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Microbiological and Sensory Profile of Collagen Supplemented Milk with Pretreatment and Pulsed Electric Field Pasteurization Process

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A B S T R A C T

Collagen-supplemented milk products are susceptible to damage due to high-temperature processing of Pulsed Electric Field (PEF) pasteurization is carried out with a pretreatment temperature of <65°C to maintain quality. This study aims to determine the total microbe, the level of microbial decline and the intensity of the sensory profile of collagen-supplemented milk by pretreatment in the PEF pasteurization process. This study used a completely randomized design with a factorial pattern of 2 factors and 3 replications, the first factor was the pretreatment temperature (30, 45 and 60°C), and the second factor was the pretreatment time (10, 20, 30 minutes) which was then pasteurized using the PEF method with a voltage of 15 kv/cm for 120 seconds. Furthermore, these 9 treatments were compared with PEF pasteurized milk without pretreatment and Low Temperature Long Time / LTLT pasteurized milk (pasteurized at 65°C for 30 minutes). The results showed that pretreatment with a temperature of 60°C for 20 and 30 minutes resulted in a total microbial count of 3.406±0.19 CFU/ml and 3.395±0.146 log CFU/ml, respectively. The average total microbe of collagen supplemented milk in the combination of PEF pasteurization with pretreatment is smaller than the pretreatment process alone with a value below the SNI standard so it is safe for consumption. The average microbial decrease was 2.88 log cycles and was not significantly different (P<0.05) with LTLT thermal pasteurization. The results of the sensory profile intensity test showed that the pretreatment with a temperature of 60°C for 10, 20 and 30 minutes had a sensory profile value that was closest to LTLT pasteurization on the attributes of uncooked odor, milky odor, and milky flavor.

1. INTRODUCTION

1.1. *Research Background*

Cow's milk is a product with high nutrition and benefits in fulfilling community nutrition because it has casein protein (76%) and whey protein consisting of lactalbumin (52%), lactoglobulin (18%) [1]. as well as complete essential amino acids, especially methionine by 18.4% and lysine by 21.5% and equipped with various vitamins and minerals with high bioavailability [2].

To make milk a commercial product that is needed by the community, other components are often added to increase the functional value and product development. One of the additional components that can increase health benefits in the body is collagen hydrolyzate [3]. The recommended amount of collagen for consumption is 2% to 30% to have a positive effect on health [4].

Milk processing method using high temperature (thermal) causes changes in chemical components such as protein and a decrease in micronutrient content because it has perishable food [5]. Changes in the structure of whey protein/aggregation in milk can affect the characteristics of milk at heating temperatures > 70°C [6]. Collagen products can undergo a gelation transition due to damage to the triple helix hydrogen bonds which leads to the formation of collagen degradation into gelatin at temperatures > 45°C [7].

Nutrition and quality attributes in collagen supplemented milk can be maintained through proper processing. One of the processing methods to improve food safety by reducing pathogenic microbes without changing the quality is the Pulsed Electric Field (PEF) pasteurization method. The PEF method is a non-thermal food preservation technology that results in the transfer of electrical energy and can reduce the growth of pathogenic microbes [8].

1.2. *Literature Review*

1.2.1. *Pulsed Electric Field Pasteurization*

PEF technology is more considered a pasteurization method for foodstuffs because it can kill pathogenic microbes by minimizing damage to the physical properties of food and organoleptic changes [9]. The PEF process is based on the application of high-voltage short pulses (20-80 kV/cm) with very short pulse times (approximately 1 second) on liquid food between two electrodes. PEF preservation has the potential to inactivate microbes due to damage to the microbial cell membrane (electroporation) [10]. Research using coconut water samples as a test material gave the results of an electric field shock that was effective in reducing total microbial pathogens [11].

The PEF method has drawbacks, it is necessary to test the combination method between pasteurization and heating and the PEF method. The combination of the pasteurization method with a certain temperature can accelerate the rate of death of pathogenic bacteria and reduce the degradation of nutrients and organoleptic properties of dairy products so that quality dairy products are obtained. research conducted with heating treatment at 55°C combined with PEF voltage of 35 kV/cm effectively reduced E.coli and P.fluorescens up to 6.0 Log CFU/mL and on other pathogenic microorganisms [12].

