Statistical Analysis of Inference on Total Microbes in Hydrolyzed Bovine Collagen Milk with Different Pasteurization Treatments Using Pulsed Electric Field

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Abstract

Boiling raw milk and other pasteurization methods have an excellent food safety consumption and maintain the food quality. The nutrition and microbiological qualities in milk are easily damaged, thus proper handling is required. A way to handle the milk properly by considering the pasteurization method and its condition, such as temperature and time in the pasteurization process. Therefore, the purpose of this study is to determine the effect of pasteurization method using individually heating and combine with pulsed electric field using various temperature and pre-heating time on the total amount of microbiological in dairy products enriched with hydrolyzed bovine collagen and determine a proper treatment. Completely Randomized Design (CRD) was used as an experimental design with temperature variables (35 ºC, 45 ºC, 55 ºC, and 65ºC) while the pre-heating time used was (10 minutes, 20 minutes, 30 minutes, and 40 minutes). A proper treatment was obtained from pre-heating conditions with a temperature of 55ºC and a time of 30 minutes by using inference statistics method with paired T-test and resulting total plate count (TPC) of 3.30 ± 0.01 log cfu/ml, viscosity of 4.48 ± 0.08 mPa.S, and emulsion stability of 12.33 ± 1.73%. Hopefully, this research can be an innovation in the combination process of thermal and non-thermal milk enriched with hydrolyzed bovine collagen. The product result is safer due to decreasing number of microorganisms in milk.

Keywords
Paired T-test, Milk, Pre-heating, Pulsed Electric Field.

1. Introduction
Milk is one of the most important food in human life and is a source of animal protein which contains high nutritional value. The nutrient contents of milk consists of protein, fat, vitamins, and minerals that are useful for human health (Widyatama 2018). Milk is a beverage that is suitable for animals and humans due to easily digested nutrients. Apart from being a source of animal protein, milk is also very good for bacterial growth. In addition, milk is a medium for several microorganisms that can change the chemical composition of milk during storage. Pasteurization is one of the milk processing businesses by heating to maintain the quality and safety of milk. This effort is a process of eradicating pathogenic bacteria that may still be present in milk. Pasteurized milk is another form of fresh milk and as an effort to extend its durability (Resnawati 2020).

Pasteurization using thermal is one method that can reduce the population of microorganisms present in milk, but this method also reduce nutrients from milk. Therefore, proper processing of fresh cow's milk to extend the shelf

life while maintaining its quality is required. Pulsed electric field (PEF) is one of the non-thermal food processing techniques using short pulses with high-intensity electric shocks that are applied in liquid foods to maintain the quality of food (Priyanto et al. 2021). This method is one of alternative techniques to avoid greatly reduction of quality due to high temperature using heating conventional method. The advantage of this technique can maintain the original color, texture and aroma as well as the nutritional value during the preservation process (Ariantini et al. 2017). In addition, it has been reported that utilization of various temperature treatment with various heating temperatures (30, 45, and 60°C) and diverse heating time (10, 20, and 30 min) which combined using PEF reveals significant effect on the sensory properties (Esfandiar et al. 2022). PEF can also be combined with the initial heat before the electric shock is conducted which is called the initial temperature of pre-heating (Putranto et al. 2022).

The addition of hydrolized bovine collagen can improve the functional properties of food products. Hydrolyzed collagen is a food ingredient that is high in peptide content. Peptides consumed regularly can increase the inhibitory activity of angiotensin-I converting enzyme (ACE) (Priyanto et al. 2021), ant-inflammatory, anti-oxidative, antimicrobial, and anti-obesity (Marcone et al. 2017). Previous studies have been reported that processing condition has an influence on microbial population in milk. Therefore, the experiments of statistical analysis using paired samples T test is big attracting research from pasteurized milk using individually thermal and combined with PEF with various temperature and pre-heating time on the total of microbiology.

1.1 Objectives
The purpose of this study was to determine the effect of different pasteurization method, mainly individually pre-heating and combination of pre-heating be equipped with PEF on microbiological number in dairy products enriched with hydrolyzed bovine collagen. In addition, selected pasteurization was determined from both method which further investigation were various temperature and pre-heating time towards the microbial numbers.

