Pulsed electric field application on pasteurization of orange milk from low grade orange: study on nutritional, physical, chemical properties, and total microorganism

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Abstract. Oranges with low grade categories have a low price, so one of the utilization efforts is combine with milk into orange milk products.. Orange milk, extracted from orange of siam cultivate and added with milk, was pasteurized using a pulsed electric field (PEF) method. The aim of this research was to analyze the changes on nutritional, physical and chemical properties and total microbes from orange milk after pasteurization. The research was conducted from July until September 2021 located in Jember. The PEF treatment was carried out using treatment time variation for 5 minutes (1 cycle), 10 minutes (2 cycles) and 15 minutes (3 cycles). Several parameters of Vitamin C, pH, dissolved solid and total microbes were observed. The result showed that PEF treatment did not significantly change nutritional (vitamin C), physical (dissolved solid), and chemical properties (pH), but total microbes very significantly change after pasteurization with PEF in compared with no treatment pasteurization. The best treatment was found in the variation of 15 minutes (3 cycles) with degradation microbial killing effectiveness reached 94.58%.

1. Introduction
Siam oranges are a type of orange. It is widely developed in Indonesia because the production is high and favored by consumers. Stay at the time of harvest, oranges with low grade categories have a harvest potential of about 15%. Low grade siamese oranges have a low price, thus affecting farmers' incomes[1]. Orange-based combination drinks can be an alternative for orange processed fruit as it has a great public demand. It also be combined with milk. In fresh condition, orange juice and milk cannot stand long at room temperature. For this reason, further processing is needed to make it last longer. Heating is a conventional processing technology to reduce microbial contamination in food. This process ensures product safety, but the possibility of damage to the taste, nutrition and physicochemical properties cannot be avoided [2]. For this reason, the food processing industry continues to develop alternative of preservation technologies to minimize damage caused by excessive heat treatment. Pulsed Electric Fields (PEF) is a non-thermal technology that is possible to inactivate microorganism with no significant damage cause to the taste, color, and nutrients in foods [3].
The PEF process is based on the short pulses application at high voltage (20-80 kV/cm) to a food placed between two electrodes [4]. The PEF application has been widely used by many researchers such as [5], [6] and [7]. Research conducted by [8] and [9], shows that the combination of heating and electric field shock was effective to inactivate Pseudomonas and E. coli in milk and coconut water. This study aims to use a pulsed electric field to inactivate total microbes in a combination drink of milk and orange juice with time variations at a constant voltage, so that it is expected to provide knowledge about these applications for food processing.

2. Research methods

2.1 Materials and tools

The material used is siam orange juice obtained from the extraction of siam oranges without the addition of other ingredients. Siam oranges were purchased from Tanjung market-Jember. Other materials are alcohol 95%, cotton and sterile bottles. Pure cow’s milk purchased from a cow farmer in Kemuning lor village. The tools used are a series of HPEF tools, high voltage voltmeter, pH meter, petri dish, tds meter, juice extractor, and cool box.

2.2 Research methods

The research was carried out from June to August 2021 at TEFA Canning State Polytechnic of Jember. The method used is descriptive experimental method. Testing is done by varying the processing time. Processing time is 0, 5, 10, and 15 minutes. The volume of apple cider entered in the treatment chamber is 3 liters.

For each processing time with HPEF, 3 (three) replicates of samples is taken and then be analyzed for changes in nutritional value (vitamin C), chemical properties (pH and total dissolved solids) and the number of microbes (total microbes and coliforms) contained in the HPEF will be calculated. Siam orange juice processed with HPEF. The high-voltage pulse generator used in the pasteurization of Siam orange milk with HPEF method consists of several blocks, which are the keypad block, microcontroller, display, flyback converter circuit, high voltage transformer and chamber. The keypad is used to enter the high voltage setting and time for treatment requirement.