1.2.2. Sensory Profile of PEF Pasteurized

Based on research [13] the pasteurization process of milk using PEF at an electric field strength of 15-30 kV/cm for 800 μs showed that the concentration of volatile components in the aldehyde group (pentanal, hexanal, and nonanal) of milk was higher when compared to pasteurized milk (temperature 75°C for 15 minutes). second). The increase in aldehyde group components such as pentanal, hexanal, nonanal, and 2(5H)- Furanone components showed the intensity of the milk caramel aroma, which was characterized by the distinctive aroma of "fatty" and "waxy" raw milk which was not found in heat-treated milk. The formation of the characteristic aroma and taste of raw milk is due to the electrochemical reaction of the milk electrode interface in the processing of pasteurized milk using PEF [14].

Thermal pasteurized milk, 2-Heptanone component intensity is higher than PEF milk where this component produces a "fruity" and "milky" aroma that can be found in commercial milk [13]. The aldehyde groups such as pentanal, hexanal, and nonanal are the result of fatty acid auto-oxidation that occurs in products high in fat and protein [15]. So that the application of mild heat treatment during the PEF pasteurization process can prevent the formation of components that can reduce consumer acceptance [15].

1.3. *Research Objective*

This study aims to determine the total microbe, the level of microbial decline, and the intensity of the sensory profile of collagen-supplemented milk at the pretreatment temperature and time treatment with the PEF pasteurization process and to compare the PEF pretreatment samples with PEF pasteurized milk (without pretreatment) and LTLT pasteurized milk 60oC, 30 min.

2. MATERIALS AND METHODS

2.1. *Materials and Tools*

Samples of fresh cow's milk were obtained from fresh milk farmers in Taman District, Sidoarjo Regency, East Java. Hydrolyzed bovine collagen powder (Halavet brand) and white crystal sugar. Media Plate Count Agar (PCA), 85% NaCl, aquades.

Pasteurization using a PEF based machine with a double jacket heating modification in a stainless steel treatment chamber and LTLT pasteurization using a hotplate magnetic stirrer, Laminar Air Flow Cabinet, incubator, autoclave, vortex, micropipette, blue tip, yellow tip, petridish, measuring cup, cubicle taster and questionnaire sheet for sensory evaluation.

2.2. *Design Experiment and Analysis*

The research design used a Completely Randomized Design with a factorial pattern with 2 factors and 3 replications. The factor I pretreatment temperature (30°C, 45°C, and 60°C). Factor II pretreatment time (10, 20, and 30 minutes). Samples with PEF pretreatment temperature and time were compared with control samples of PEF pasteurization (without pretreatment) and LTLT pasteurized (65°C for, 30 minutes).

2.3. *Implementation of Research*

2.3.1. Collagen Supplemented Milk Process

1000 ml of fresh milk samples were mixed with 2% (w/v) collagen and 3% (w/v) sugar in the treatment chamber. Furthermore, pretreatment was carried out according to variations in temperature (30°C, 45 °C, 60 °C) and time variations (10, 20, 30 minutes). After the pretreatment process, all samples were pasteurized PEF using a voltage of 15 kV/cm with a shocking time of 120 seconds.

For comparison, used PEF pasteurized milk (without pretreatment) using a PEF machine, with the same stress and shock time. While LTLT pasteurized milk (as comparison II), the pasteurization process was carried out at a temperature of 65oC for 30 minutes, using a magnetic hotplate.

2.3.2. Total Microbes using the Drop Plate Method [16]

Tests were carried out on milk samples after pretreatment temperature and time (before pasteurization of PEF) and milk after pasteurization of PEF (with pretreatment). One ml of the sample was homogenized in 9 ml of 0.85% NaCl solution which was then used at 10-1 to 10-5 dilutions. A total of 10 l samples from each dilution solution were dropped on the surface of sterile PCA media and incubated at 38°C for 18 hours. The growth of bacterial colonies was calculated using Colony Forming Units per mL (CFU/ml) of the sample using the formula:

CFU = number of colonies x 1000/10 x dilution factor

2.3.3. Organoleptic intensity profile [17]

Intensity rating test or sensory profiling is intended to determine the intensity of the sensory profile of the sample against the control sample. The number of panelists is 40 trained people. Each panelist was asked to state how strong the sample intensity is on sensory attributes in all treatments on a scale used of 0-5. And the sensory attributes tested on the intensity rating

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test are uncooked odor, savory aroma, milk aroma, viscosity, and aftertaste.

