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EVALUATION OF MICROBIAL NUMBERS AND PHYSICAL PROPERTIES OF MILK PRESERVED WITH DIFFERENT TIME OF PRE-HEATING AND PULSED ELECTRIC FIELDS (PEF) EXPOSURE

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ABSTRACT

Milk production is intended for human consumption; hence preservation technology is required while maintaining the sensory and nutritional attributes. Some of the technologies applied in milk are the use of pre-heating combined with pulsed electric fields (PEF) exposure. The study aimed to examine the effect of combination milk pasteurization process using pre-heating and PEF with various conditions. The raw milk first received the pre-heating treatment at 70 °C, followed by PEF exposure. A factorial experiment, completely randomized design (CDR), was applied in this research. It used two factors, namely factor I (pre-heating duration: 10, 20, and 30 minutes), and factor II (PEF exposure duration: 2, 4, and 6 minutes) with three replications, resulting in nine (9) treatments. The results reveal that there was a bacterial reduction within the range of 0.70-1.06 log CFU/mL. Besides, the increasing duration of pre-heating combined with PEF exposure decreases the values of viscosity, emulsion stability, and lightness of raw milk samples. Moreover, the most effective treatment observed in the raw milk-treated with pre-heating of 10 minutes and PEF of 2 minutes, with the level of TPC, viscosity, emulsion stability, and lightness of 1.88 log CFU/mL, 14.65 cP, and 95.65%, respectively

Key words: Emulsion stability; lightness; milk; total plate count; viscosity

INTRODUCTION

Milk is a liquid produced by animals used for the nourishment of their offspring. In Indonesia, milk is mainly produced from cows (cattle milk). According to data from The Indonesian Central Bureau of Statistics, the production of milk from dairy cows in 2018 was approximately 135 million liters with an economic value of around 774 million Indonesia Rupiahs (IDR). Then, it increased in the year 2020, reaching a production volume of just above 221 million liters with an economic value of nearly 725 million IDR (Indonesian Central Bureau of Statistics, 2021).

Besides, milk production is intended for human consumption due to the presence of high nutrients, including water, lipid, protein, lactose, and some minerals, irrespective of the variety of species. The lipid content in cow's milk mainly consists of saturated fatty acids (55.7-72.8%) and monounsaturated fatty acids (22.7-30.3%), while the major protein fractions are caseins (24.6-28.0 g/L) and whey proteins (5.5-7.0 g/L) (Roy et al., 2020). The composition of lactose and mineral components in cow's milk are 48.0 g/L and 7.0 g/L, respectively (Adams et al., 2016) However, the milk is subjected to be one perishable food as the presence of those nutrients supports the microorganisms to grow and proliferate, including the microorganisms, caused foodborne diseases and food spoilage. Moreover, there are microbial, physical, and chemical parameters that also affect the quality of the milk, including total plate count (TPC), emulsion stability, viscosity, and color/lightness. The lower number of TPC indicates the effectiveness of pasteurization process, which is affected by several factors including pasteurization temperature, cooling process, storage time, and milking method (Andriani et al., 2021). Regarding the emulsion stability, it was affected by the presence of oil phase. The increasing concentration of oil phase in the milk would results in the unstable emulsions (Braun et al., 2019). Moreover, the measurement of viscosity in milk would give an impact on rheological properties of milk, while the lightness would cause an enzymatic change affecting the milk color, which eventually affect the consumers' preference.

Regarding that, several effective processing methods are required to ensure milk quality upon consumption and extend the shelf life (Fatih et al., 2021). Heat treatment of milk has been the common technique in milk processing, which includes thermalization, pasteurization, ultra-high temperature (UHT) treatment, and in-container sterilization (Deeth and Lewis, 2017).

Despite microbial reduction and extended shelf-life of the milk, there are some drawbacks resulting from heat treatment, some of which are nutritional value loss and undesirable flavor changes (Shabbir et al., 2021) These have been major concerns from consumers' perspective whose expectation is related to the sensory quality, functionality, and nutritional value of products (Nowosad et al., 2021). Therefore, non-thermal treatments are needed to achieve those targets.

