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Pulsed electric field and pre-heating treatment effect on free fatty acid (FFA), pH, vitamin C, and organoleptic properties of milk

Anugerah Dany Priyanto[1, 6], Teti Estiasih[3], Angky Wahyu Putranto[4], Widyasari[5] and Pardi Sampe Tola[2*]

[1] Department of Food Technology, Faculty of Engineering, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia
[2] Department of Physics, Faculty of Engineering, University of Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia
[3] Departement of Food Science and Biotechnology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
[4] Department of Biosystems Engineering, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia
[5] Department of Visual Communication Design, Faculty of Architecture and Design, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia
[6] Innovation Center of Appropriate Food Technology for Lowland and Coastal Area, University of Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, Indonesia

KEYWORDS

Free fatty acids
Preheating
Pulse electric field
Organoleptic

ABSTRACT

The pulsed electric field (PEF)has been extensively studied in milk processing. However, treatment conditions still need to be improved to meet safety for consumption. This study aimed to determine optimum conditions by varying PEF and preheating treatment time. Free fatty acids (FFA), pH, and vitamin C are used to assess the milk quality. Preheating temperature is fixed at 70°C and the treatment time: 10, 20, and 30 minutes. PEF parameters are 1.5×10^5 V/m electric field, 66 µs pulse width, and the applied time of 2, 4, and 6 minutes. The pH value and preheating treatment time show a negative linear relationship in the range of 6.75-7.15, while PEF treatment time did not significantly affect pH. The FFA linearly increases as preheating treatment time increases from 0.0053-0.0097%. Vitamin C decreases as preheating treatment time increase from 0.003-0.0049 mg/ml. PEF treatment significantly affects vitamin C, where vitamin C decreases as treatment time increases. Preheating and PEF treatment time show positive effects on the organoleptic properties of milk. The organoleptic properties values are flavor 3.93, aroma 4.00, and color 3.9 out of 5.

Introduction

Milk is one comestible that requires careful handling to prevent its nutrition and organoleptic parameter, in addition to reducing its microorganism (pathogen). According to the Centers for Disease Control and Prevention, fresh milk contains various types of microorganisms: brucella, campylobacter, cryptosporidium, Escherichia coli, listeria, and salmonella that imperil human health (Centers for Disease Control and Prevention). The microorganism content of milk is the fundamental cause of quality degradation and fragile characteristic of milk at ambient exposure. Heating treatment is the common method used to decrease or deactivate microorganisms. Low temperature and ultrahigh temperature pasteurization are commonly used to reduce milk's microorganisms to extend milk's shelf-life. However, heating treatment not only affects microorganism but also change the chemical structure of milk nutrition and essential mineral that determine organoleptic properties of milk, such as protein, vitamin, milk glucose, and lactic acid (Fellows, 2017; Qian et al., 2017).

Casein is the major nutrition (protein) of milk, where cow's milk contains about 80% of casein. Heating at high temperatures above 75°C could drive irreversible structure changes of casein, e.g., casein's denaturation, which has negative effects on milk products (Qian et al., 2017). Formation of milk skin indicates casein denaturation, and continuous denaturation of casein could lead to coagulation. Low-temperature heating can prevent significant irreversible structural changes in large

*Corresponding author
E-mail address: pardi.sampe.fisika@upnjatim.ac.id
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molecules such as protein, carbohydrates, and fat (Mckinnon et al., 2009; Morand et al., 2011). However, small-molecule such as vitamins easily lose their nutritional value even heated at low temperatures due to structural change (Qian et al., 2017; Jones et al., 2016). Careful handling of milk is required to maximize microorganism deactivation and preserve milk nutrition. Several pasteurizations have been introduced and continuously improved to effectively decreases microorganism content, sustain nutrition and enhance the organoleptic properties.

Minimal or non-thermal milk processes have been reported to not only deactivate microorganisms but also retain milk'a nutritional quality (i.e., vitamins, minerals, and essential flavors) (Maged et al., 2012). Several energy sources, such as mechanical pressure, magnetic field, electric field, gamma ray, microwave, UV light, and vibration (ultrasound), can potentially be applied as replacements for thermal processing (Butz and Tauscher, 2002). These external energies are expected to only disturb or interact with milk's microorganisms without or with minimal effects on the chemical structure of essential minerals. Recent studies reported that high applied voltage or pulsed electric field (PEF) processing shows promising results as an alternative for milk processing (Maged et al., 2012; Priyanto et al., 2021). The PEF process is extensively used for medical treatment and promises to be implemented in food processing to deactivate microorganisms. Liquid-based foods reported already treated with PEF are milk, milk product, egg products, and juice.

