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Efficacy of a pilot-scale ultrasonication system for pasteurization of milk

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ABSTRACT

This study aimed to test a pilot-scale continuous ultrasonication (US) system to pasteurize whole milk in terms of its properties, energy consumption, and inactivation of alkaline phosphatase (ALP), total mesophilic aerobic bacteria (TMAB), total coliform (TC), total Enterobactericeae (TE), and Escherichia coli. Except for those treated by 90% amplitude for 20 and 30 min, the samples were found negative for ALP activity. US processing with different amplitude, temperature, and treatment time provided significant inactivation on the mean initial numbers of TC, TMAB, TE, and E. coli. The maximum cost was estimated for the US system and plate heat exchanger to pasteurize 20.000 L milk as 2.778,00 € and 3.624,00 €, respectively. 96.52% amplitude, 40 min, and 53.50 °C were determined jointly as the optimal operational settings.

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1. Introduction

Due to its high water activity and lower acidity, milk is excellent medium for spoilage microorganisms and foodborne pathogens as well as enzymes causing deterioration. Although the thermal treatment of milk from pasteurization to ultra-high temperature (UHT) provides the inactivation of microorganisms and enzymes, recent concerns have been with both its safety and the preservation of its physical, nutritional, and sensory properties. The thermal treatment was reported to decrease its folic acid content and adversely affect its color, aroma, and odor (Pegu and Arya, 2021; Prasantha and Wimalasiri, 2019). Ultrasonication (US), as a novel processing technology, has emerged as an alternative to the heat processing of milk. US with frequencies of 20-100 kHz and energy intensities of 10-100 kHz W cm_{-2} generates acoustic cavitation, and thus, a localized pressure of 100-5000 bar with a high temperature of 500-15000K. Free radicals, shockwaves, liquid microjets, and interfacial turbulence are formed by the collapse of cavities (Carpenter et al., 2016; Gogate and Pandit, 2001), thus pasteurizing liquid foods (Asaithambi et al., 2019; Milly et al., 2007; Milly et al., 2008). Depending on the US intensity, the treatment disrupts casein micelles and milk fat globules (Martini, 2013) and reduces their size (Nguyen and Anema, 2017; 2010). Also, whey proteins undergo partial cleavage on hydrophobic interactions (Chandrapala et al., 2012).

In related literature, there still exists a knowledge gap about how the US processing impacts whole milk. Thus, the objectives of the study were to (1) determine changes in technological properties, microbial inactivation, and energy consumption in response to the US processing of whole milk; and (2) jointly optimize the process parameters.

2. Materials and methods

2.1. Materials

Raw milk was purchased from local producers and brought to Pinar Dairy Products Inc. (Izmir, Turkey) in cold chain. All milk samples were kept at 4°C until processing.

2.2. Pilot-scale ultrasonic system

A pilot-scale ultrasonic system; BSP-1200 Ultrasonic Processor (Sonomechanics, Florida, USA) was used to process milk samples. The system generates 1200 W ultrasonic waves with a piezoelectric transducer. The detailed scheme of the system is given in Figure 1.

The system properties are: maximum 500 L h^{-1} flow rate, 1200 W ultrasonic power generation, 20 kHz work frequency, 20-100% amplitude, and cooling-heating chamber cooperated with ultrasonic temperature range of 15-100°C. The fluid material can be heated or cooled to provide optimum condition before feeding and is continuously ultrasonicated at an optimum temperature. Milk was heated or cooled in the system at a desired treatment temperature. Ultrasonication process (200 kHz, 700 W) was applied using

the three amplitudes (90, 95, and 100%), six temperatures (40, 45, 50, 53, 55, and 57 °C), and three durations (20, 30, and 40 min).