One of the emerging non-thermal treatments applied in the food processing industry is pulsed electric fields (PEF). In Indonesia, this technology is used in the small milk processing plant located in Ponorogo, East Java for pasteurization

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(Priyanto et al., 2020). The PEF technology uses short pulses of high electric fields with an intensity of 10-80 kV/cm with a duration of microseconds (Pal, 2017). Such pulse electric fields would result in membrane electroporation (1-10 kV/cm) which can be used for further processing steps, for the example biorefinery process of bioactive compounds (phenol, flavonoid, anthocyanin, and carotenoid) from food waste, drying, freezing, as well as microbial inactivation (Dewi et al., 2019; Izza et al., 2016; Putranto et al., 2014, 2018, 2020) The PEF technology can also be used in combination with antimicrobial agents, membrane filtration, and heat (Nowosad et al., 2021)

The use of PEF treatment under a specific condition in milk has been reported to effectively reduce the number of microbes. When the PEF with a field strength of 28 kV/cm with a treatment time of 101 microseconds is used, the microbial reduction could reach up to 6 log CFU/mL for *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria innocua* individually (Sharma et al 2014). In addition, the implementation of electric fields is conserving the biological activity of bioactive molecules that are naturally present in milk (Mathys et al., 2014). However, such potential could only be seen if the field strength above 25kV/cm is combined with another hurdle technology with a processing temperature above 50 °C (Soni et al., 2021) Another study also stated that the enhancement of safety and the quality of fresh milk and milk products can be achieved by applying the combination of PEF with mild heating (below pasteurization condition) (Alirezaie et al., 2020). Moreover, there was a 4.67 log inactivation of *Bacillus subtilis* in a pre-heating of saline water (70 °C) exposed to a pulse electric field with a flow rate of 5 L/h, a frequency of 150 Hz, and an energy input of 226.5 kJ/kg (Reineke et al., 2015).

The application of PEF in combination with heat treatments needs further research to determine which specific conditions would result in the best microbiological, physical, and chemical characteristics of milk. Furthermore, it would not only provide the dairy industry with effective processing technologies but also lead to an increase in the effectiveness of cost and energy use. To our knowledge, the research about a specific condition in implementing the combination of PEF and heat treatment, in particular pre-heating, have been remained insufficient. Therefore, this research aims to examine the effect of combination food preservation techniques using pre-heating and PEF with various conditions.

MATERIALS AND METHODS

Materials

Fresh raw milk was purchased from CV Milkinesia Nusantara, Ponorogo District, East Java, Indonesia, and then was transported to the laboratory using mobile cold storage. For total plate count (TPC) analysis, the materials used were plate count agar (Himedia M091, India), distilled water, cotton, alcohol, and wrapping plastics (Cling Wrap, Indonesia). This research did not require IRB/IACUC approval because there were no human or animal participants.

Methods

The experimental design applied in this research was a completely randomized design (CDR) using two factors, namely factor I (pre-heating duration: 10, 20, and 30 minutes), and factor II (PEF exposure duration: 2, 4, and 6 minutes) with three replications, resulting in nine (9) treatments.

Besides, there were also milk samples without PEF exposure provided in this research as a comparison with milk samples exposed to PEF technology in terms of microbiological characteristics (Total Plate Count). Each pre-heating treatment was prepared ~~for~~ 3 times which would have been compared with the milk sample treated with different duration of PEF exposure (10, 20, and 30 minutes). After that, several following procedures were conducted.

Pre-Heating Process followed by PEF Treatment

The fresh raw cow's milk with a volume of 2.5 liters was placed in a cylindrical treatment chamber of PEF equipped with a double jacket heating system and stirrer (Putranto et al., 2020), then the temperature control was set to 70 °C. After reaching the targeted temperature for starting the pre-heating treatment (70 °C), the timer was started for 10-30 minutes. The exposure to the PEF was conducted after the pre-heating process was completed. The specific condition applied to all milk samples were a PEF with a field strength of 15 kV/cm, a frequency of 8.197 kHz, a pulse width of 66 microseconds, and duration exposure of 2, 4, and 6 minutes.

Characterization of Milk

After receiving the treatment, all the milk samples (raw and treated samples) were characterized physically, chemically, and microbiologically (Naghili et al., 2013), viscosity (Zhao et al., 2020), emulsion stability (Panesar and Shinde, 2012), and lightness. Analysis of TPC was initiated by preparing the plate count agar. Subsequently, 1 ml of milk sample that had been pasteurized was diluted to the 9 mL of NaCl solution until reaching the dilution factor of 10. Then, all those samples were poured into PCA agar and were incubated at 37 for 24 hours, following which the number of colonies formed was counted.