PEF process exhibit flexibility, where microorganism respond affected by several parameters, i.e., applied voltage, pulse width, homogeneity of induction field, and the distance between electrodes (Barba et al., 2015; Golberg et al., 2016; Pal, 2017). Depending on the PEF parameter, the membrane of the microorganism could be in a reversible or permanently damaged state (irreversible state). The irreversible PEF process is widely applied for medical treatment, but also possible to be applied for milk processing (Barba et al., 2015). Moreover, the PEF process was reported to enhance drying rates, increase extraction yields, preserve organoleptic properties, and extend the shelf-life of food (Taiwo et al., 2002; Bajgai and Hashinaga, 2001).

The PEF process is based on the induction of a potential difference across a biological cell, or transmembrane potential difference, by applying a short high-voltage pulse between two electrodes (Jaeger and Knorr, 2017; Schoenbach et al., 2004; Zimmermann et al., 1974). The induced potential difference between the interior and exterior of a biological cell would provide energy for ions to penetrate the cell membrane forming ion channels that allow ions to move across the membrane and ion concentration gradients across the cell membrane (Toepfl et al., 2006; Barba et al., 2015). Formation of ion channels and accommodation of cell ions transport are key factor inactivation of microorganisms. Careful design of PEF parameters in combination with low-temperature processing may reduce microorganisms effectively while preserving milk's nutritional value and organoleptic properties.

This study aimed to determine optimum preheating and PEF treatment conditions by varying treatment time. Specific parameters of preheating and PEF milk processing have remained insufficient. Therefore, it is necessary to figure out the specific condition of preheating and PEF process. In this study, several combinations of preheating and PEF treatment time were carried out, and the optimal physicochemical quality of processed milk was determined in terms of free fatty acids (FFA), pH, and organoleptic.

Research Methods

Fresh milk obtained from SW Dairy Farm, Kletek, Sidoarjo, was milked at 05.00 AM and transported to the laboratory in mobile cold storage. Fresh milk was subsequently analyzed 3 hours after milked and repeated two times. The fresh milk parameters are: negative alcohol content, 1.028 g/mL density, and 6.6 pH, which meets The Indonesian national standard (SNI) for fresh milk (SNI 01-3141-1998). The density of fresh milk used in this study was lower than in our previous study (Priyanto et al., 2022). The total plate counts (TPC) value of fresh milk we use in this study is 5.267 ± 0.017 log cfu/mL (Esfandiar et al., 2022), below the maximum value of fresh milk according to SNI, 6.00 log cfu/mL.

The pasteurization process was conducted using a PEF machine built at Food Analysis Laboratory, UPN Veteran Jatim. The PEF machine had a 12 L cylinder treatment container capacity and a double jacket heating system. The electrodes and mixing system adopted the PEF model developed by Putranto et al. (2020), where the cathode was placed outside of the treatment chamber and the anode inside the treatment chamber. Electrodes were made of food-grade stainless steel 304 type. Before preheating and the PEF process, 2.5 L fresh milk mixed with collagen powder 2% w/v (Halavet, Turkey) and sugar cane

3% w/v (Gulaku, Indonesia). The homogeneity of milk was maintained by stirring at 50 rpm. Pre-heating time was counted after reaching the temperature set point; then the PEF process was conducted. Compared to other studies where preheating temperatures vary below 70°C (30, 45, and 60°C) with a shocking time of 2 minutes and only 1 L mixed fresh milk was used (Esfandiar et al., 2022). In this study, the preheating temperature was fixed at 70°C, while the heating duration varies at 10, 20, and 30 minutes. The PEF time also varies, where electric pulsed applied for 2, 4, and 6 minutes, while the applied voltage constant of $1.5 \times 10^5\,\mathrm{V/m}$, frequency of 8.197 kHz, pulse width of 66 μs.

Several parameters were used to assess the quality of pasteurized milk e.g., free fatty acids (FFA), pH, and vitamin C content. Free fatty acids content was calculated using the AOAC method (Firdaus, 2015): 50 mL of neutral alcohol 95% mixed with 50 mL milk by stirring and heated for 10 minutes, 3-5 drops of 1% phenolphthalein (PP) indicator added after milk cooled down, followed by titration with 0.1 N NaOH solution. This process was repeated three times, and the final FFA content (%) was calculated with Equation 1.

$$\% \, FFA = \frac{ml\ NaOH\ x\ N\ NaOh\ x\ BM\ As.fat\ (aq)}{sample\ mass\ gr\ x\ 1000}\ x\ 100\ \% \quad (1)$$

The pH of milk was measured with a pH meter, previously calibrated and neutralized. Vitamin C content was measured with the acid-base titrations method: 3 drops of PP were added to 10 mL milk, followed by titration with 0.1 N NaOH until the color changed (turned pink).

