Food Science of Animal Products

Research Article

Effects of induced electric field and high-pressure microjet sterilization on the physicochemical properties of milk

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Abstract: This study aimed to investigate the difference between low-temperature sterilization methods including induced electric field (IEF) technology and the high-pressure microjet (HPM) and traditional thermal treatment including pasteurization and ultra-high temperature (UHT) sterilization on the sterilization effect and physicochemical properties of milk. The number of harmful colonies, particle size, thermal properties, protein secondary structure, and microstructure were characterized. Results demonstrated that low-temperature sterilization methods were equally effective as thermal sterilization methods. Differential scanning calorimetry results indicated that UHT treatment had enhanced the thermal stability of proteins in milk, while IEF treatment had improved the thermal stability of fats in milk. IEF sterilizing milk (IM) sample exhibited the largest particle size, followed by UHT sterilization milk (UM), raw milk (RM), and pasteurization milk (PM) according to the results of microstructure and size distribution. HPM sterilizing milk (HM) had the smallest particle size due to high pressure. Fourier transformed infrared spectra revealed that UHT sterilization changed the structure of milk protein due to the interactions between proteins, and protein-lactose. Conversely, the low-temperature sterilization methods had less effect on the protein structure. In conclusion, IEF and HPM have the similar sterilization effects to that of thermal sterilization methods, but have less effects on heat sensitive proteins, which has reference significance for the updating of sterilization technology in food field.

Keywords: induced electric field; high-pressure microjet; sterilization methods; milk; physicochemical properties

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1 Introduction

Dairy foods have attracted significant interests because of their vital contribution to human dietary health, especially regarding quality and safety[1]. Milk, serving as the main ingredient in dairy food, is abundant in premium proteins, fats, lactose, and an assortment of vitamins and minerals[2]. It is also rich in various bioactive immune proteins, including immunoglobulins, lactoferrin, and lactoperoxidase, which are crucial for boosting immune performance and health promotion.

Sterilization plays a crucial role in dairy production to kill harmful microorganisms and extend the shelf life of products. The traditional thermal sterilization methods include pasteurization (Figure 1A)[3], ultra-high temperature (UHT) sterilization (Figure 1B)[4], etc, among which pasteurization is widely used in industry[5]. However, although these techniques can successfully remove dangerous microbes, alter the milk's taste[2,6], they may also lead to the depletion of key bioactive components, especially immunoglobulins and lactoferrin, which are especially vulnerable to harm in thermal processing[7]. Thus, investigating innovative sterilization techniques is crucial to avert their deactivation.

As low-temperature and non-thermal processing methods, induced electric field (IEF) (Figure 1C) and high-pressure microjet (HPM) (Figure 1D) demonstrate significant promise in sterilization[8–9]. The fundamental concept of IEF involves introducing a fluctuating magnetic field to the food medium through electromagnetic induction, which in turn generates a alternating voltage, leading to the destruction of the cell membrane due to electrical failure[10]. Through the pasteurization, UHT, IEF, and HPM device diagrams shown in Figure 1, the differences in different sterilization methods can be intuitively seen.

IEF is a new sterilization technology. Yang et al.[8] found that IEF treatment could kill the harmful microorganisms through electrical breakdown. IEF treatment is operated at a lower temperature than heat sterilization, which can effectively reduce the damage of active proteins e.g. lactoferrin caused by heat treatment, and is conducive to the retention of nutrients. The IEF can also effectively sterilize the juice and protect its internal active components. After IEF treatment, apple juice had stable quality during storage, and there was no adverse effects on phenolic, flavonoid content, color and volatile compounds[11–13]. He et al.[10] also reported that kiwi juice had the least color change and the least loss of total phenols and total flavonoids after IEF treatment, compared to heat sterilization. IEF

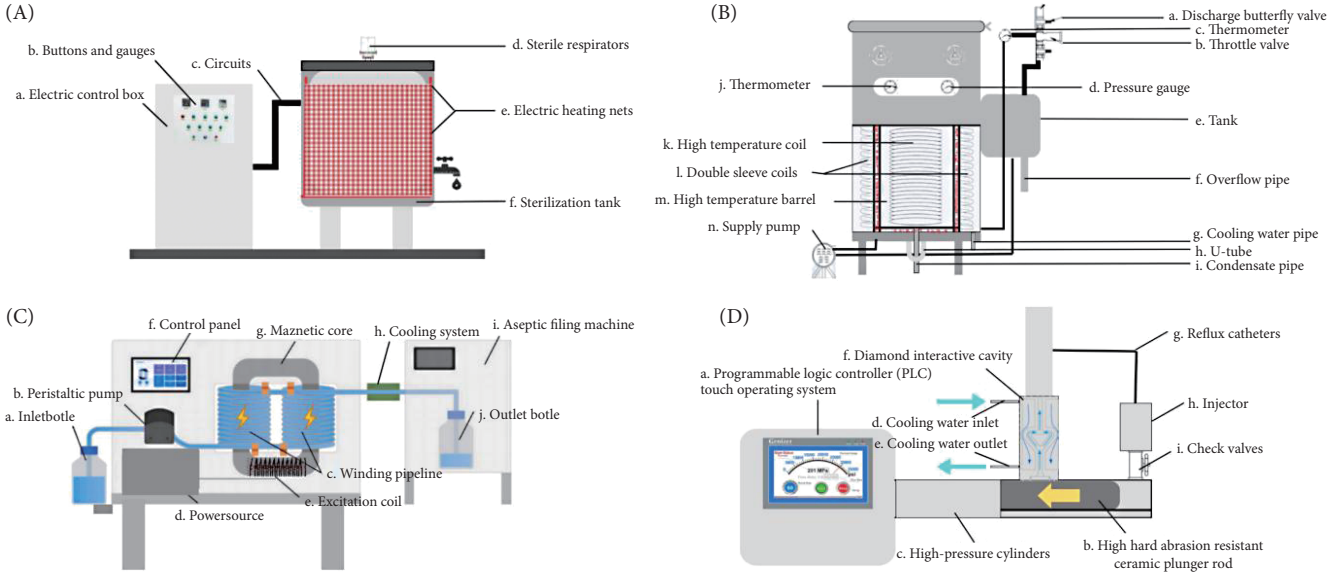

Figure 1 Diagram of sterilization devices of (A) pasteurization, (B) UHT, (C) IEF, and (D) HPM.

also exhibited little effect on carotenoids and trace active components in orange juice[14]. So IEF boasts an extensive area for development and research in treatment and sterilization.