For testing the viscosity, as much of 100 mL of milk sample was placed in a beaker glass. Then, the whole spindle of viscometer was submerged into the milk sample. After that, the value was generated in in the viscometer's display. Lastly, the emulsion stability was measured by preparing 10 mL of milk sample placed in an Eppendorf tube, then it was centrifuged with the velocity of 3000 rpm for 15 minutes. After that, the volume of supernatant and sediment were measured according to the following formula:

$$viscosity\ (\%) = \frac{volume\ of\ sediment\ (ml)}{total\ volume\ of\ sample\ (ml)} \times 100\%$$

Data Analysis

Data were analyzed by using SPSS Software to run the Analysis of Variance (ANOVA) test with a confidence level of 95% and LSD Test was performed when there was a significant difference among treatments.

RESULTS AND DISCUSSION

Characteristics of Raw Milk

The physical and chemical properties of the milk were examined and presented in **Table 1**. The density of raw milk used in this research (1.06 g/mL) was slightly higher compared to a quality standard of raw cow's milk regulated in an Indonesian National Standard, namely SNI 3141.1:2011. This is probably due to several factors including the different time intervals of the milking process. Vidyanto et al. (2015) examined that the density of the raw milk produced from the shorter interval time of the milking process was higher than the longer interval time of milking process. Also, the pH value as well as the presence of catalase and peroxidase in raw milk samples were in accordance with Indonesia National Standard, namely SNI 3141.1:2011 (BSN 2011). Catalase is one of the antioxidative enzymes in milk that play a crucial role in the decomposition of hydrogen peroxide (Lindmark-Månsson and Åkesson 2000). Likewise, peroxidase also acts as an antioxidative enzyme by catalyzing the reactive oxygen species (ROS) generated during metabolism and converting the ROS into harmless molecules (Koksal, et al. 2016).

Moreover, there was no coagulation observed in the raw milk during alcoholic and boiling tests, indicating that the milk proteins are heat stable. The heat stability is affected by the farm factors, including seasons, altitude, total annual rainfall, herd size by the number of cows, milk yield, cow breed, type of milking, and summer grazing application (Hanuš et al. 2021).

Table 1. Physical and chemical characteristics of raw milk

Parameter(s)	Current Research
Density	1.06 g/mL
pH	6.5
Catalase	0.33 cc
Peroxidase	Positive
Alcoholic Test	Negative
Boilling Test	Negative

Total Plate Count

In this research, the number of bacteria in raw milk treated with merely pre-heating and pre-heating combined with PEF were assessed. The result showed that the range of total plate count in raw milk receiving pre-heating treatment was 1.82-2.94 log CFU/mL, while the range of raw milk receiving pre-heating combined with PEF exposure was 1.12-1.88 log CFU/mL (Table 2). In other words, the combination treatment could reduce the number of bacteria in raw milk by as many as 0.70-1.06 log CFU/mL. Those ranges are lower than the number maximum TPC value set in SNI 3141.1:2011, which is 6 log CFU/mL.

Table 2. Data of TPC of milk treated with pre-heating only as well as pre-heating and PEF

Treatments	TPC (log CFU/mL)	Treatments	TPC (log CFU/mL)
Pre-heating 10 minutes, PEF 0 minutes	2.86 ± 0.11^{a}	Pre-heating 10 minutes, PEF 2 minutes	1.88 ± 0.03^{a}
Pre-heating 10 minutes, PEF 0 minutes	2.87 ± 0.04^{a}	Pre-heating 10 minutes, PEF 4 minutes	1.75 ± 0.05^{b}
Pre-heating 10 minutes, PEF 0 minutes	2.94 ± 0.05^{a}	Pre-heating 10 minutes, PEF 6 minutes	1.63 ± 0.05^{c}
Pre-heating 20 minutes, PEF 0 minutes	2.20 ± 0.08^{b}	Pre-heating 20 minutes, PEF 2 minutes	1.65 ± 0.01^{c}
Pre-heating 20 minutes, PEF 0 minutes	2.29 ± 0.10^{b}	Pre-heating 20 minutes, PEF 4 minutes	1.51 ± 0.07^{d}
Pre-heating 20 minutes, PEF 0 minutes	2.26 ± 0.10^{b}	Pre-heating 20 minutes, PEF 6 minutes	1.31 ± 0.07^{e}
Pre-heating 30 minutes, PEF 0 minutes	1.95 ± 0.04^{c}	Pre-heating 30 minutes, PEF 2 minutes	1.28 ± 0.02^{ef}
Pre-heating 30 minutes, PEF 0 minutes	1.85 ± 0.06^{cd}	Pre-heating 30 minutes, PEF 4 minutes	1.26 ± 0.02^{f}
Pre-heating 30 minutes, PEF 0 minutes	1.82 ± 0.05^{d}	Pre-heating 30 minutes, PEF 6 minutes	1.12 ± 0.03^{g}