Milk pasteurization parameters, i.e. pre-heating and PEF treatment time, were separated into three level which resulting on 9 combination of pasteurization parameter. The pH, FFA, and vitamin C content measurements were carried out triplicate for every parameter combination and the average value was used as the final result for analysis. Organoleptic characteristics were obtained by conducting a survey from 30 panelists for every parameter combination. The scale used in this study was between 1 to 5, equivalent to strongly dislike to really like. The organoleptic properties we evaluate in this study include flavor, aroma, and color.

Results and Discussion

The average FFA content of milk after preheating and the PEF process with different treatment times is shown in Figure 1. The PEF treatment time shows negligible effect on FFA content, while FFA content increases linearly with pre-heating time. Increasing FFA content indicates cell membrane change due to external energy exposure. The FFA content significantly increases as preheating time increases in the range of 0.0053 to 0.0097%, where PEF treatment time not showing a significant effect on % FFA. The increasing FFA content in milk indicates that microorganism membranes were damaged during preheating. High FFA content caused by lipolysis could reduce milk quality. Milk with high FFA concentration is impressionable to oxidation and leads to the development of offensive taste and flavor, damaging the organoleptic properties of milk.

The results show that PEF treatment time relatively does not affect the FFA content of milk. It was assumed that the milk's FFA content is affected more by the applied electric field than treatment time. Equation 1 shows that the transmembrane potential difference, ΔV_m is directly proportional to the electric field E. In contrast, ΔV_m is a negative exponential function of applied electric field time (PEF treatment time), t, where the longer the electric field is applied transmembrane potential difference will reach saturation condition. As a result, the effect of PFE treatment time on FFA content is not significant.

The transmembrane potential difference as a function of time, $\Delta V_m(t)$, Equation 1 can be expressed as follows (Ehrenberg et al., 1987).

$$\Delta V_m(t) = f \cdot E(t) \cdot a_m \cdot \cos\theta \cdot \left(1 - e^{-t/\tau_m}\right) \quad (2)$$

Where f is dependent on cell shape (1.5 for spherical cell), $E(t)$ is the external applied electric field, a_m is cell radius, θ is the electric field angle with the specified point, t is treatment time, and τ_m is membrane charging time.

It can be seen that the applied electric field, $E(t)$, is linearly proportional with ΔV_m as described by Equation 1. While ΔV_m is negative exponent function of applied electric field time, treatment time (t). The characteristic of τ_m is on the order of 1 μs (Ruiz-Fernández et al., 2022, and Daniel et al., 2016). Hence, at a millisecond pulse of the applied electric field, transmembrane potential has reached saturation condition. As aresult, the PEF treatment time has no effect on FFA. In this study, we apply an electric field with a pulse width of 66 μs. The saturation condition is illustrated in Figure 2. The results show that the applied field has a significant effect on ΔV_m at narrow pulse width, while above 5 μs ΔV_m reach saturation condition.

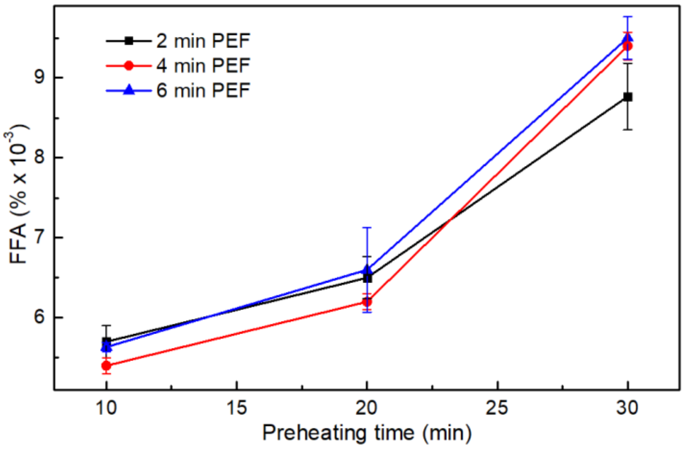

Figure 1. Average FFA content as function of pre-heating and PEF treatment time.