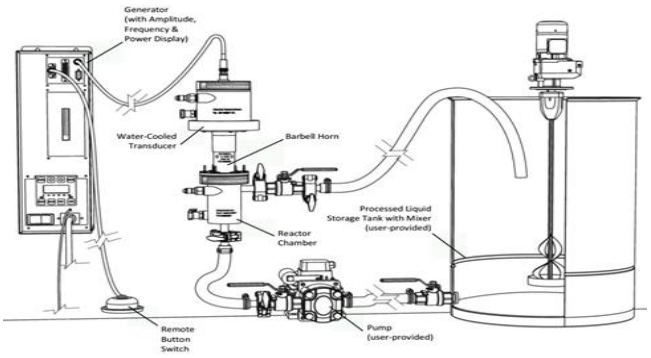

Figure 1. Pilot scale continuous ultrasonic system scheme

2.3 Approximate composition analysis

Protein, fat, and non-fat dry matter content were determined using a Milkoscan FT1 (MilkoScan, Hillerød, Denmark). Results were expressed as amount % in milk.

2.4 pH measurement

pH was measured at room temperature using a WTW ProfiLine pH 3110 (Germany).

2.5 Viscosity measurement

Viscosity of the milk samples was determined using a DV-1 model Brookfield Digital Viscometer (Brookfield Engineering Labs Inc.).

2.6. Alkaline phosphatase analysis

Alkaline phosphatase (ALP) activity was measured using a Sensobiz® (Ankara, Turkey) sensor.

2.7. Microbiological analysis

Total Enterobacteriaceae (TE), total coliform (TC), and Escherichia coli were enumerated according to the Anonymous (2016) method. Total mesophilic aerobic bacteria (TMAB) count was performed using ISO 4833-1 (2013).

2.8. Energy consumption

Energy consumption (kWh) of the US system was measured using a TT T-ECHNI-C Watt meter (Acrel, Shanghai, China). Heat treatment performed at 72°C for 15 sec was used for energy calculation.

2.9. Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests were conducted (Minitab 17, Minitab, Inc., State College, PA) for determination of differences among the treatments. The best-fit artificial neural network (ANN) was constructed to predict (1) the multiple responses of microbial inactivation as a function of amplitude, treatment time, and treatment temperature. The activation function of the hyperbolic tangent (TanH) (a sigmoid function), one hidden layer with seven neurons, a learning rate of 0.1, and a squared penalty method were selected in the ANN through a trial-and-error approach by monitoring the improvements on RMSE and R2 values based on a random 5-fold cross-validation. The joint optimization was based on the composite desirability (D) of 0 to 1 (optimal). The simultaneous optimization was implemented using the best-fit ANN according to the target function of minimization for the responses. Interaction effect and predictor importance were determined using the Monte Carlo simulation of independent resampling. All the analyses were performed using JMP Pro 16.

3. Results and discussions

Initial trials were conducted with different amplitudes, temperatures, and sonication times to determine the processing parameters for milk pasteurization. Regardless of the treatment temperature and time, the amplitudes of 70-85% were not effective for microbial inactivation. The treatments conducted with 90, 95, and 100% amplitudes were effective for both microbial and ALP inactivation. Similarly, since the treatment times below 20 min were not effective, the experimental design in Table 1 was applied after the initial runs.

The temperature increased slightly during the US processing. With application of different amplitude and treatment times, the processing temperature varied between 42 °C with 90% amplitude for both 20 and 30 min and 61 °C with 100% amplitude for 40 min. pH of the control samples (6.68 ± 0.04) ranged from 6.61 ± 0.10 with 100% amplitude for 40 min to 6.70 ± 0.07 with 90% amplitude for 20 min. Fat (3.58 ± 0.04%) content of the samples changed from 3.50 ± 0.06 with 100% for 30 min to 3.67 ± 0.06 with 90% amplitude for 30 min; whereas protein (3.18 ± 0.06%) content of the samples changed from 3.10 ± 0.07% with 100% amplitude for 40 min to 3.26 ± 0.08 with 90% amplitude for 30 min, respectively. Viscosity of the control samples (0.062 ± 0.06 kg ms-1) was in the range of 0.062 ± 0.07 by 90% amplitude for 20 min at 42 °C and 0.071 ± 0.08 by 100% amplitude for 40 min at 61 °C (Table 1). Overall, the applied US parameters did not cause significant changes in the pH, TSS, viscosity, and approximate composition of whole milk ($p > 0.05$).