2. Literature Review

2.1 Pasteurization
Pasteurization of milk is an act of heating (heat transfer) milk to a certain temperature and maintain a stable temperature for determined time (Prasetyo 2020). This treatment is an eliminating process of pathogenic bacteria that may still be present in milk. Simply pasteurization is conducting by heating milk in polyethylene (PE) plastic packaging using a vessel filled with water at a temperature of 75^0C. This pasteurization technique can suppress the number of microorganisms. Therefore, it could maintain the quality as well as the shelf life of milk to extend more than 8 days with keeping in the refrigerator (Resnawati 2020).

2.2 Pulsed Electric Field (PEF)
Pulsed electric field (PEF) is a non-formal food processing and preservation techniques to provide high quality foods using high-intensity electric fields (5-50 kV/cm) with short high voltage pulses (μs) (Syed et al. 2017). This technique is applied to semi-solid or liquid foods to inactivate the contamination of microorganism with the principal of electroporation process on the cell membrane. The advantage of PEF can prevent the degradation of nutrient content, color, texture, aroma during the treatment process (Ariantini et al. 2017). In addition, PEF is one of non-thermal processing methods that can be applied to kill microorganisms on fresh milk (Putranto, A.W et al. 2022). The working principle of PEF is an application of electric shock to the material by placing a sample among two electrodes and is based on electroporation in cell membrane due to electrical conductivity from high voltage applied to the material (Nazrun et al. 2021).

2.3 Inference Statistics
Inferential statistics is a method that deals with the analysis of some data to then arrive at forecasting or drawing conclusions about the entire parent data set (Eman et al. 2019). Paired t-test (paired t-test) is one method of testing the hypothesis in which the data used are not independent (pairs) (Farida et al. 2022). The characteristics that are most often found in paired cases are that one individual (object of research) gets 2 different treatments. Even though using the same individual, the researcher still obtained 2 kinds of sample data, namely data from the first treatment and data from the second treatment (Montolalu and Langi 2018). One Way Anova is an analysis that involves only one independent variable (Riadi et al. 2020). In detail, One Way Anova is used in a study which has the characteristics of involving only one independent variable with two or more categories selected and determined by the researcher not randomly. The selected category is called non-random because the researcher does not intend to

generalize the results to other categories other than those studied on that variable. For example, the gender variable only consists of two categories (male and female) (Ibnu and Freddy 2020).

3. Methods

The data collection method used is quantitative method, which in its collection emphasizes causality and the variables are arranged in statistical form. The data used in this study, namely the Paired Sample T-Test test using TPC milk collagen data, there is pasteurization time and pre-heating temperature before and after being given PEF. While the One-Way Anova test also uses TPC data for collagen milk, there are pasteurization times and pre-heating temperatures before and after being given PEF with different numbers. The observed variables are the pre-heating time and temperature which will produce data obtained from the study, then the data will be analyzed using statistical inference which consists of two tests, namely the Paired Sample T-Test test and the One-Way Anova test to determine the best treatment for total or microbiological counts in dairy products enriched with hydrolized bovine collagen.

4. Data Collection

Provision of pre-heating temperature was able to significantly reduce the number of pathogenic microorganisms in fresh milk and collagen milk. The fresh milk ingredients used in this study had a TPC value of 5.267 ± 0.017 log cfu/ml. this value is still included in the maximum TPC value of fresh cow's milk according to SNI 01-3141-1998 which is 6.00 log cfu/ml. In this study, the TPC value of collagen milk due to variations in the preheating temperature treatment and heating time was between 5.255 ± 0.000 log cfu/ml to $2,488 \pm 0.005$ log cfu/m. The following is the TPC value of collagen milk (log cfu/ml) against the pre-heating time and temperature obtained before and after being given Pulsed Electric Field (PEF).