The high voltage can be set from 20 kV to 40 kV and the length of treatment can be set in the range of 0 minutes to 15 minutes. The microcontroller is used to display the high voltage and treatment time that is set through the keypad. The flyback converter circuit will receive the output of the microcontroller in the form of square pulses that can be adjusted for the pulse width. The output of flyback converter in the form of a voltage pulse will count the input voltage of the high-voltage transformer so that the output of the transformer will be in the form of a high-voltage pulse. High-voltage transformers can produce a maximum output of 40 kV. Constant frequency is 20 kHz. All PEF components are assembled in a high-voltage generator box made of mica. The high-voltage pulses produced are directly shoted into the chamber (the treatment area) which is coated on the outside by electrodes made of copper. The treatment chamber is made of 4 mm thick stainless steel plate which is safe for all food products. The treatment chamber is in the form of a four-legged cylinder with a volume of 1.7 liters equipped with an exhaust valve. To protect the entire pulsed electric field, the outside is provided with a barrier made of a clear mica box.

3. Results and discussion

The treatment room is a container for orange milk to be pasteurized. High voltage will be fired towards the treatment chamber which is made of stainless steel in the form of a tube. The treatment room is made in a static or batch system with a diameter of 45 cm and a height of 50 cm and is provided with an iron support to keep it upright and a faucet is placed at the bottom of the tube for discharge hole. The walls’ thickness of the treatment room is 3 mm. Next to the treatment room, there are three electrodes spaced more than 5 cm apart which conduct electricity to the material. The chamber is entered in an insulator made of mica (15 x 5 x 55 cm). The electrode is mounted on one side of the insulator facing the treatment room. The results of the treatment room design are shown in Figure 1.
3.1 Vitamin C
Vitamin C is a water-soluble nutrient and vitamin that is essential for life and for maintaining health. This vitamin is also known by the chemical name of its main form, namely ascorbic acid. The vitamin C content of Siam orange juice before pasteurization is 9.792 mg/100gr. The highest level of vitamin C after pasteurization in 5 minutes treatment is 9.608 mg/100gr and the lowest in the 15 minutes treatment is 8.714 mg/100gr. The graph of changes in vitamin C levels against treatment time is shown in Figure 2.

![Figure 2. Effect HPEF on the vitamin C](image)

In Figure 2 it can be seen that the presence of nonthermal pasteurization treatment with a high pulsed electric field (HPEF) will cause a decrease in vitamin C level, but this change tends to be insignificant compared to those without HPEF. [10] stated that the higher the temperature and the longer heating, the greater the degradation of vitamin C. Oxidation of vitamin C (ascorbic acid) will convert ascorbic acid into L-dehydroascorbic acid which is chemically very unstable and can undergo further changes to L diketogulonic acid which has no vitamin C activity anymore. This is in accordance with [11], in her research which shown that the longer the heating (boiling) time, the lower the vitamin C content of cherry syrup. This is because vitamin C is a vitamin that is easily oxidized, especially by the heating process.

3.2 pH
The pH of Siam orange juice before pasteurization is 4.50. The highest pH after pasteurization in the treatment for 15 minutes of 5.55 and the lowest is in the treatment for 10 minutes that is 5.51. Graphic of changes in pH with treatment time is shown in Figure 3.

![Figure 3. Graphic of changes in pH with treatment time](image)
In Figure 3 it can be seen that the pH of non-thermal pasteurized orange juice using a pulsed electric field does not change significantly and is still in the average range with the pH before pasteurization. This is in line with research by [12] that the pH orange juice mixture and carrots treated with PEF did not change significantly. [13] also stated that the HPEF treatment did not significantly change the pH of goat’s milk. These changes in the pH value are explained, according with [14], because the migration of ions to the electrodes occurring when an external voltage is applied, which can produce changes of the fluid composition and the chemical properties such as the pH value. The application of HPEF on the orange milk did not show any effect on the pH value.

3.3 Dissolved solid

Dissolved solid total of Siam orange juice before pasteurization was 1303 ppm. The value of Dissolved solid total from orange juice after pasteurization of HPEF tends not to change, except for the 15 minute treatment of 726 ppm. The graphic change in total dissolved solid to treatment time is shown in Figure 7.

