Offline quality control for optimization of pasteurized milk production parameters

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Abstract. One of the superior livestock products with high nutritional composition is milk. Food safety must be ensured to maintain milk quality. However, milk quality can change if bacterial levels are not maintained. There are total plate count levels that often exceed standardized levels in milk production. As a case study, one community cooperative producing pasteurized milk find that the total plate count was exceeded the required standard of 3 × 10⁴ CFU/mL. The method proposed in this study is the Taguchi experimental design, an offline quality control monitoring method, with orthogonal array L₉(2⁷). There are 7 parameters used as control factors in this study, such as storage temperature and cooling process. The results showed the combination of each parameter to get the most optimal level of total plate count in pasteurized milk. In addition to improving food safety, it is hoped that this study can improve good health and well beings, as well as improve economic prosperity and local livelihoods.

1. Introduction
Consumers are increasingly critical in selecting, assessing and determining the products to be purchased. It is the reason for an industry to win the market competition to meet the needs and desires of consumers [1, 2]. One product that has a high potential demand is a product produced by the livestock sector, particularly milk. The high need for milk makes milk consumption experience significant growth every year [3]. However, until now the existing population of dairy cattle and milk production is not enough to supply the needs of the national milk, causing milk imports [4].

Currently the production of fresh processed milk, either in the form of pasteurized milk and ultra high temperature (UHT) is still a little produced. The milk processing industry prefers to increase the production of processed milk products compared to fresh processed products because the high protein, glucose, lipid, mineral and vitamin content in fresh milk makes bacteria easy to grow and develop [5, 6]. The high number of bacteria in the fresh milk can lead to decreased quality and expiration process becomes fast so make consumers think twice if they want to buy fresh milk [7].

Availability of milk should be considered to meet the nutritional adequacy level for the community. In fulfilling the availability of milk must be accompanied by efforts to improve the quality and safety of products, because no matter how high nutritional value of a food will not matter if the food is not good for health [8]. Quality and nutritious milk for consumers is one that meets food safety requirements. Microbiological quality of a food product is determined by the amount and type of microbes contained in a food. This microbiological quality will determine the shelf life of a product that can be evaluated from microbial damage and product safety as determined by the pathogenic species [9].
One of the milk processing industries is cooperatives that connect farmers and large industries. The cooperative does not have a private farm but cooperates with farmers who are scattered in several surrounding areas with large industries as their consumers [10]. Based on preliminary studies, the problem faced by cooperatives is the failure of milk production which has a negative impact on the cooperative. The condition of fresh milk and milk from healthy cows does not guarantee it is safe for consumption.

In processing milk it also does not guarantee milk is not contaminated with microbes [11, 12]. One of the milk processing that is often done is the pasteurization process. This process has the aim of slowing down microbes and killing harmful organisms such as bacteria, protozoa, molds, and yeasts [13]. However, there are some microbes are likely still growing in pasteurized milk [14, 15]. Parameters that can be used to measure the concentration of microbes in milk products is the level of total plate count (TPC) [16, 17]. The maximum permissible TPC content according to the provisions of the Indonesian National Standard (SNI) of $3 \times 10^4$ CFU/mL [18]. Excessive levels of microbial contamination can affect the quality of milk because it is contaminated with bacteria, so the milk cannot be sold to consumers. Several factors in the process of pasteurized milk production are thought to affect TPC levels.

Therefore, this study was conducted with the aim to control the quality of pasteurized milk through experiments by determining the factors that can influence TPC levels. The purpose of the experiment is to understand how to reduce and control the variability of a product or process, then determine the parameters that affect the performance of a product or process [19, 20]. The approach that can be used in quality control is off-line and online. In this study, off-line quality control was conducted because it identifies the source of variations and determines the optimal design and process through experiments. One method that is included in the offline quality control and efficient to use is the Taguchi Method [21, 22].