Efficacy of the US processing on milk properties significantly depended on the applied processing parameters. The US processing by 200, 300, and 400 W for 4, 6, and 8 min slightly increased the total soluble solid (TSS) and viscosity of the control samples. The cavitation effect of the US treatment increased TSS, and thus, the viscosity. The increased viscosity can be also related to the increased number of fat globules due to the size reduction and casein adsorption on their surface (McSweeney and Fox, 2009; Pegu and Arya, 2021). The combination of US with the pre-heat treatment increased the heat stability of the dairy ingredients and gelling properties (Ashokkumar et al., 2010; Pegu and Arya, 2021). No significant difference was reported for the protein or lactose content of the raw and pasteurized milk processed by 2.5, 5.0, 6.0, 7.5 and 10.0 min at 100% amplitude (Cameron et al., 2010).

Except for those treated by 90% amplitude for 20 and 30 min at 42 °C, the samples were found negative for ALP activity. An increase in both amplitude and temperature provided inactivation on ALP activity (Table 1). ALP, naturally occurring endogenous enzyme of raw milk, is bound to membranes of fat globules (Griffiths, 1986; Shamsi et al., 2008). As a heat sensitive enzyme, its denaturation occurs during the heat treatment at 56 °C for 30 min.

Table 1. Ultrasonication treatments of raw milk

Amplitude (%)	Treatment time (min)	Temperature (°C)	US sample temperature (°C)	Fat (%)	Protein (%)	Alkaline phosphatase activity	pH	Non-fat dry matter	Viscosity (kg ms^{-1})
0	0	4	4	3.58 ± 0.04^{ab}	3.18 ± 0.06^{a}	+	6.68 ± 0.04^{a}	8.31 ± 0.76^{a}	0.062 ± 0.06^{a}
90	20	40	42	3.61 ± 0.01^{ab}	3.11 ± 0.09^{a}	+	6.66 ± 0.06^{a}	8.29 ± 0.82^{a}	0.062 ± 0.07^{a}
90	30	40	42	3.60 ± 0.02^{ab}	3.14 ± 0.06^{a}	+	6.67 ± 0.04^{a}	8.40 ± 0.90^{a}	0.064 ± 0.08^{a}
90	20	45	47	3.60 ± 0.03^{ab}	3.13 ± 0.08^{a}	-	6.66 ± 0.04^{a}	8.06 ± 0.68^{a}	0.069 ± 0.08^{a}
90	30	45	49	3.67 ± 0.06^{ab}	3.12 ± 0.08^{a}	-	6.66 ± 0.06^{a}	7.97 ± 0.80^{a}	0.068 ± 0.07^{a}
90	20	50	53.1	3.61 ± 0.02^{a}	3.25 ± 0.10^{a}	-	6.70 ± 0.07^{a}	7.94 ± 0.69^{a}	0.066 ± 0.08^{a}
90	30	50	54.6	3.58 ± 0.03^{ab}	3.26 ± 0.08^{a}	-	6.69 ± 0.06^{a}	8.03 ± 0.58^{a}	0.069 ± 0.09^{a}
95	20	50	52.8	3.52 ± 0.03^{b}	3.22 ± 0.08^{a}	-	6.64 ± 0.08^{a}	8.16 ± 0.46^{a}	0.066 ± 0.08^{a}
95	30	50	54.2	3.52 ± 0.02^{b}	3.22 ± 0.09^{a}	-	6.65 ± 0.08^{a}	8.07 ± 0.48^{a}	0.065 ± 0.07^{a}
95	20	53	55.5	3.64 ± 0.03^{a}	3.18 ± 0.06^{a}	-	6.63 ± 0.10^{a}	7.96 ± 0.56^{a}	0.066 ± 0.07^{a}
95	30	53	58	3.57 ± 0.04^{a}	3.19 ± 0.08^{a}	-	6.68 ± 0.07^{a}	8.11 ± 0.70^{a}	0.063 ± 0.05^{a}
95	20	55	57	3.62 ± 0.03^{a}	3.24 ± 0.06^{a}	-	6.65 ± 0.05^{a}	8.04 ± 0.67^{a}	0.066 ± 0.07^{a}
100	30	50	54	3.53 ± 0.03^{ab}	3.16 ± 0.06^{a}	-	6.67 ± 0.06^{a}	8.07 ± 0.71^{a}	0.068 ± 0.05^{a}
100	40	50	55	3.53± 0.02^{ab}	3.10 ± 0.07^{a}	-	6.66 ± 0.04^{a}	8.15 ± 0.80^{a}	0.063 ± 0.09^{a}
100	30	53	57	3.50 ± 0.06^{ab}	3.21 ± 0.06^{a}	-	6.68 ± 0.12^{a}	8.02 ± 0.77^{a}	0.065 ± 0.07^{a}
100	40	53	58	3.52 ± 0.07^{ab}	3.21 ± 0.08^{a}	-	6.63 ± 0.10^{a}	7.90 ± 0.65^{a}	0.068 ± 0.05^{a}
100	30	55	59	3.52 ± 0.06^{ab}	3.22 ± 0.09^{a}	-	6.65 ± 0.09^{a}	7.96 ± 0.66^{a}	0.064 ± 0.07^{a}
100	40	55	60	3.52 ± 0.02^{ab}	3.22 ± 0.06^{a}	-	6.63 ± 0.08^{a}	7.98 ± 0.59^{a}	0.069 ± 0.06^{a}
100	30	57	60	3.63 ± 0.03^{ab}	3.20 ± 0.06^{a}	-	6.68 ± 0.08^{a}	8.03 ± 0.66^{a}	0.070 ± 0.08^{a}
100	40	57	61	3.63 ± 0.06^{ab}	3.19 ± 0.08^{a}	-	6.61 ± 0.10^{a}	8.07 ± 0.70^{a}	0.071 ± 0.08^{a}