2.4. *Analytical Methods*

Observational data on microbiological parameters were statistically analyzed using Analysis of Variance (ANOVA) with a statistically significant definition as $p<0.05$ at an error rate interval of 5%. and continued with Duncan's real difference test on the SPSS Statistics version 22. program. Organoleptic profile intensity data using expressed in absolute values and spider web diagram using Microsoft Office Excel 2019.

3. RESULT AND DISCUSSION

3.1. *Total Microbes*

Total microbes in samples of fresh milk, collagen supplemented milk, pretreatment temperature and time without PEF, PEF with pretreatment, PEF pasteurization without pretreatment and LTLT pasteurization can be seen in Table 2. The average total microbes in fresh milk before processing was 5.396±0, 13 log cfu/ml. the number of safe microbes in pasteurized milk is 4.0 log cfu/ml [26] so that fresh milk must be processed to reduce the total microbes.

Treatment of temperature and time of pretreatment of collagen supplemented milk showed a decrease in the total microbial count of fresh milk before processing. Pretreatment at 45°C for 20 and 30 minutes showed significantly different total microbes ($p<0.05$) compared to fresh milk. While the pretreatment temperature of 60°C for 20 and 30 minutes resulted in the highest reduction in total microbes. The average total microbes at pretreatment temperature of 60°C for 20 and 30 minutes were 3.406±0.19 log CFU/ml and 3.395±0.146 log CFU/ml, respectively. Pretreatment at 60°C for 20 and 30 minutes was able to reduce the average total microbe, respectively, up to 1.99 and 2.05 log cycles from the total microbe of fresh milk before processing.

Milk that is processed using mild heat with a temperature range of 45-60°C for a certain time is able to reduce the average number of microbes in milk but [18]. In Bacillus cereus spores, heating treatment at 30-40°C can induce the growth of inactive spores [19]. Based on research using liquid whey protein, the effectiveness of mild heating temperature with microbial death of L. innocua is less than optimal [20]. Therefore, the addition of heating pretreatment at a certain time in the PEF pasteurization process can also increase the efficiency of energy use by increasing more optimal microbial death [21].

The average total microbe in the combined PEFpretreatment and pasteurization process was lower than in the pretreatment process alone (without PEF). Milk supplemented with collagen with PEF pretreatment at 45°C for 20 and 30 minutes and PEF pretreatment at 60°C for 10 minutes (fig 1.) showed an average decrease in the total microbial count of 2.0 log cycles and statistically showed no significant difference ($p>0.05$). While the pretreatment temperature of 60°C for 20 and 30 minutes resulted in a higher average decrease in total microbes, respectively 2.520±0.4 log CFU/ml and 2.551±0.13 log CFU/ml and did not differ significantly with the pasteurization treatment. LTLT. PEF pasteurized milk with pretreatment from all the above treatments is safe for consumption because it complies with the SNI standard for pasteurized milk.

Pasteurization of PEF combined with thermal treatment resulted in a decrease in the number of pathogenic microbes by 2.5 log cycles [10]. Reduction of E.coli ATCC 11775 by heating 55oC with 30 kV PEF treatment can reduce up to 3 log cycles [14]. Likewise, the microbes S. aureus and P. fluorescent treated with PEF 40 kV and mild heating 45°C were able to inactivate both microbes an average of 5 log cycles [22].

3.2. *Microbial Reduction*

Pretreatment combination of PEF pasteurization can help increase microbial death. the processing of the PEF method is influenced by several factors, one of which is the provision of a hurdle concept or a combination of treatments such as heating temperature with a certain time and high voltage electric shock [22]. The increase in the effectiveness of microbial inactivation in the PEF and mild heat treatment processes is related to the increase in the fluidity of the cell membrane due to the influence of temperature and damage to the formation of pores in the microbial cell membrane [21]. Microbial inactivation by electric shock is caused by changes that occur in proteins and phospholipids that function in cell wall synthesis [27]. This instability is due to the high stress on the material. High stress is directly proportional to the total microbial death [28].