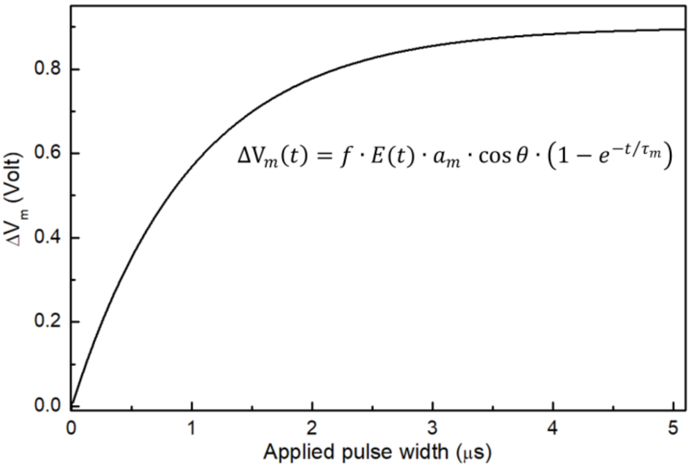

Figure 2. Transmembrane potential difference as a function of time, $\Delta V_m(t)$, where $f = 1.5$ for spherical cell, $E = 1.5 \times 10^5\ V/m$, $a_m \approx 0.4$ μm, and applied field assumed perpendicular to cell surface, $\cos\theta = 1$.

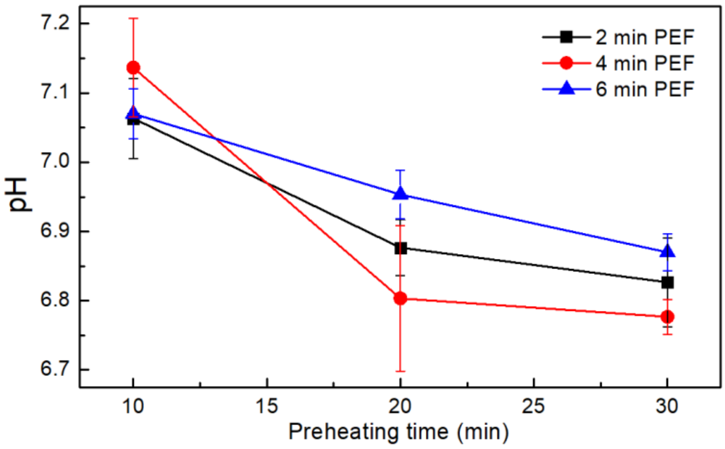

Figure 3. Average pH value as function of pre-heating and PEF treatment time.

The average pH value of milk after preheating and the PEF process with different treatment times is shown in Figure 3. Preheating and PEF treatment time variations showed a reduction trend in milk's pH values. The preheating time significantly affected pH reduction, where the time increment is large enough, 10 minutes. Increasing preheating time provides a large amount of thermal energy that can increase the number of reactions. Such conditions could change the chemical structure of milk molecules or microorganisms, thus affecting the pH value. The pH value and preheating time show a negative linear relationship in the range of 6.75 to 7.15. On the other hand, PEF treatment time has relatively shown a negligible effect on pH value, and this may be due to the small time increment of PEF treatment, i.e., 2 minutes.

The pH decreases linearly as temperature increases reported by Ma and Barbano (2003). They conclude that pH decreases related to the thermal death of bacteria during pasteurization. Meanwhile, Chandrapala et al. (2010) reported that pH reduction as temperature increases are related to calcium and phosphate activity during heating. During heating, calcium phosphate's solubility decreases, leading to the formation of a precipitation phase which induces re-equilibrium between HPO_4^{2-} and $H_2PO_4^-/PO_4^{3-}$ and produces H^+ ions as the result of pH decrease. The precipitation and equilibrium reactions are shown below:

$$H_2PO_4^-{}_{(aq)} \leftrightarrow HPO_4^{2-}{}_{(aq)} + H^+{}_{(aq)}$$

$$HPO_4^{2-}{}_{(aq)} \leftrightarrow PO_4^{3-}{}_{(aq)} + H^+{}_{(aq)}$$

$$Ca^{2+}{}_{(aq)} + 0.7H_2PO_4^-{}_{(aq)} + 0.2PO_4^{3-}{}_{(aq)} \leftrightarrow Ca(HPO_4)_{0.7}(PO_4)_{0.2}{}_{(s)}$$

Thus, the decreases in pH as the preheating time increase in the present study are due to the thermal death of bacteria and/or calcium and phosphate activity during preheating and PEF treatment.