Thus, ALP, as a universal indicator of high temperature short time (HTST) pasteurization, is generally used for the thermal processing (Pegu and Arya, 2021). The US processing was also effective for ALP inactivation, and a 14% reduction was obtained at 400 W for 8 min (Pegu and Arya, 2021). Changes in enzyme activity is related to disruptions on hydrogen bonds and van der Waals interactions in the polypeptide structure (Munir et al., 2019). Denaturation rate of ALP increased with the extension of exposure time and intensity as more bubble formation and collapsing were both favored with local hotspots and micromechanical shock. Temperature increase as a result of increase in exposure time and intensity has an important role in denaturation of ALP activity (Pegu and Arya, 2021). US processing parameters of 2.5, 5.0, 6.0, 7.5 and 10.0 min at 100% amplitude, on the other hand, were not effective for the deactivation of alkaline phosphatase and lactoperoxidase activities (Cameron et al., 2010).

The mean initial TE, TC, TMAB, and total Escherichia coli counts were 5.38 ± 0.45, 5.41 ± 0.37, 6.02 ± 0.00, and 7.81± 0.36 log cfu mL-1, respectively. A complete inactivation was observed for TE, TC, and total E. coli counts with 100% amplitude for both 30 and 40 min at 57.0 °C. The mean initial TMAB count reduced to 2.12 ± 0.08 log cfu mL-1 under the same processing conditions (Figure 2).