The total value of dissolved solid is the sum total content of all substances, both inorganic and organic which dissolved in food. The components in the fruit consist of water-soluble components, such as glucose, fructose sucrose, and water-soluble protein (pectin). The decrease in total dissolved solid value is assumed as the material contained in the siam orange juice is not degraded due to HPEF processing, so that it can reduce the total of dissolved solid in siam orange juice. This is in accordance with the opinion of [15] which states that the decrease in lactose is due to lactose being degraded to glucose and galactose which in turn becomes lactic acid.
3.4 Total microbes
The total of microbe’s milk in siam orange juice before pasteurization is 1.50x10^5 cfu/ml. The total of these microbes is not in accordance with the Indonesian National Standard (SNI) where the maximum total of microbes contained in fruit juice drinks is 2x10^2 cfu/ml. The highest microbes total after pasteurization in the 5 minutes treatment is 7.40x10^4 cfu/ml and the lowest is 8.13x10^3 cfu/ml after the pasteurization treatment. The graphic of the decrease in microbes total with time changes is shown in Figure 8.

From Figure 8 it can be calculated the effectiveness value of microbial killing which is the difference between the number of microbes before treatment and the number of microbes after treatment divided by the number of microbes before treatment multiplied by 100 percent. The highest killing effectiveness value at 15 minutes is 94.58% and the lowest at 5 minutes is 50.66%. In Figure 8 it can be seen that the longer the pasteurization treatment with PEF, the less the total microbes present in the orange juice milk. In general, the total microbes of orange juice milk after pasteurization have not met the SNI standard, where the maximum limit of total microbes allowed is 2x10^2 cfu/ml. Therefore, it is necessary to increase the PEF pasteurization time. The longer processing time will result in a greater decrease in total microbes.

![Graph showing reduction of total microbes with treatment time](image)

**Figure 4. Reduction of total microbes with treatment time**

The analysis results showed the presence of coliforms in the fresh siam orange milk. At the time after pasteurization is still found the presence of coliform. Referring to the Indonesian National Standard (SNI) where the maximum permissible coliform limit is 20 APM/ml, then Siam orange milk juice after HPEF pasteurization treatment is included in SNI, which is still below 20 APM/ml.

The length of treatment also affects the amount of microbial decline, where the longer the treatment time, the greater the microbial mortality. It is suspected that the largest total microbes contained in orange milk juice have a high susceptibility to electric current, causing a high mortality rate. Meanwhile, the remaining total living microbes is assumed to have high resistance to electric shocks, so that the number of subsequent electric shock treatments does not give too much effect in killing the remaining of total microbes. According to [16], spore death by electrocution is limited by the strength electric field generated, the duration of the shock, the number of shocks applied and the cell size. Meanwhile, according to [17], high-voltage electricity for a long time has a greater effect in reducing the number of spores contained in the media.

Non thermal Pulsed Electric Field (PEF) method is one of the non-thermal treatment methods for food preservation, as PEF has the potential to inactivate microbes without changing the taste and nutritional richness of food. The high intensity pulsed electric field process is based on the application of high voltage short pulses (20-80 kV/cm) for a very short time (approximately 1 second) to liquid food placed between two electrodes [18]. PEF technology is more considered than heat treatment of food, because PEF can kill more microbes, avoid or reduce damage in taste, physical properties of food and organoleptic damage [19] and [20]. Microbial inactivation performed with PEF is associated with electro mechanical instability of the cell membrane. The cell membrane protects microbes from environmental condition by working as a semipermeable wall, for example, the membrane regulates the entry of nutrients into the cell and regulates the release of end products of cell metabolic activity [2]. If the cell membrane is ruptured, there is a discharge of fluid from the cell and a loss of cell metabolic activity.
4. Conclusion
The conclusions from this study results are:
1. Chemical properties of pasteurized orange milk juice with PEF, namely pH and dissolved solid do not change significantly.
2. The vitamin C content of pasteurized orange milk juice with PEF, in which the pH does not change significantly.
3. The highest microbial killing effectiveness reached 94.58%.

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