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