The processing of whole milk at 24 kHz for 0, 2, 4, 8, and 16 min provided a 1 log reduction in the total viable count (TVC) while a 1.5 log reduction in psychrotrophic counts (PC) was achieved only after 16 min, respectively (Chouliara et al., 2010). Similarly, TVC fell by 0.2, 0.6, and 2.9 logs after the continuous flow US process with the increased temperatures of 48.6, 62, and 76 °C, respectively (De Jong and Villamiel, 2000). The US processing at 200, 300, and 400 W for 4, 6, and 8 min resulted in 0.73 log reduction in total psychotrophic count and 0.79 log reduction in yeast and mold count after 400 W for 8 min (Pegu and Arya, 2021). It has been reported that microbial inactivation obtained by US can be provided by cavitation having mechanical effect including shear stress, generation of turbulence, shock waves, and liquid jet; chemical effect including generation of free radicals; and thermal effects including creation of local high-temperature (Asaithambi et al., 2019; Salve et al., 2019; Tao et al., 2009). Microbial inhibition provided by US depends on free radical formation occurred by the disruption of chemical bonds and oxidation reactions in the bubble-liquid interface of the bulk liquid resulting with the cell membrane disruption and microbial inactivation (Bermúdez-Aguirre et al., 2011). US processing of milk (41 °C) at 0, 108 and 216 µm amplitudes for 3 min provided 0.64, 0.53 and 3.37 log inactivations, respectively (Ganesan et al., 2015).

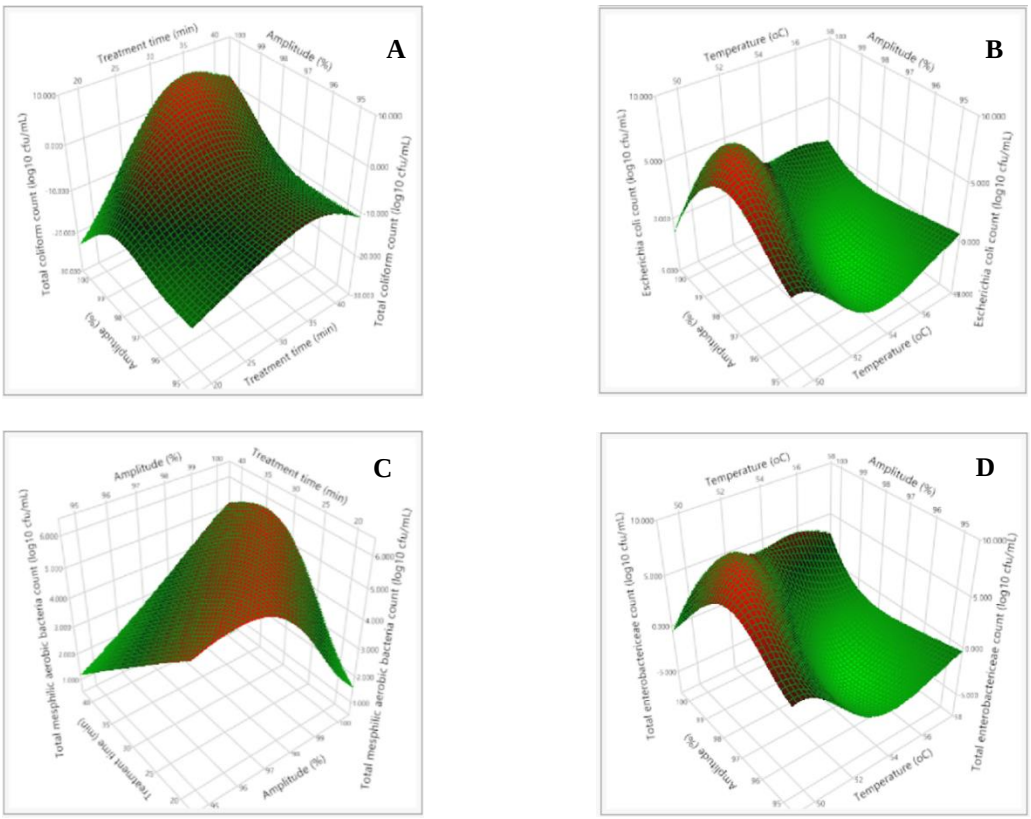

Figure 2. Microbial inactivation by US processing parameters. Inactivation of A) total coliform B) Escherichia coli, C) Total mesophilic aerobic bacteria, and D) Total Enterobactericeae as a function of amplitude and treatment time

Increased US processing temperature to 72 °C at 180 µm amplitude with processing times of 1 and 5 min resulted in 3.04 and 4.28 log reductions, respectively (Pegu and Arya, 2021). The processing of raw milk by US with 200, 300, and 400 W for 4, 6, and 8 min resulted in 0.9 and 0.7 log reductions on total plate count and total yeast and mold count (Pegu and Arya, 2021).