HPM, a prevalent method for liquid homogenization[15], is extensively utilized in food processing. This technology employs high-pressure pumps to channel milk into microchannels to refine and standardize it with the created intense shear, impact, and hole forces during rapid flow[9]. Previous study revealed that the diameter of the fat sphere in HPM-treated milk could be minimized to less than a micron to enhance the emulsion's physical stability[16–17], which could make dairy products' flavors gentler and softer, while also improve the digestibility and absorption speed of the nutrients in milk[18]. The brief duration of treatment and cooler temperatures enable HPM to maintain the function of milk's bioactive components more effectively[19–20], particularly certain heat-sensitive substances like immunoglobulin and lactoferrin[21]. HPM is also capable of maintaining the synergistic techno-functionality of perilla protein isolate[22]. Besides the application in homogenization, HPM may also be a useful method for sterilization. Nonetheless, the study of HPM in sterilization remains limited, which is meaningful for further study.

This study aimed to make sure the sterilization effect of IEF and HPM, and compare the sterilization effect and physicochemical properties of milk treated by both thermal sterilization and low-temperature sterilization methods. Bovine milk samples treated by pasteurization, UHT, IEF, and HPM were prepared, and the total number of colonies, particle size, thermal properties, protein secondary structure, and microstructure were determined. The results can provide theoretical basis for the evaluation of new sterilization technology.

2 Materials and methods

2.1 Materials

Raw milk (RM), pasteurization sterilized milk (PM), and UHT sterilized milk (UM) for commercial use were provided by Heilongjiang Feihe Dairy Co., Ltd. (Harbin, China). Milk was transported to the laboratory preserved at 4 °C. Colony counting tablets were purchased from Guangdong Dayuan Oasis Food Safety Technology Co., Ltd. (Guangzhou, China). The chemical reagents used were sourced from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 IEF, pasteurization, HPM, and UHT treatments of milk

Partial of blended RM was served as control group. PM and UM were heated at 72 °C for 15 s and at 130 °C for 5 s, respectively according to the previous methodology[23]. IEF sterilized milk (IM) was prepared by a IEF apparatus (NIF-10, INDUC Scientific Co., Ltd., Wuxi, China) according to the method of Yang et al.[8] with some modifications. The flow rate, excitation voltage, and frequency were set as 8 L/h, 800 V, and 50 kHz according to preliminary experiment. The temperature of IM was 55 °C. HPM sterilized milk (HM) was prepared by a laboratory-scale high pressure processor (HPP.L2-600/2, Suzhou Microflow Nanobiotechnology Co., Ltd., Suzhou, China) for 60 min at 25 °C. The pressure rose at a rate of 500 psi, eventually reached a pressure release of 40 000 psi. All samples were kept at 4 °C for further analysis.

2.3 Microbiological analysis

The microorganisms of samples including total colony count, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* counts under different sterilization treatment were measured according to the method of Bastin et al.[24] with minor adjustments in the extremely sterile table aseptic environments. The specific culture process was to complete the process of diluting the bacterial solution and sampling the colony counting tablets in a super-clean table, and then transferred to the incubator for 18–24 h at 34 °C. The whole process ensures that there is no interference from miscellaneous bacteria and pollutants. Samples were diluted with sterilized distilled water to the proper concentration, and placed into colony counting tablets for total colony count, *E. coli*, *S. aureus*, and *Salmonella* counts, to incubate for 18–24 h. Each concentration was repeated for three times.

2.4 Particle size measurement

A Malvern 3000 zetasizer (Malvern Instruments, Worcestershire, UK) was used to determine the particle size of milk samples with different sterilization treatment at 25 °C following the method of Sun et al.[25]. Each sample was measured at least three measurements.

2.5 Differential scanning calorimetry (DSC) measurement

The thermal characteristics of milk samples were assessed using a differential scanning calorimeter (Mettler Toledo, DSC 3, Zurich, Switzerland) with the slightly modified method of Sun et al.[23]. The samples were freeze-dried using a freeze-dryer (Alpha 1–2, Marin Christ Inc., Osterode, Germany) prior to being measured. The specimen (5 mg) was heated from 30 to 200 °C at a pace of 10 °C/min, using a sealed blank disk as a benchmark.

2.6 Fourier transformed infrared (FTIR) spectroscopy measurement

FTIR spectra of samples were obtained to track milk's structural changes under various sterilization methods using a Rheonaut rheometer (Thermo Scientific HAAKE, MARS 40, Waltham, USA) equipped with a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Waltham, USA). The infrared spectra of freeze dried samples were documented between 400–4 000 cm^{-1}, with a total of 32 scans.