It is known that the TPC value of collagen milk (log cfu/ml) against the time and temperature of pre-heating is at 35^0C with a time of 10 minutes resulting in the number of microbes as much as 5.255 ± 0.000 in Pre-heating and as much as 4.498 ± 0.010 at Pre-heating + PEF, with 20 minutes resulted in the number of microbes as much as $4,914 \pm 0.008$ on Pre-heating and as much as 4.484 ± 0.717 on Pre-heating + PEF, with a time of 30 minutes the number of microbes as much as $44,736 \pm 0.006$ on Pre-heating and as much as 4.736 ± 0.006 on Pre-heating + PEF, and with a time of 40 minutes the number of microbes as much as 4.615 ± 0.004 on Pre-heating and as much as 4.371 ± 0.013 on Pre-heating + PEF. At a temperature of 45^0C with a time of 10 minutes the number of microbes as much as $5,218 \pm 0.019$ on Pre-heating and as much as 4.455 ± 0.718 on Pre-heating + PEF, with a time of 20 minutes the number of microbes as much as $4,895 \pm 0.004$ on Pre-heating and as much as 4.423 ± 0.012 on Pre-heating + PEF, with a time of 30 minutes the number of microbes as much as $4,568 \pm 0.215$ on Pre-heating and as much as 4.371 ± 0.013 on Pre-heating + PEF, and with a time of 40 minutes the number of microbes as much as $4,544 \pm 0.018$ on Pre-heating and as much as $4,322 \pm 0.029$ on Pre-heating + PEF. At a temperature of 55^0C with a time of 10 minutes the number of microbes as much as $5,130 \pm 0.023$ on Pre-heating and as much as 4.423 ± 0.012 on Pre-heating + PEF, with a time of 20 minutes the number of microbes as much as $4,874 \pm 0.492$ on Pre-heating and as much as $4,369 \pm 0.011$ on Pre-heating + PEF, with a time of 30 minutes the number of microbes as much as $4,541 \pm 0.004$ on Pre-heating and as much as 3.299 ± 0.003 on Pre-heating + PEF, and with a time of 40 minutes the number of microbes as much as $4,525 \pm 0.009$ on Pre-heating and as much as 4.272 ± 0.010 on Pre-heating + PEF. While at a temperature of 65^0C with a time of 10 minutes the number of microbes as much as $4,985 \pm 0.003$ on Pre-heating and as much as 4.312 ± 0.015 on Pre-heating + PEF, with a time of 20 minutes the number of microbes as much as $4,803 \pm 0.005$ on Pre-heating and as much as $4,243 \pm 0.018$ in Pre-heating + PEF, with a time of 30 minutes resulted in the number of microbes as much as 4.455 ± 0.011 in Pre-heating and as much as $3,498 \pm 0.010$ in Pre-heating + PEF, and with a time of 40 minutes the number of microbes was $3,512 \pm 0.009$ in Pre-heating. heating and as much as $2,488 \pm 0.005$ on Pre-heating + PEF.

Based on the results of statistical analysis, there was a significant difference between the preheating treatment with preheating and PEF on variations in temperature and preheating time as indicated by the notation next to the TPC value. The higher the heating temperature, the lower the total microbial value in collagen milk. This is because pathogenic microbes when in milk are enclosed in protein compounds. Therefore, giving the preheating temperature in this study also aims to remove protein compounds that protect the microbial cell membrane in milk.

5. Result and Discussion

5.1 Numerical Result
a. Paired Sample T-Test

The research was conducted to determine the effect of pre-heating time and temperature on the total or amount of microbiology in dairy products. There are two different types of use, namely pre-heating without Pulsed Electric Field and pre-heating with added Pulsed Electric Field. The study used 4 samples and produced 32 data with a time of 10 to 40 minutes and temperatures (35^0C, 45^0C, 55^0C, dan 65^0C). It can be seen that protein compounds are denatured above 50°C, so the process of electrophoration and breakdown of microbial cell membranes will occur quickly when the cell membrane is not covered by protein. However, the provision of this temperature must be below the denaturation temperature of several important compounds in milk such as vitamins, antioxidants and so on, namely a maximum temperature of 70°C. This is so that the process of giving preheating remains constant.