The average vitamin C content of milk after pre-heating and PEF process with different treatment time is shown in Figure 4, where the reduction of vitamin C concentration is a function of increasing preheating time and PEF time. Preheating and PEF treatment duration showed similar effects on of vitamin C content of milk, where vitamin C decreases as preheating and PEF treatment time increases. Vitamin C is a small molecule highly affected by the external energy e.g. thermal or electric energy. This study indicates that, as treatment time increases, more energy is absorbed by vitamin C molecules. Thus, it changes the chemical structure, causing a reduction in vitamin C concentration.

The average value of four organoleptic properties with 9 different pasteurization parameters combination labeled P_1 to P_9 is shown in Table 1. The panelist preference level, determined by organoleptic properties, increases as preheating and PEF time increases. The highest preference value of flavor is 3.93 out of 5, which increases as pre-heating and PEF treatment time increase. This may be due to the increased formation of the Maillard component, which is important for flavor formation. The highest value of aroma is 4.00 out of 5; our survey shows flavor and aroma have the same tendency, where those parameters were highly related. The highest value of color is 3.93 out of 5, with preheating and PEF treatment time not showing a significant effect on panelist appraisement of color. All samples with different treatment conditions give relatively the same value. This implies that preheating and PEF treatment time has a low effect on milk's color. Our organoleptic survey showed that panelists tend to prefer milk with longer preheating and PEF treatment times.

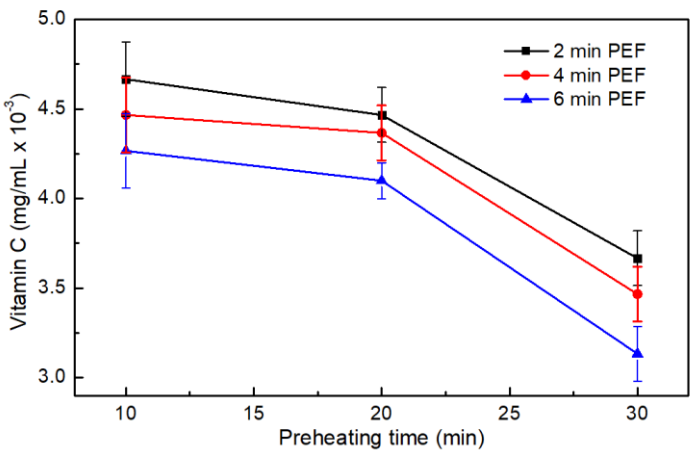

Figure 4. Average vitamin C content as function of pre-heating and PEF treatment time.

Table 1. Average value of organoleptic properties of milk with different combination of pre-heating and PEF treatment time.

Parameter	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9
Flavor	3.60	3.73	3.67	3.87	3.47	3.53	3.33	**3.93**	3.73
Aroma	4.00	3.80	3.73	3.73	3.40	3.53	3.53	3.87	**4.00**
Color	3.73	3.73	3.73	3.47	3.87	3.67	3.93	3.60	**3.93**

Note: P_1 to P_3 are treatment with 2 minutes PEF and preheating time of 10, 20, and 30 minutes; P_4 to P_6 are treatment with 4 minutes PEF and preheating time of10, 20 and 30 minutes; P_7 to P_9 are treatment with 6 minutes PEF and preheating time of 10, 20 and 30 minutes.

Conclusion

FFA content increases as preheating time increases, while pH and vitamin C exhibit the opposite properties. These parameter changes suggest that increasing preheating time can provide energy to deactivate bacteria and induce chemical reactions or molecular changes in milk components. On the other hand, PEF treatment time variation did not show a significant effect on FFA and pH. This indicates that our applied field pulse width, 66 µs, is far above membrane charging time (τ_m = 1 µs). Vitamin C concentration reduces as pre-heating and PEF treatment time increases, which confirms that the structure of the small molecule is easily changed by external energy. The FFA content varies between 0.0053-0.0097% and pH between 6.75-7.15. According to SNI standards, these values are in the range of safety values for consumption. The survey results indicate that increasing of preheating and PEF treatment time relatively increases organoleptic properties of milk. For further study, careful design of preheating and PEF parameter are important to meet the demand of high-quality milk (e.g., minimum microorganism concentration and minimum nutrition reduction during processing). Variations of applied electric field amplitude and pulse width around τ_m also need to be considered to improve efficiency of PEF treatment.

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Declarations

Conflict of interests The authors declare no competing interests.

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