2.7 Confocal laser scanning microscope (CLSM) observation

The microstructure of milk samples was measured by a FV3000 CLSM (Olympus, Oberkochen, Japan). Samples with 1 g/mL protein were stained using a hybrid fluorescent solution, including 0.02 g/mL fat red for fat visualization, and 0.012 g/mL rhodamine B for protein detection. Approximately 300 μL of dyed emulsions were applied to the microscope slides, followed by the application of cover slips over the concave slides. The argon krypton laser stimulated rhodamine B and fat red at wavelengths of 543 and 633 nm. Observations were conducted with a 63 × oil immersion lens. A minimum of three samples from every sample were examined to capture typical micrographs of the samples[25]. Measurements were conducted three times, selecting the most representative vision for analysis.

2.8 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

SDS-PAGE was used to confirm the distribution of protein molecular weights among milk samples with different sterilization treatment[23]. The samples were diluted with deionised water to the protein concentration of 5 mg/mL. The loading buffer and samples were mixed at a ratio of 1:4 (*V*/*V*), and heated in boiling water for 5 min. Then, 10 μL of the sample and the marker were loaded onto a polyacrylamide precast gel and separated under the following electrophoresis conditions: concentrated gel working constant voltage, 80 V; separation gel working voltage, 120 V; and electrophoresis was stopped when the dye moved to the bottom of the precast gel (1 cm). After electrophoresis, the gels were stained with Coomassie brilliant blue, destained with distilled water.

2.9 Statistical analysis

Data were carefully analyzed by SPSS 22.0 software (IBM SPSS Inc., Chicago, USA). The analysis was conducted with a confidence interval of 95% to confirm its statistical relevance. If not specifically stated, every experiment was conducted thrice (n = 3) to confirm the accuracy of the measurements. A one-way analysis of variance, employing a standard linear modeling approach, was used to identify variations in average values. Significant differences were marked by distinct letters ($P < 0.05$).

3 Results and discussion

3.1 Sterilization effect of different treatments

As shown in Table 1, it could be observed that the total colony count, *S. aureus*, *Salmonella*, and *E. coli* bacteria for PM, UM, IM, and HM were nearly 0, which were significantly lower than those of RM. It demonstrated that low-temperature sterilization technologies such as IEF and HPM can achieve the same sterilization effectiveness as traditional heat treatment methods. Results were in accordance with previous studies which also reported that the non-thermal effects generated by electromagnetic fields had sterilization effect[8,26].

Table 1 Total colony count, *E. coli*, *S. aureus*, and *Salmonella* count of milk samples treated by different sterilization methods (CFU/mL).

Group	Total colony count	*E. coli* count	*S. aureus* count	*Salmonella* count
RM	7.4×10^5	1.21×10^4	1×10^2	3.1×10^3
PM	0	0	0	0
UM	0	0	0	0
IM	0	0	0	0
HM	0	0	0	0

3.2 Particle size of milk

As shown in Figure 2, HM exhibited the smallest particle size compared with the other groups. The dissolution of milk components caused by HPM may be responsible for the decrease in milk particle size[27]. The hydrophobic interaction between the molecules of micelles can be reduced by high pressure. Therefore, casein micelles are destroyed under high pressure and micellar calcium phosphate[28–29]. Within the particle size range of 1–10 μm, the particle size peak strength of PM was the highest, followed by RM, UM, and IM. For particle sizes greater than 20 μm, the particle size peak strength of IM was significantly larger than those of the UM, RM, and PM samples, which was attributed to the protein aggregation or the binding of fat and protein caused by electric field[30]. Furthermore, UM showed larger particle size than those of RM and PM. This is because that high temperature treatment can lead to the denaturation and aggregation of milk protein[31]. Sun et al.[23] reported the similar results that the particle sizes of UHT infant formula protein model samples were larger than those of pasteurized samples.

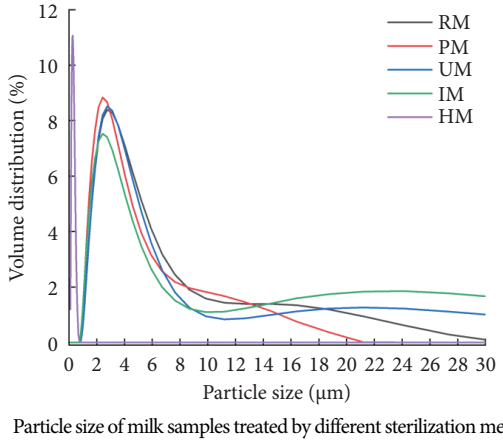

Figure 2 Particle size of milk samples treated by different sterilization methods.

3.3 DSC analysis of milk

As shown in Figure 3, each sample exhibited two endothermic peaks in the 25–50 °C range, which represent the melting process of solid fat in milk as reported by Szulc et al.[32] and Brozek et al.[33]. The two endothermic peak temperatures of RM are 32.8 and 37.2 °C, respectively. PM showed the similar endothermic peak temperatures (32.7 and 37.4 °C) to those of RM. The decreased temperatures of UM (28.0 and 36.6 °C) and HM (27.8 and 35.8 °C) and the increased temperatures of IM (32.6 and 39.9 °C) of the endothermic peaks indicated that IEF can improve the thermal stability of milk fat, while UHT and HPM can reduce its thermal stability. The fat in milk typically exhibits polymorphism during melting and crystallization processes[34], so IEF treatment can transform the crystal structure of milk fat into a more stable form.

The endothermic peaks in the 75–125 °C range shown in Figure 3 represented the denaturation of milk proteins according to the previous studies[32]. Compared to RM (92.7 °C), the protein denaturation temperature of PM, IM, and HM shifted towards lower values (90.4, 86.4, and 86.9 °C). Conversely, UHT treatment increased the temperature of protein denaturation with the endothermic peak to 120.9 °C, which was related to the Maillard reaction between proteins and lactose molecules in milk under heat treatment conditions[23]. Previous studies by Liu et al.[35] also demonstrated that glycation reactions can enhance the thermal stability of proteins.