b. One Way Anova

Research was also conducted to determine the effect of pre-heating time and temperature on the total or amount of microbiology in dairy products both before being given a Pulsed Electric Field and after being given a Pulsed Electric Field. The study used 4 samples and produced 32 data with the time used ranging from 10 minutes to 40 minutes and the temperatures were (35^0C, 45^0C, 55^0C, dan 65^0C), with a different calculation mechanism. It can be seen that the comparison of the value between pre-heating without PEF and pre-heating added PEF has a different value with a slight difference and also with a large difference. Similarly, the provision of this temperature must also be below the denaturation temperature of several important compounds in milk such as vitamins, antioxidants and so on, namely a maximum temperature of 70°C. This is so that the process of giving preheating remains constant. (Figure 1)
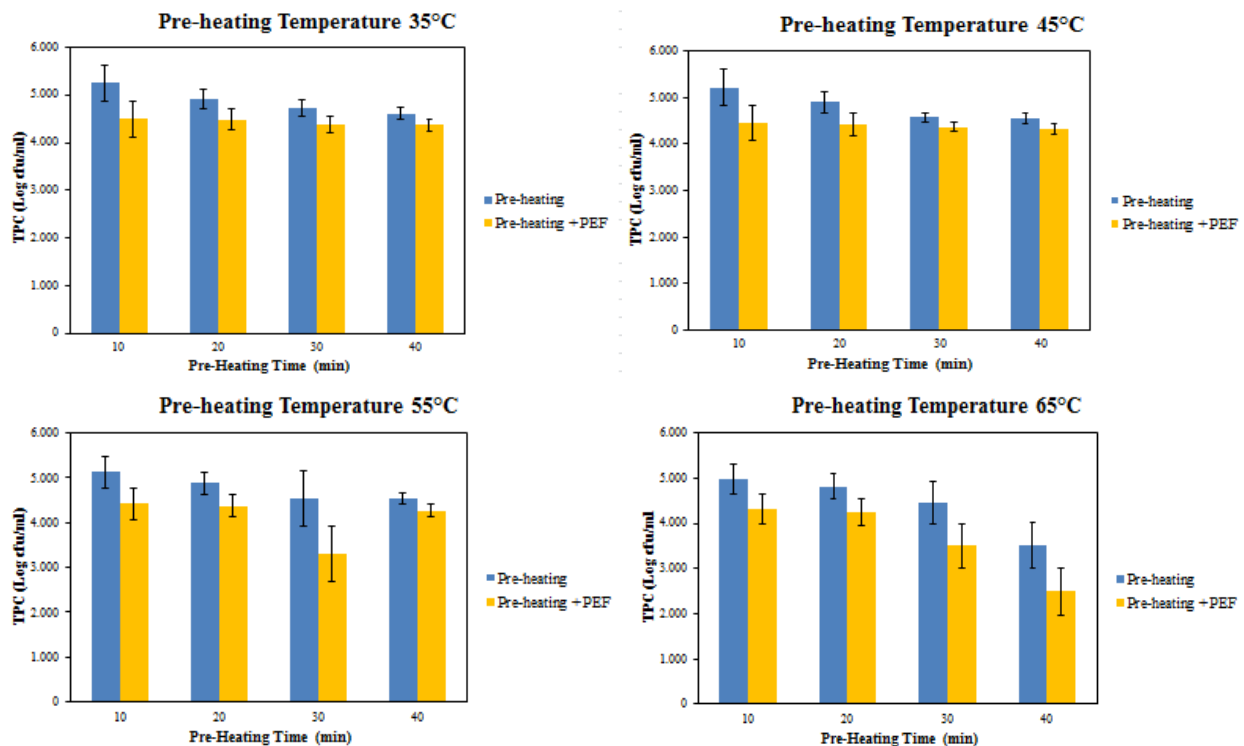

Figure 1. Bar Chart Of TPC Milk Collagen Value (Log Cfu/Ml) Against Time And Temperature

5.2 Graphical Results

Below is the calculation output using the Paired Sample T-Test method and with the help of the Minitab software. (Figure 2)

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Pre-Heating 35	4	4,880	0,279	0,139
Pre-Heating+PEF 35	4	4,437	0,064	0,032

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0,443	0,223	0,111	(0,089; 0,797)