Remarks: *Mean values within a column followed by the different letters are significantly different at $p < 0.05$ according to LSD Test.

This is in accordance with the study conducted by Sharma *et al*. (2014), showing that the PEF treatments results in the reduction of *Escherichia coli* and *Listeria innocua* number from 8.3 log CFU/mL to less than 2 log CFU/mL. Similarly, there was a significant reduction in total bacterial viability and coliforms of raw milk after PEF treatment as many as 2.43 and 0.9 log CFU/mL, respectively (Šalaševičius et al., 2021). Another study from Emanuel *et al*. (2021) also indicated that after PEF treatment in a brain-heart infusion (BHI) medium, there was a reduction of 3.2 and 4.8 log CFU/mL of *Staphylococcus aureus* and *Pseudomonas putida*, respectively. However, the level of microbial reduction in this study was lower compared to previous studies due to the difference in the use of PEF specific conditions, for instance the electric field strength, exposure duration, and the initial number of microbial present in the raw milk.

Another study reported that the effect of preheating pasteurized milk products at 70°C for 10 minutes, followed by PEF for 3.9 minutes, 15 kV/cm was able to reduce the total microbial pathogens with a TPC value optimum of 2.126 log CFU/ml (Putranto, et al., 2022). According to Table 2, it is also observed that the number of bacteria decreased significantly ($p < 0.05$) as the duration of PEF exposure increased. This is because the PEF exposure on microbial cells would lead to disturbance on the integrity of the cell membrane transiently or permanently (Nowosad et al., 2021). Takaki *et al*. (2018) suggested that the PEF exposure would cause the charging up of the membrane, which further critically affected by the electric field strength and exposure period, leading to irreversible breakdown.

Viscosity

As one of crucial physical properties in dairy products, viscosity influences the appearance of dairy products and is important in all production stages, including manufacture, processing, and storage (Postnov et al., 2018). Viscosity of raw milk treated with pre-heating combined with PEF within various conditions were presented in Table 3.

Table 3. Data of viscosity, emulsion stability, and lightness of milk treated with pre-heating and PEF

Treatments	Viscosity (cP)	Emulsion Stability (%)	Lightness (%)
Pre-heating 10 minutes, PEF 2 minutes	5.07 ± 0.38^{a}	14.65 ± 0.10^{a}	95.65 ± 1.51^{a}
Pre-heating 10 minutes, PEF 4 minutes	5.13 ± 0.25^{a}	14.23 ± 0.08^{b}	92.15 ± 1.45^{b}
Pre-heating 10 minutes, PEF 6 minutes	4.97 ± 0.57^{a}	13.86 ± 0.11^{c}	90.51 ± 0.52^{b}
Pre-heating 20 minutes, PEF 2 minutes	4.23 ± 0.06^{b}	13.72 ± 0.03^{d}	88.21 ± 0.15^{c}
Pre-heating 20 minutes, PEF 4 minutes	4.33 ± 0.25^{b}	13.62 ± 0.06^{d}	85.42 ± 0.45^{d}
Pre-heating 20 minutes, PEF 6 minutes	4.17 ± 0.12^{b}	13.50 ± 0.01^{e}	84.34 ± 0.87^{de}
Pre-heating 30 minutes, PEF 2 minutes	3.97 ± 0.15^{bc}	12.48 ± 0.03^{f}	83.46 ± 1.56^{e}
Pre-heating 30 minutes, PEF 4 minutes	3.87 ± 0.12^{bc}	12.25 ± 0.07^{g}	81.64 ± 0.54^{f}
Pre-heating 30 minutes, PEF 6 minutes	3.60 ± 0.30^{c}	12.06 ± 0.05^{h}	76.96 ± 0.31^{g}

Remarks: *Mean values within a column followed by the different letters are significantly different at $p < 0.05$ according to LSD Test.