3.4 FTIR spectra analysis of milk

As shown in Figure 4A, the wavenumber of the first characteristic peak of RM is 3 281.91 cm^{-1}. The characteristic peaks of PM, UM, IM, and HM increased to 3 281.36, 3 285.16, 3 289.15, and 3 288.1 cm^{-1}, respectively. The shifted peaks in the range of 3 000 to 3 500 cm^{-1} indicated the presence of O–H stretching vibrations and suggested the formation of hydrogen bonds between molecules in the milk samples[36].

The peaks in the wavenumber range of 1 600–1 700 cm^{-1} represent amide I vibration caused by the stretching of C=O bonds, while the peaks in the wavenumber range of 1 500–1 600 cm^{-1} represent amide vibration caused by the C–N stretching coupled with N–H bending vibration[37]. As shown in Figure 4A, the characteristic peak positions of amide I vibration for RM, PM, UM, IM, and HM were 1 644.96, 1 645.32, 1 640.48, 1 645.36, and 1 645.38 cm^{-1}, respectively. The characteristic peak positions of amide II vibration for RM, PM, UM, IM, and HM were 1 539.34, 1 539.39, 1 537.33, 1 539.23, and 1 539.39 cm^{-1}, respectively. The shift of characteristic peaks of PM, IM, UM, and HM samples suggested that different sterilization treatments have a certain effect on the protein structure. The characteristic peak shift of HM might be caused by protein depolymerization under high pressure. The absorption peak positions of PM and UM in the 1 500–1 700 cm^{-1} range shifted compared to RM, which was due to the Maillard reaction between lactose and proteins[38]. This also explains the C=O stretching vibrations and N–H bending vibrations[39]. Sereechantarerk et al.[40] mentioned that electric field technology might alter the protein structure in milk, and the shift in absorption peak positions in the 1 500–1 700 cm^{-1} range for IM compared to RM supported this point. Additionally, in this study, all five samples exhibited two distinct absorption peaks in the 2 800–3 000 cm^{-1} range, with varying positions, related to –CH stretching vibrations, suggesting that different sterilization methods altered the hydrocarbon chain disorder in the samples[41].

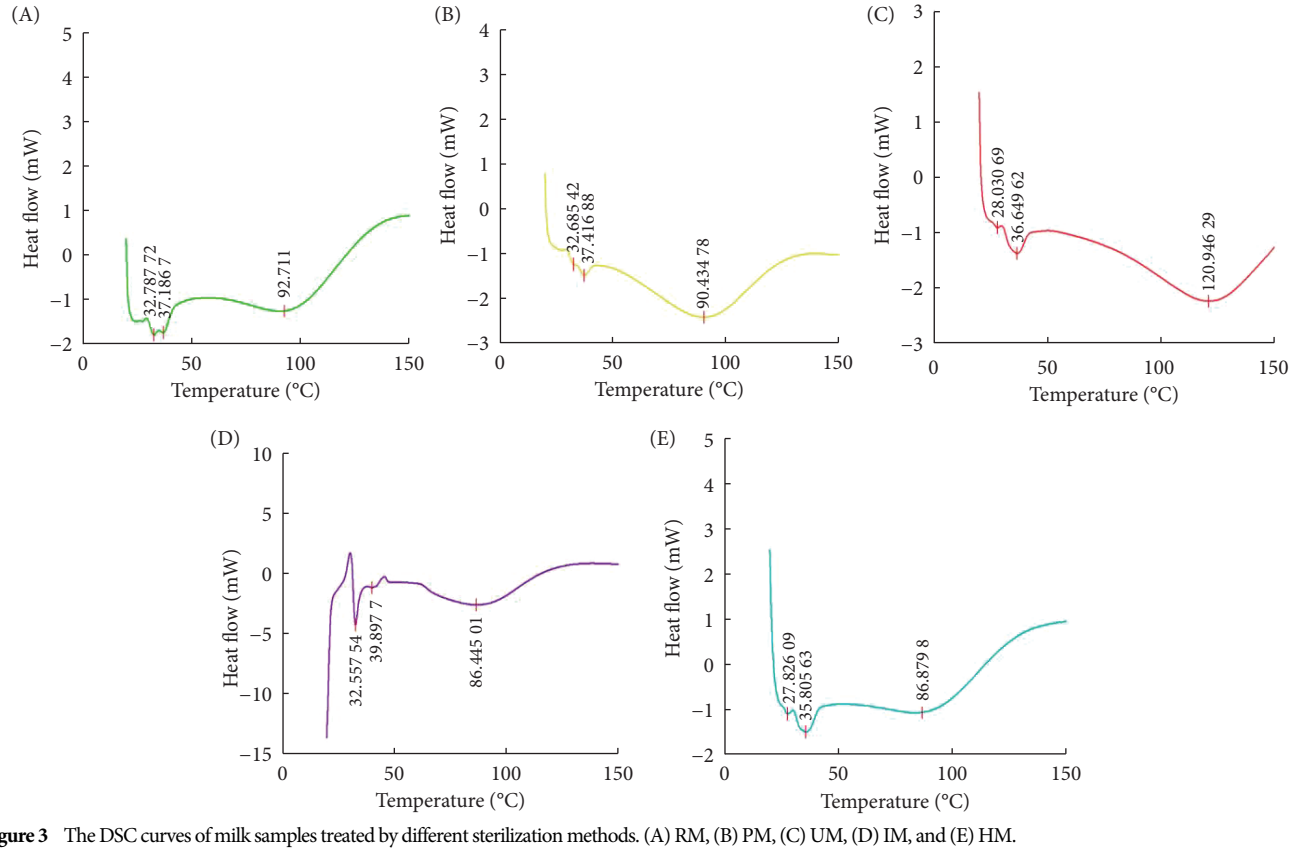

Figure 3 The DSC curves of milk samples treated by different sterilization methods. (A) RM, (B) PM, (C) UM, (D) IM, and (E) HM.