μ_difference: mean of (Pre-Heating 35 - Pre-Heating+PEF 35)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
3,98	0,028

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Pre-Heating 55	4	4,768	0,290	0,145
Pre-Heating+PEF 55	4	4,091	0,532	0,266

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0,677	0,420	0,210	(0,008; 1,345)

μ_difference: mean of (Pre-Heating 55 - Pre-Heating+PEF 55)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
3,22	0,049

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Pre-Heating 45	4	4,806	0,318	0,159
Pre-Heating+PEF 45	4	4,393	0,059	0,029

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0,413	0,264	0,132	(-0,007; 0,834)

μ_difference: mean of (Pre-Heating 45 - Pre-Heating+PEF 45)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
3,13	0,052

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
Pre-Heating 65	4	4,439	0,656	0,328
Pre-Heating+PEF 65	4	3,635	0,849	0,424

Estimation for Paired Difference

Mean	StDev	SE Mean	95% CI for μ_difference
0,803	0,222	0,111	(0,449; 1,158)

μ_difference: mean of (Pre-Heating 65 - Pre-Heating+PEF 65)

Test

Null hypothesis	H_0: μ_difference = 0
Alternative hypothesis	H_1: μ_difference ≠ 0

T-Value	P-Value
7,22	0,005

Figure 2. Value of Paired Sample T-Test

From the output of the Paired Sample T-Test above, it was found that the mean, standard deviation, and mean SE of the pre-heating and pre-heating + PEF data at a temperature of 35^0C were 0,443 ; 0,223 and 0,111 with a T-Value of 3.98 and a P-Value of 0.028 . The mean, standard deviation, and mean SE of the pre-heating and pre-heating + PEF + PEF data at a temperature of 45^0C are 0,413 ; 0,264 and 0,132 with a T-Value of 3.13 and a P-Value of 0.052. The mean, standard deviation, and mean SE of the pre-heating and pre-heating + PEF data at a temperature of 55^0C are 0,677 ; 0,420 and 0,210 with a T-Value of 3.22 and a P-Value of 0.049. Meanwhile, the mean, standard deviation, and mean SE of the pre-heating and pre-heating + PEF data at a temperature of 65^0C are 0,803 ; 0,222 and 0,111 with a T-Value of 7.22 and a P-Value of 0.005.