Tabel 1. Comparison of the total microbial mean between PEF collagen supplemented milk (with temperature treatment and pretreatment time) with fresh milk, PEF pasteurized (without pretreatment) and LTLT pasteurized

Treatment	Temp of Pretreatment (°C)	Mean of Total Microbial (Log CFU/ml)					
		Time of Pretreatment (Min)			Raw Milk	PEF Pasteurization	LTLT Pasteurization
		10	20	30			
Pretreatment	30	5.509±0.12[a]	5.420±0.31[a]	5.434±0.2[a]			
	45	5.542±0.09[a]	4.563±0.11[b]	4.511±0.12[b]			
	60	4.200±0.04[b]	3.406±0.19[c]	3.343±0.14[c]	5.396±0.13[a]	4.396±0.20[b]	2.450 ±0.23[d]
Combination of Pretreatment+PEF	30	4.633±0.26[b]	4.467±0.16[b]	4.450±0.16[b]			
	45	4.504±0.16[b]	3.417±0.21[c]	3.392±0.18[c]			
	60	3.314±0.23[c]	2.520±0.40[d]	2.551±0.37[d]			

Notes: Values accompanied by unequal letters show a significant difference = 0.05

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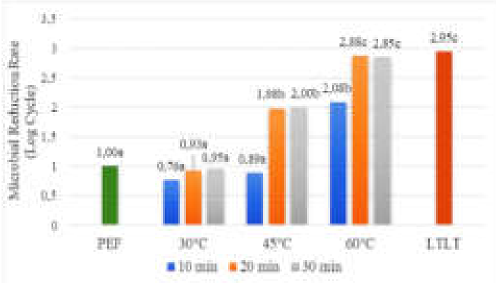

Fig 1. Comparison of the total microbial reduction rate (Log Cycle) between collagen-supplemented PEF milk (with temperature treatment and pretreatment time) with PEF pasteurized milk (without pretreatment) and LTLT pasteurized milk

PEF pasteurization method (without pretreatment) compared with the pretreatment in Fig 1. Combination of temperature and pretreatment time in the PEF process has a fairly good microbial reduction rate as shown in Fig 1. In the 60°C temperature treatment for 20 and 30 minutes the level of microbial reduction was not significantly different (P<0.05) with pasteurization using heat (thermal process) such as LTLT (65°C for 30 minutes). The combination between preheating and PEF was able to reduce microbes up to 2.5 log cycles in fresh milk at a voltage of 100 kv/cm at 75°C; 15 sec [10].

Microbial inactivation carried out by the PEF pasteurization method is associated with the stability of the cell membrane which functions to protect microbes from environmental conditions by working as a semipermeable wall [26]. Damage to cell membranes due to electrical breakdown due to pressure on the membrane by an electric voltage which causes large holes in the membrane so that the membrane cell undergoes irreversible breakdown which is also known as the electroporation phenomenon [10].

3.3. Sensory Profile Intensity Rating Test

The intensity of the PEF pretreatment compared to PEF and LTLT pasteurization on sensory attributes is presented in the form of a spider web, which can be seen in Figure 3. Based on the test results, the PEF treatment sample showed variations in the pretreatment treatment at 60°C for 10, 20, and 30 minutes and had the same average intensity profile. closest to the LTLT pasteurized sample on the sensory attributes of uncooked odor, savory aroma, milk aroma, and milk taste. The results of research conducted by treating temperature and pre-heating time can increase consumer sensory acceptance of taste, aroma, and mouthfeel and can improve the organoleptic value of collagen milk with PEF pasteurization [23]. The sweet taste profile of all samples did not show any difference with the LTLT and PEF pasteurized samples.

Oxidation and hydrolysis reactions affect the development of off-flavors in dairy products such as rancidity accompanied by an uncooked odor and tallowiness [24]. The PEF pretreatment with variations of heating at 45°C and 60°C showed a lower intensity of uncooked odor than the pretreatment with heating at 30°C because mesophyll microbes and enzyme activity created chemical changes such as smaller fat and protein in milk.