It is obvious that increasing duration of pre-heating treatments significantly reduces ($p < 0.05$) the viscosity of raw milk. This is mainly because the heat treatment cause denaturation of whey protein that is poor thermal stability in raw milk (Wijayanti et al., 2014). It is supported with a study conducted by Qian *et al*., (2017) suggested that as heat treatment temperature rise (65 °C above), both the denaturation degree of whey protein and the combination degree of whey protein and casein increased to more than 20%. Meanwhile, the different exposure time of PEF does not affect the milk viscosity.

The PEF technology affects minimally to the sensory and nutritional qualities of food products, including thermolabile compounds (Syed, 2017). Besides, this could be due to the use of low field strength

(15kV/cm) within short time (2-6 minutes), so that the PEF exposure was not sufficient to change the physical properties, especially the flow of raw milk, which influence the viscosity.

Emulsion Stability

Milk and milk proteins (caseins by around 80% and whey proteins by approximately 20%) are widely used as a natural emulsifier with high oil content. A stabilization of the fat phase in milk can be achieved via different mechanisms of emulsification (Braun et al., 2019). In this research, it can be clearly observed that the pre-heating combined with PEF exposure in raw milk results in significant decrease ($p < 0.05$) of emulsion stability of raw milk (Table 3).

This is because the heat treatment leads to conformational changes on the milk proteins, especially in the long period of exposure (Takaki et al., 2021). This might be due to the absorption of energy by polar groups of protein during PEF treatment generating the free radicals. These free radicals can influence the interaction between protein molecules, including hydrogen bonds, disulfide and salt bridges, and Van der Waals forces (Han et al., 2018). Priyanto et al., (2021) stated that the observation of emulsion stability can be performed by assessing the emulsion separation as a result of the centrifugation process.

Lightness

Colour is another sensory quality that affect consumer perception on food products, including milk. There are two types of colour measurement system for dairy and dairy products, one of which is the Hunter measurement system (L, a, and b) or called chromatic components. In this research, the color of raw milk samples treated with pre-heating combined with PEF was represented with the Lightness values (Table 3).

This is because the lightness value dominates in milk and other dairy products and the application of a thermal process can cause changes in the lightness parameter (Milovanovic *et al*., 2020). It can be clearly seen that the increased duration of pre-heating treatment as well as PEF exposure cause significant reduction ($p < 0.05$) in Lightness values. The decreased lightness value in pasteurized milk indicates that the color of the milk tends to be cloudy white and can even brownish color, while a fresh or raw cow's milk has a value of 81% indicating bright white color (Milovanovic *et al*., 2020).

This is caused by the pre-heating treatment temperature (70 °C) for 30 minutes that contribute to the form of Maillard reaction. During Maillard reaction, the lactose reacts with the free amnio acid side chains of milk proteins to produce various substances (Shimamura & Hiroyuki, 2012). In the case of milk, it leads to the form of brownish appearance, thus reducing the Lightness of raw milk itself. Moreover, Cheng *et al*., (2019) summarized that the effect of heat had the second largest effect on color of milk protein beverages with regards to instrumental color and sensory attributes.

CONCLUSION

Considering all parameters explained previously, the treatment of pre-heating at a temperature of 70 °C combined with PEF exposure would reduce the total plate count of raw milk as well as affect the viscosity, emulsion stability, and lightness of the raw milk. Besides, it can be concluded that the most effective treatment observed in the raw milk-treated with pre-heating of 10 minutes and PEF of 2 minutes, with the level of TPC, viscosity, emulsion stability, and lightness of 1.88 log CFU/mL, 14.65 cP, and 95.65%, respectively. For further research, it is highly recommended to investigate the possible recovery mechanism of injured PEF-treated bacteria, which would be beneficial to anticipate foodborne outbreaks due to the consumption of contaminated milk.

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