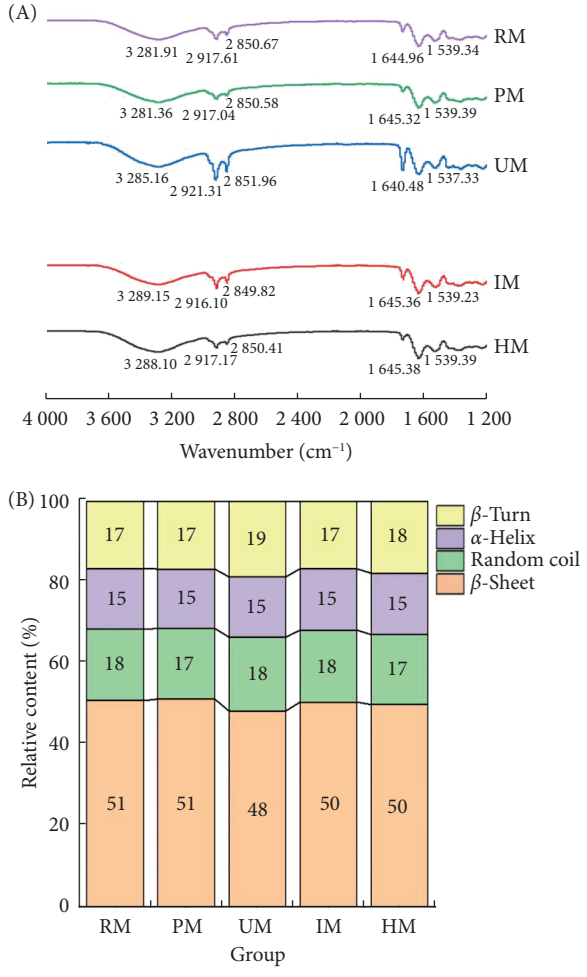

Figure 4 (A) FTIR spectra and (B) secondary structure relative content of milk samples treated by different sterilization methods.

This study referenced the method by Gao et al.[42], employing OMNIC and Peakfit software to perform Fourier self-deconvolution and second derivative operations on the amide I band data of various samples. The relative contents of β-sheet (1 600–1 640 cm^{-1}), random coil (1 640–1 650 cm^{-1}), α-helix (1 650–1 660 cm^{-1}), and β-turn (1 660–1 700 cm^{-1}) were shown in Figure 4B. The β-sheet is considered a major component of protein secondary structure[43]. There were no significant differences in secondary structure relative content between PM, IM, HM, and RM. However, the β-sheet relative content in UM was significantly reduced compared to RM, indicating a decrease in hydrogen bonding between molecules. Combined with the conclusions drawn from Figure 4A, it could be concluded that the Maillard reaction occurring between lactose and protein has led to partial depolymerization of the protein molecules.

3.5 CLSM analysis of milk

As shown in Figure 5, there were significant differences in the CLSM images of milk samples treated with various sterilization methods. The particles in HM could hardly be observed in CLSM images due to high pressure, so result was not given here. After pasteurization and UHT treatment, the size of the fat globules in the samples significantly increased, while the PM samples exhibited some larger protein particles, but most protein particles were smaller and more densely packed. The UM samples had larger protein particles but were more loosely distributed. This was due to the aggregation and denaturation of proteins during the Maillard reaction between proteins and lactose under heat treatment conditions[44], which was consistent with previous DSC analysis results. Additionally, irregular shapes of fat globules were observed in the UM samples, due to the destruction of the membrane structure on the surface of the fat globules caused by ultra-high temperature[45]. The IM samples had larger protein particles but smaller fat globules, and they were more dispersed compared to UM and PM. The reason for this phenomenon is that the electric field causes adsorption on certain charged groups of protein molecules, leading to protein aggregation[46]. As the temperature increases, proteins in milk gradually aggregated on the surface of fat globules and combine with fat. Therefore, fat globule particles in PM and UM were larger, while IEF, as a low-temperature pasteurization method, resulted in fat globules in IM samples being finer and more dispersed[47]. The findings reported by Yang et al.[8] were consistent with this study.