Below is the calculation output using the One Way Anova method and with the help of the Minitab software. (Figure 3)

```
Factor  Levels  Values                        Factor Information
Factor       2  Pre-heating, Pre-heating + PEF
                                              Factor  Levels  Values
                                              Factor       2  Pre-heating, Pre-heating + PEF
Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value  Analysis of Variance
Factor   1  0.3929  0.39294     9.63    0.021
Error    6  0.2449  0.04081                    Source  DF  Adj SS   Adj MS  F-Value  P-Value
Total    7  0.6378                             Factor   1  0.3420  0.34196     6.55    0.043
                                               Error    6  0.3132  0.05220
                                               Total    7  0.6552
Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)       Model Summary
0.202022  61.61%     55.21%      31.75%
                                                     S    R-sq  R-sq(adj)  R-sq(pred)
                                               0.228481  52.19%     44.23%      15.01%
Means

Factor              N    Mean   StDev      95% CI     Means
Pre-heating         4   4.880   0.279  ( 4.633,  5.127)
Pre-heating + PEF   4  4.4368  0.0636  (4.1896, 4.6839)  Factor              N    Mean   StDev      95% CI
                                                          Pre-heating         4   4.806   0.318  ( 4.527,  5.086)
                                                          Pre-heating + PEF   4  4.3928  0.0585  (4.1132, 4.6723)
Pooled StDev = 0.202022
                                                          Pooled StDev = 0.228481


Factor Information                             Factor Information

Factor  Levels  Values                         Factor  Levels  Values
Factor       2  Pre-heating, Pre-heating + PEF Factor       2  Pre-heating, Pre-heating + PEF


Analysis of Variance                           Analysis of Variance

Source  DF  Adj SS   Adj MS  F-Value  P-Value  Source  DF  Adj SS   Adj MS  F-Value  P-Value
Factor   1  0.9160   0.9160     4.99    0.067   Factor   1   1.291   1.2912     2.24    0.185
Error    6  1.1004   0.1834                     Error    6   3.453   0.5754
Total    7  2.0164                              Total    7   4.744


Model Summary                                  Model Summary

      S    R-sq  R-sq(adj)  R-sq(pred)               S    R-sq  R-sq(adj)  R-sq(pred)
0.428250  45.43%     36.33%       2.98%         0.758570  27.22%     15.09%       0.00%


Means                                          Means

Factor              N   Mean  StDev      95% CI     Factor              N   Mean  StDev      95% CI
Pre-heating         4  4.768  0.290  (4.244, 5.291) Pre-heating         4  4.439  0.656  (3.511, 5.367)
Pre-heating + PEF   4  4.091  0.532  (3.567, 4.615) Pre-heating + PEF   4  3.635  0.849  (2.707, 4.563)

Pooled StDev = 0.428250                         Pooled StDev = 0.758570
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Figure 3. Value of One Way Anova