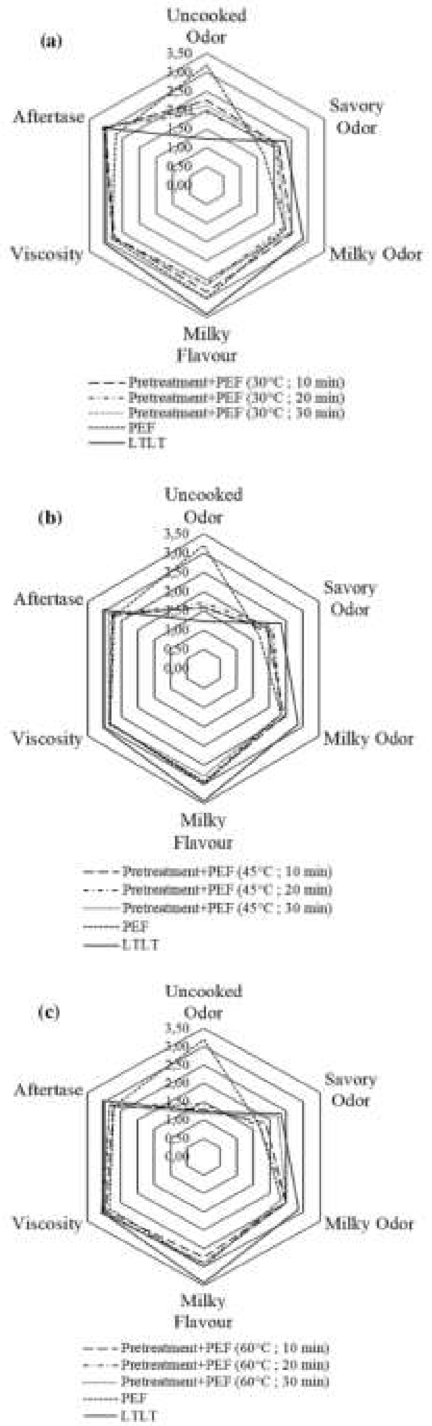

Fig 2. Sensory profile between PEF collagen supplemented milk with pretreatment temperature (a) 30°C, (b) 45°C, (c) 60°C compared to PEF pasteurized milk (without pretreatment) and LTLT pasteurized milk

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Intensity of milky odor and milky flavour showed that the 30°C treatment value was closer to the intensity of the PEF pasteurization (without pretreatment). Aroma and taste components of milk caramel were more detectable in the PEF treatment alone, while in thermal pasteurization they were not detected [13]. This is due to the presence of a 2(5H)-Furanone component which has a waxy caramel odor while in thermal treatment it gives a more distinctive milky aroma with the marked "Cooked Milk" and "Creamy" odor. The distinctive aroma of cooked milk can be detected at 45°C and 60°C treatment variations which are close to the intensity of the LTLT pasteurization profile.

Addition of collagen in this study did not have a significant effect when compared to commercial dairy products. Research shows that the addition of 2.0% collagen has a sensory profile closest to that of powdered milk without collagen. Sensory attributes that are close to the control sample are, milky odor, milky flavour, sweet taste, savory taste, and viscosity [25].

4. CONCLUSION

Pretreatment of collagen supplemented milk can help reduce total microbial and affect to intensity of sensory profile. Total number of microbes in collagen supplemented milk was the lowest in pretreatment 60°C for 20 minutes which resulted 3,406 log CFU/ml, while the combination of pretreatment and pasteurization PEF resulted in a smaller total of microbes compared to the pretreatment process alone with total microbes below SNI 3951:2018. The combination of pretreatment with PEF pasteurization was able to reduce the total microbes by 2.88 log cycles so as to produce the final microbial total in collagen-supplemented milk of 2.520±0.40 log CFU/ml. Treatment at 60°C for 10, 20 and 30 minutes had a sensory profile that was closest to LTLT pasteurization on the attributes of uncooked odor, milky odor, and milky flavour and had a better sensory profile than PEF pasteurization alone.

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