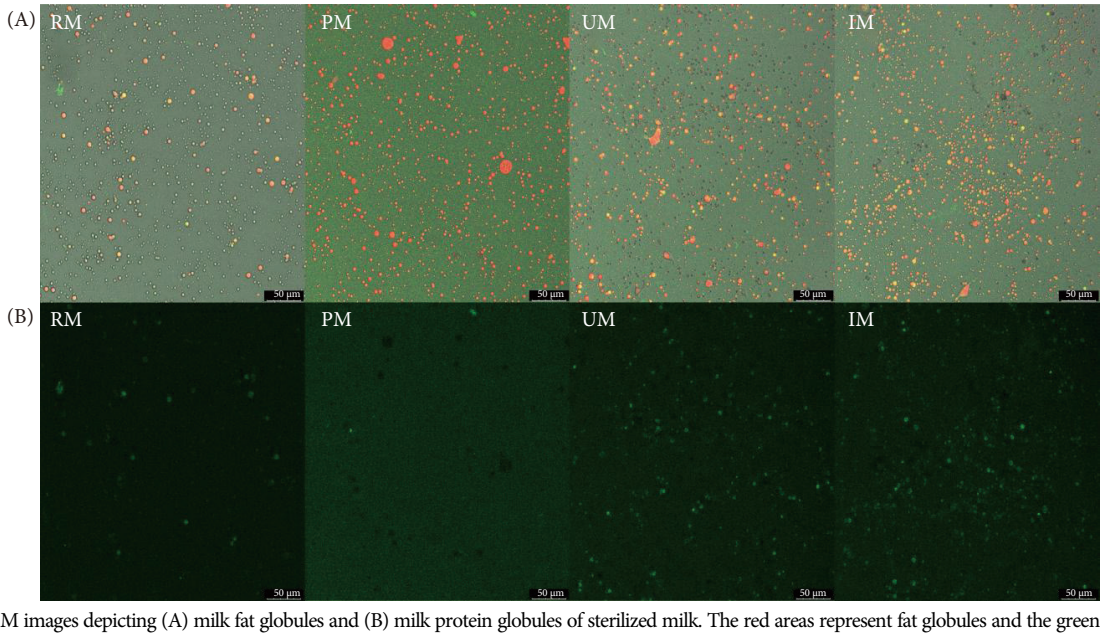

Figure 5 CLSM images depicting (A) milk fat globules and (B) milk protein globules of sterilized milk. The red areas represent fat globules and the green areas represent proteins.

3.6 SDS-PAGE analysis of milk

As shown in Figure 6, β-lactoglobulin (β-Lg), casein, and bovine serum albumin (BSA) are the main components of whey protein. There were no significant differences in the bands corresponding to the main proteins among the samples, indicating that different pasteurization methods do not significantly affect the content of these proteins in cow's milk, which was similar to the results observed by Yang et al.[8]. For the bands corresponding to protein molecular weights in the range of 70–180 kDa, the bands in the UM sample were less prominent compared to other samples, which might be due to the denaturation of protein structures under ultra-high temperature treatment, leading to the Maillard reaction between lactose and proteins, resulting in a reduction in protein molecular weight[48].

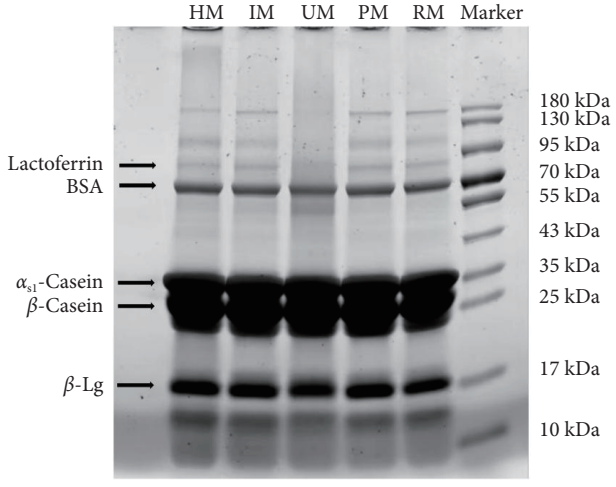

Figure 6 The SDS-PAGE images of milk samples treated by different sterilization methods.

4 Conclusion

The low-temperature IEF, and HPM technologies had similar sterilization effects to that of traditional thermal sterilization methods including pasteurization and UHT, the thermal stability of proteins in milk was improved by UHT, while IEF enhanced the thermal stability of fats in milk. HM had the smallest particle size due to high pressure. Thermal sterilization can change the secondary structure of milk protein by Maillard reaction as well as protein aggregation. Under high temperatures, fat globules also aggregate on the protein surface, increasing the particle size, whereas IEF treatment helps disperse the fat globules in milk. Currently, the specific mechanisms by which technologies such as IEF, HPP, UHT, and pasteurization affect the proteins and fats in milk during the sterilization process are not well understood. Therefore, further theoretical research is needed to explore how these sterilization technologies impact the nutritional components in milk and how they can be applied to create operational dairy food.

Conflict of interest

No conflicts of interest are declared for any of the authors.

Acknowledgements

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References

[1] A. R. Al-Hilphy, H. I. Ali, S. A. Al-Iessa, et al., Optimization of process variables on physicochemical properties of milk during an innovative refractance window concentration, J. Food Process. Pres. 44(10) (2020) e14782. https://doi.org/10.1111/jfpp.14782.

[2] P. Vashisht, A. Sharma, N. Awasti, et al., Comparative review of nutri-functional and sensorial properties, health benefits and environmental impact of dairy (bovine milk) and plant-based milk (soy, almond, and oat milk), Food Hum. 2 (2024) 100301. https://doi.org/10.1016/j.foohum.2024.100301.

[3] A. Rashid, I. Javed, M. Ayaz, et al., Probing the physicochemical and sensorial properties of pasteurized milk via nanoparticles, Int. J. Food Prop. 22(1) (2019) 825–842. https://doi.org/10.1080/10942912.2019.1607374.

[4] N. Liang, J. Koh, B. J. Kim, et al., Structural and functional changes of bioactive proteins in donor human milk treated by vat-pasteurization, retort sterilization, ultra-high-temperature sterilization, freeze-thawing and homogenization, Front. Nutr. 9 (2022) 926814. https://doi.org/10.3389/fnut.2022.926814.

[5] M. Al-Farsi, I. Al-Gharibi, A. Al-Abri, et al., Evaluating the shelf-life of pasteurized milk in Oman, Heliyon 7(3) (2021) e06555. https://doi.org/10.1016/j.heliyon.2021.e06555.

[6] T. Yu, X. Zhang, R. Feng, et al., Comparison of the effects of high hydrostatic pressure and pasteurization on quality of milk during storage, Foods 11(18) (2022) 2837. https://doi.org/10.3390/foods11182837.

[7] A. Aceti, L. Cavallarin, S. Martini, et al., Effect of alternative pasteurization techniques on human milk's bioactive proteins, J. Pediatr. Gastroenterol. Nutr. 70(4) (2020) 508–512. https://doi.org/10.1097/mpg.0000000000002598.