From the output of the One way Anova above, it was found that at a temperature of 35^0C the mean and standard deviation values from the pre-heating data were 4.4880 and 0.279 while the pre-heating + PEF were 4.4368 and 0.0636 with an F-Value of 9.63 and P-Value 0.021. At a temperature of 45^0C the mean and standard deviation values from the pre-heating data were 4.806 and 0.318 while the pre-heating + PEF were 4.3928 and 0.0585 with F-Value of 6.55 and P-Value of 0.043. At a temperature of 55^0C the mean and standard deviation values from the pre-heating data were 4.768 and 0.290 while the pre-heating + PEF were 4.091 and 0.532 with F-Value of 4.99 and P-Value of 0.067. At a temperature of 65^0C the mean and standard deviation values from the pre-heating data were 4.439 and 0.656 while the pre-heating + PEF were 3.635 and 0.849 with an F-Value of 2.24 and a P-Value of 0.185.

5.3 Proposed Improvements
The proposed improvement in this research is in the pasteurization process by using graphs or plots to make it easier to determine a good temperature. (Figure 4)
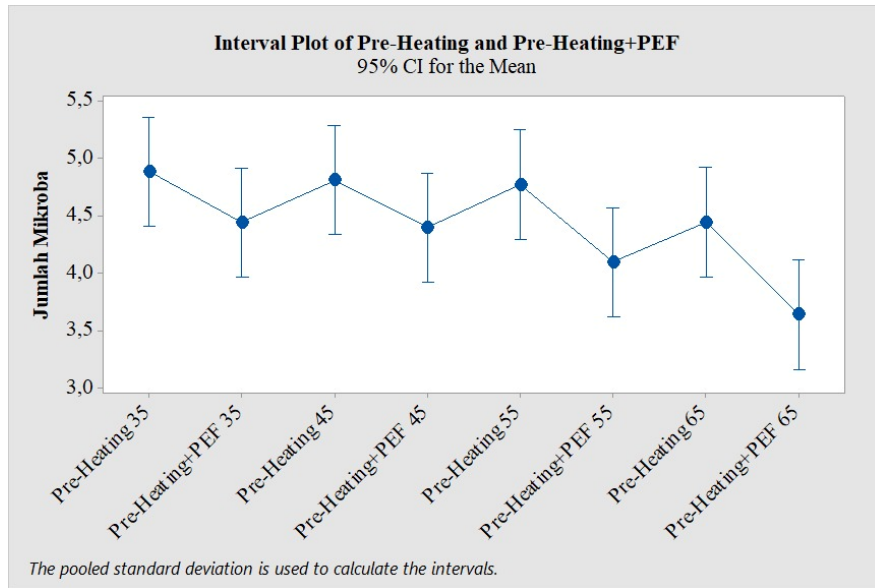
Figure 4. Value of One Way Anova

From the pre-heating plot interval above, data obtained with a temperature of 35^0C of 4800 and pre-heating +PEF of 4400. Pre-heating with a temperature of 45^0C of 4800 and pre-heating +PEF of 4400. Pre-heating with a temperature of 55^0C of 4800 and pre-heating of 4800 -heating+PEF of 4200. Pre-heating with a temperature of 65^0C of 4400 and pre-heating+PEF of 3200.

5.4 Validation

Paired Sample T-Test on pre-heating and pre-heating+PEF data at 35^0C obtained a mean value of 443, a standard deviation value of 223, and a mean SE value of 111. While the Paired Sample T-Test test on pre-heated data -heating and pre-heating+PEF at a temperature of 45^0C, the mean value is 414, the standard deviation value is 264, and the mean SE value is 132. Also, the T-count value < T-table = 0.052 < 3.98 so H0 is rejected. and P value < 0.05 = 0.028 < 0.05 so that H0 is also rejected, which means that there is a significant difference in the mean of pre-heating and pre-heating+PEF data with temperatures of 35^0C and 45^0C, so it can be illustrated through the graph below. (Figure 5)
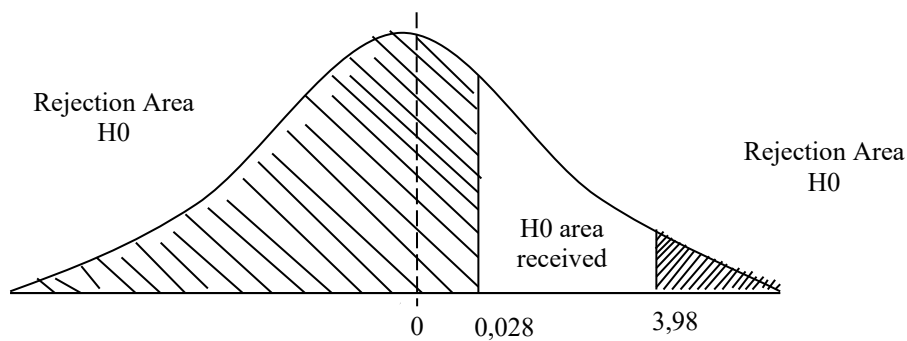

Figure 5. Graph of Paired Sample T-Test

For the One Way Anova test, it is found that the pre-heating plot interval with a temperature of 55^0C is 4800 and pre-heating+PEF is 4400. Pre-heating with a temperature of 650C is 4800 and pre-heating+PEF is 4400. Pre-heating with a temperature of 550C is 4800 and pre-heating+PEF of 4200. Pre-heating with a temperature of 65^0C was 4400 and pre-heating+PEF of 3200. In addition, the value of F-table <F-count = 0.049 < 3.22 so that H0 was rejected. and P value < 0.05 = 0.005 < 0.05 so that H0 is also rejected, which means that there is a difference in the

average data of pre-heating and pre-heating+PEF with temperatures of 55^0C and 65^0C, so that it can be illustrated through the graph below. (Figure 6)
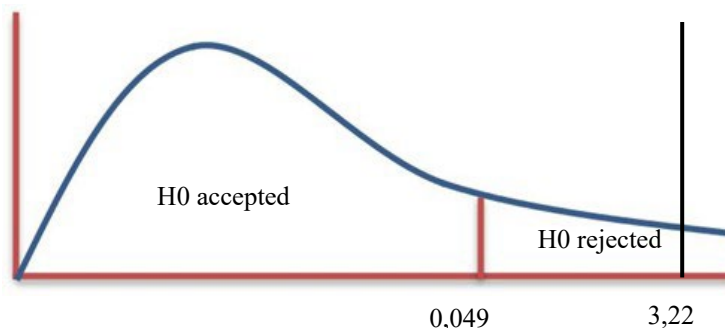

Figure 6. Graph of One Way Anova

6. Conclusion

From the research data, it can be concluded that the use of minitab software is very helpful in calculating inferential statistics accurately and quickly. In this case it includes Paired Sample T-Test and One Way Anova. These calculations are presented in several outputs showing that milk enriched with hydrolized bovine collagen which was given pre-heating treatment at 55^0C for 30 minutes followed by pasteurization using PEF was the best treatment. It is evident from H0 which is received more than the others. The hope of this research is to become literate for the processing of electric shock pasteurization, especially in dairy products enriched with good and correct hydrolized bovine collagen.

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