Table 2. Energy constumption and cost of pasteurization of milk by ultrasonication process.

Amplitude (%)	Treatment time (min)	Temperature (°C)	Energy consumption (kW)	Cost (€)	Energy Need of 1 L milk (kW)	Cost of 1 L milk processing (€)	Pasteurization cost of 20.000 L milk (€)	Pasteurization cost of 20.000L milk with HTST (€)
90	20	50	0.170	0.0085	0.00185	0.000096	2.564	3.624
90	30	50	0.254	0.0126	0.00185	0.000096	2.564	3.624
95	20	45	0.172	0.0086	0.00185	0.000096	2.564	3.624
95	30	45	0.258	0.0124	0.00185	0.000096	2.564	3.624
95	20	53	0.175	0.0087	0.00185	0.000096	2.564	3.624
95	30	53	0.263	0.0126	0.00185	0.000096	2.564	3.624
95	20	56	0.178	0.0089	0.00185	0.000096	2.564	3.624
95	30	56	0.267	0.0134	0.00185	0.000096	2.564	3.624
100	30	57	0.27	0.0136	0.00191	0.000128	2.695	3.624
100	40	57	0.272	0.0142	0.00191	0.000130	2.778	3.624

Energy consumption and cost of US and heat pasteurization are given in Table 2. Energy consumption by US was changed by the applied amplitude, treatment time and temperature. Cost of milk pasteurization by US changed from 2.564 € to 2.778 € for 100% amplitude, 40 min treatment time at 57 °C treatment temperature; whereas it was calculated as 3.624 € for HTST treatment (72 °C for 15 sec). The energy cost of the US processing was lower than that of the heat pasteurization. Similar to present study, it is also reported that ultrasound provides significant energy saving than that of the heat treatment even though US processing takes longer time than that of the HTST process (Chemat et al., 2011; Gogate and Pandit, 2001).

The joint optimization showed 96.52%, 40 min, and 53.50 °C as the optimal processing parameters (D = 0.995). Amplitude, temperature and treatment time were the main effects with for the inactivation of TE, TC, E. coli and TMAB, with amplitude and temperature having greater impact on microbial inactivation.

4. Conclusion

A continuous pilot-scale US processing of whole milk with 90, 95 and 100% amplitude, 20, 30, and 40 min, and 45, 45, 50, 53, 55, and 57 °C did not adversely affect pH, non-fat dry matter, and fat and protein contents of whole milk. Overall, the applied US processing parameters were successful on the denaturation of ALP enzymes, except the samples treated by 90% for 20 and 30 min at 42 °C. The initial numbers of E. coli, TMAB, and TE significantly decreased with the US processing ($p < 0.05$). 96.52%, 40 min, and 53.50 °C were the optimal operational conditions with 0.995 desirability. The US processing unit was comparable to the HTST processing in terms of energy efficacy and cost. The maximum cost was estimated at 2.778 € for the US processing at the highest amplitude and temperature as well as the longest treatment time and at 3.624 € for the HTST unit to pasteurize 20.000 L of milk. This study was conducted to determine effect of pilot-scale US system on microbial inactivation and some properties of raw milk; but it is also important to investigate effect of US on properties of cheese made from US-treated milk. Moreover, further studies need to be conducted to determine the effect of the pilot-scale US system on protein denaturation, sensory analyses, nutritional content, and shelf-life extension of milk in comparison with the HTST processing.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Gulsun Akdemir Evrendilek: Project administration, data analyses, writing manuscript. **Anil Bodruk**: Data collection, project administration, editing manuscript. **Furkan Acar**: Data collection, conducting test and analyses, editing manuscript. All authors accepted the manuscript.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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