[8] N. Yang, Z. Zheng, Y. Jin, et al., Effect of induced electric field on microorganism inactivation, nutrients and physicochemical properties of milk, Food Biosci. 58 (2024) 103708. https://doi.org/10.1016/j.fbio.2024.103708.

[9] A. J. Bucci, D. L. van Hekken, M. H. Tunick, The effects of microfluidization on the physical, microbial, chemical, and coagulation properties of milk, J. Dairy Sci. 101(8) (2018) 6990–7001. https://doi.org/10.3168/jds.2017-13907.

[10] C. He, N. Yang, Y. Jin, et al., Application of induced electric field for inner heating of kiwifruit juice and its analysis, J. Food Eng. 306 (2021) 110609. https://doi.org/10.1016/j.jfoodeng.2021.110609.

[11] M. Zhang, N. Yang, L. Guo, et al., Physicochemical properties of apple juice influenced by induced potential difference (induced electric field) during disposable continuous-flow treatment, J. Food Eng. 234 (2018) 108–116. https://doi.org/10.1016/j.jfoodeng.2018.04.013.

[12] S. Wu, X. Xu, N. Yang, et al., Inactivation of *Escherichia coli* O157: H7 in apple juice via induced electric field (IEF) and its bactericidal mechanism, Food Microbiol. 102 (2022) 103928. https://doi.org/10.1016/j.fm.2021.103928.

[13] S. Wu, N. Yang, Y. Jin, et al., Effects of induced electric field (IEF) on the reduction of *Saccharomyces cerevisiae* and quality of fresh apple juice, Food Chem. 325 (2020) 126943. https://doi.org/10.1016/j.foodchem.2020.126943.

[14] N. Yang, Y. Jin, Y. Zhou, et al., Effect of induced electric field parameters on physiochemical characteristics and microorganism of orange juice, J. Food Process Eng. 47(8) (2024) e14709. https://doi.org/10.1111/jfpe.14709.

[15] X. Wang, S. Wang, W. Wang, et al., Comparison of the effects of dynamic high-pressure microfluidization and conventional homogenization on the quality of peach juice, J. Sci. Food Agric. 99(13) (2019) 5994–6000. https://doi.org/10.1002/jsfa.9874.

[16] S. P. Heffernan, A. L. Kelly, D. M. Mulvihill, et al., High-pressure-homogenised cream liqueurs: emulsification and stabilization efficiency, J. Food Eng. 95(3) (2009) 525–531. https://doi.org/10.1016/j.jfoodeng.2009.06.018.

[17] P. Paquin, Technological properties of high pressure homogenizers: the effect of fat globules, milk proteins, and polysaccharides, Int. Dairy J. 9(3/6) (1999) 329–335. https://doi.org/10.1016/S0958-6946(99)00083-7.

[18] Z. F. Bhat, J. D. Morton, A. E. D. A. Bekhit, et al., Processing technologies for improved digestibility of milk proteins, Trends Food Sci. Technol. 118 (2021) 1–16. https://doi.org/10.1016/j.tifs.2021.09.017.

[19] Z. Zhang, M. Zhang, Z. Gao, et al., Effect of dynamic high-pressure microfluidization on the quality of not-from-concentrate cucumber juice, Foods 13(13) (2024) 2125. https://doi.org/10.3390/foods13132125.

[20] T. K. Koley, J. Nishad, C. Kaur, et al., Effect of high-pressure microfluidization on nutritional quality of carrot (*Daucus carota* L.) juice, J. Food Sci. Tech. Mys. 57 (2020) 2159–2168. https://doi.org/10.1007/s13197-020-04251-6.

[21] D. C. C. Vidotto, R. A. A. Mantovani, G. M. M. Tavares, High-pressure microfluidization of whey proteins: impact on protein structure and ability to bind and protect lutein, Food Chem. 382 (2022) 132298. https://doi.org/10.1016/j.foodchem.2022.132298.

[22] A. Kamboj, R. Chopra, P. K. Prabhakar, et al., Perilla protein isolate exhibits synergistic techno-functionality through modification via sequential dynamic high-pressure microfluidization and enzymatic hydrolysis, Innov. Food Sci. Emerg. Technol. 94 (2024) 103683. https://doi.org/10.1016/j.ifset.2024.103683.

[23] X. Sun, L. Wang, S. Wang, et al., Effects of thermal treatments on physicochemical properties and peptide profiles of infant formula protein model based on peptidomics, Int. J. Dairy Technol. 77(3) (2024) 949–960. https://doi.org/10.1111/1471-0307.13069.

[24] B. Bastin, N. Klass, E. Crowley, et al., Evaluation of the MC-Media Pad® rapid aerobic count device for the enumeration of total aerobic counts in a variety of foods: collaborative study, first action official method SM 2019.02, J. AOAC Int. 103(5) (2020) 1318–1325. https://doi.org/10.1093/jaoacint/qsaa040.

[25] X. Sun, Y. Tan, C. Duan, et al., Effects of processing on component interactions and peptide profiles of preterm infant formula based on peptidomics by Q Exactive plus analysis, LWT-Food Sci Technol. 189(1) (2023) 115429. https://doi.org/10.1016/j.lwt.2023.115429.

[26] H. Xiang, Z. Jiang, W. Tao, et al., Insight into structural changes in heat-induced whey protein-fucoidan hydrogel by SR-IR and molecular docking techniques, Food Res. Int. 197 (2024) 115222. https://doi.org/10.1016/j.foodres.2024.115222.

[27] M. Ayyash, A. Abdalla, B. Abu-Jdayil, et al., Rheological properties of fermented milk from heated and high pressure-treated camel milk and bovine milk, LWT-Food Sci. Technol. 156(15) (2022) 113029. https://doi.org/10.1016/j.lwt.2021.113029.

[28] T. Huppertz, A. L. Kelly, C. G. de Kruif, Disruption and reassociation of casein micelles under high pressure, J. Dairy Res. 73(3) (2006) 294–298. https://doi.org/10.1017/S0022029906001725.

[29] V. Orlien, J. C. Knudsen, M. Colon, et al., Dynamics of casein micelles in skim milk during and after high pressure treatment, Food Chem. 98(3) (2006) 513–521. https://doi.org/10.1016/j.foodchem.2005.05.082.

[30] M. Walkling-Ribeiro, T. Jacob, L. Ahrne, Impact of pulsed electric field intensity on the cream separation efficiency from bovine milk and physico-chemical properties of the cream, Food Res. Int. 180 (2024) 114074. https://doi.org/10.1016/j.foodres.2024.114074.

[31] J. Kinsella, D. Whitehead, Proteins in whey: chemical, physical, and functional properties, Adv. Food Nutr. Res. 33 (1989) 343–438. https://doi.org/10.1016/S1043-4526(08)60130-8.

[32] K. Szulc, J. Nazarko, E. Ostrowska-Ligeza, et al., Effect of fat replacement on flow and thermal properties of dairy powders, LWT-Food Sci. Technol. 68 (2016) 653–658. https://doi.org/10.1016/j.lwt.2015.12.060.

[33] O. M. Brozek, K. Kielczewska, K. Bohdziewicz, Fatty acid profile and thermal characteristics of ovine and bovine milk and their mixtures, Int. Dairy J. 129 (2022) 105339. https://doi.org/10.1016/j.idairyj.2022.105339.

[34] L. Bouzidi, S. S. Narine, Relationships between molecular structure and kinetic and thermodynamic controls in lipid systems. Part II: phase behavior and transformation paths of SSS, PSS and PPS saturated triacylglycerols: effect of chain length mismatch, Chem. Phys. Lipids 165(1) (2012) 105–119. https://doi.org/10.1016/j.chemphyslip.2011.11.004.

[35] G. Liu, Q. Zhong, Thermal aggregation properties of whey protein glycated with various saccharides, Food Hydrocolloids 32(1) (2013) 87–96. https://doi.org/10.1016/j.foodhyd.2012.12.008.

[36] M. Karbasi, G. Askari, Modification of whey protein microgel particles with mono-oligo-and polysaccharides through the Maillard reaction: effects on structural and techno-functional properties, Food Struct. 28 (2021) 100184. https://doi.org/10.1016/j.foostr.2021.100184.

[37] X. Sun, C. Wang, H. Wang, et al., Effects of processing on structure and thermal properties of powdered preterm infant formula, J. Food Sci. 83(6) (2018) 1685–1694. https://doi.org/10.1111/1750-3841.14162.

[38] A. Golkar, A. Nasirpour, J. Keramat, Improving the emulsifying properties of β-lactoglobulin-wild almond gum (*Amygdalus scoparia* Spach) exudate complexes by heat, J. Sci. Food Agric. 97(1) (2016) 341–349. https://doi.org/10.1002/jsfa.7741.

[39] F. L. Gu, J. M. Kim, S. Abbas, et al., Structure and antioxidant activity of high molecular weight Maillard reaction products from casein-glucose, Food Chem. 120(2) (2010) 505–511. https://doi.org/10.1016/j.foodchem.2009.10.044.

[40] C. Sereechantarerk, P. Hongsprabhas, W. Chanput, et al., Effects of ohmic heating on structural and physicochemical changes of whey proteins, Agric. Nat. Resour. 55 (2021) 464–472. https://doi.org/10.34044/j.anres.2021.55.3.17.

[41] V. R. Kodati, M. Lafleur, Comparison between orientational and conformational orders in fluid lipid bilayers, Biophys. J. 64(1) (1993) 163–170. https://doi.org/10.1016/s0006-3495(93)81351-1.

[42] T. Gao, X. Wu, Y. Gao, et al., Construction of emulsion gel based on the interaction of anionic polysaccharide and soy protein isolate: focusing on structural, emulsification and functional properties, Food Chem.: X 22 (2024) 101377. https://doi.org/10.1016/j.fochx.2024.101377.

[43] P. X. Qi, D. Ren, Y. Xiao, et al., Effect of homogenization and pasteurization on the structure and stability of whey protein in milk, J. Dairy Sci. 98(5) (2015) 2884–2897. https://doi.org/10.3168/jds.2014-8920.

[44] M. A. van Boekel, Heat-induced deamidation, dephosphorylation and breakdown of caseinate, Int. Dairy J. 9(3/6) (1999) 237–241. https://doi.org/10.1016/S0958-6946(99)00068-0.

[45] J. Lu, J. Pickova, G. Daniel, et al., The role of key process steps on microstructural organisation of fat globules and lipid profiles in UHT milk processed in a pilot plant unit, Int. Dairy J. 109 (2020) 104741. https://doi.org/10.1016/j.idairyj.2020.104741.

[46] Y. Xie, Y. Pan, R. Zhang, et al., Modulating protein behaviors on responsive surface by external electric fields: a molecular dynamics study, Appl. Surf. Sci. 326 (2015) 55–65. https://doi.org/10.1016/j.apsusc.2014.11.078.

[47] P. Sharma, I. Oey, D. W. Everett, Interfacial properties and transmission electron microscopy revealing damage to the milk fat globule system after pulsed electric field treatment, Food Hydrocolloids 47 (2015) 99–107. https://doi.org/10.1016/j.foodhyd.2015.01.023.

[48] Y. Liu, W. Zhang, L. Zhang, et al., Characterizing the changes of bovine milk serum proteins after simulated industrial processing, LWT-Food Sci. Technol. 133 (2020) 110101. https://doi.org/10.1016/j.lwt.2020.110101.