2. Research Method
Standardized milk is milk that meets the standards of local content production [23]. Standardized milk subsequently processed in the pasteurization process. Pasteurization is the process of heating raw milk to destroy pathogenic bacteria and microorganisms that are harmful [24]. Total plate count (TPC) is a method for calculating the total negative microorganisms contained in milk by counting colonies of microorganisms that are grown on agar nutrient media. TPC levels pasteurized cow's milk maximum by SNI is $3 \times 10^4$ CFU/mL.

This study used an experimental method that provides treatment for observed objects to look for the effect of certain treatments on others under controlled conditions. This study aims to determine the control factors that influence the quality of pasteurized milk based on TPC levels. In addition, this study is also conducted to determine the optimal combination of factor parameters and the level of pasteurized milk factors.

2.1. Total Plate Count
Milk quality refers to the level of various contaminants in the milk, be it bacteria, chemicals or other contaminants that can be detected. In many South and East Asian countries, the term milk quality includes milk composition, hygiene and adulteration [25]. There are several measures of contamination of milk after production, transport and storage, for example Total Plate Count (TPC). TPC is measured in millions of colony-forming units per milliliter of milk (M/mL). Moran (2005) states that milk produced in Indonesia has a very high TPC level [26].

The TPC measures the level of bacterial contamination that occurs after milking until it reaches a milk production. This is one of the most commonly performed milk hygiene tests in the world [27]. A small sample of milk is taken and placed in an agar growth medium. This mixture is then incubated for several days and the number of bacterial colonies that grows is counted, then the name plates are counted [28]. The greater the level of contamination, the worse the quality of the milk is stored, and the less farmers are paid for the milk.
The TPC test is generally measured in a laboratory. In all laboratories, samples are enumerated using TPC with varying methods such as conventional pour plate or rapid methods. Although laboratories use different methods of TPC analysis, they are identical standard methods validated by the Association of Official Analytical Chemists (AOAC). Therefore, there is no big deal on the reliability of the results obtained from different laboratories [29]. In this study, TPC test on pasteurized milk was conducted at the Testing Laboratory of Food Quality and Food Safety, Brawijaya University. The number (N) of CFU/mL of a sample is calculated according to Eq.1 [30].

\[ N = \frac{C}{v(n_1+0.1n_2)d} \]  

Where N is number of colonies per milliliter or gram of product; C is the sum of colonies on all plates counted; v is the volume applied to each plate; n₁ is the number of plates counted at the first/lower dilution; n₂ is the number of plates counted at the second/next higher dilution; and d is the dilution from which the first count was obtained.

2.2. Experiment
The design of experiment is a simultaneous evaluation of two or more factors (parameters) on its ability to influence the average or variability of the combined results of certain product or process characteristics [31]. Sampling is obtained from fresh milk that has been pasteurized to be tested in the laboratory before shipping to consumers. From the results of samples that have been prepared, an analysis is performed using the Taguchi method to determine the factors and levels of factors that affect the quality of pasteurized milk.

2.3. Taguchi Method
Taguchi method is one method that aims to improve the quality of products and processes offline. Development of quality starts at the beginning and designing products or processes to proceed in the production phase [32]. The objective of this method is to make the product robust against noise, because it is often referred to as a robust design. This method is also developed in a scale called the signal to noise ratio (S/N) [33].

Taguchi distinguish three design-related processes during the production process, namely: system design, parameter design and tolerance design [34]. The system design stage requires in-depth knowledge of the system to be designed. The purpose of parameter design is to determine the nominal value of the optimal product or process parameter. Tolerance design aims to determine the nominal tolerance that has been determined in the design parameters. There are several parameters involved in an experiment, namely noise, control, signal, and scale factors [35].

2.4. Experimental Design
The study begins by identifying the factors that influence the milk quality based on field and literature studies. The next step is grouping influential factors into control, noise and signal factors. Moreover, it also determines the response variable in the study that is a variable whose change depends on other variables [36]. The response variable in this study is the total plate count (TPC) level in pasteurized milk.

The quality characteristics used are smaller the better. This characteristic is measured non-negative, which has an ideal condition or a zero target value (0). Quality characteristics are determined based on the parameters of the Indonesian National Standard regarding TPC levels, namely SNI 01-3951-1995. The maximum TPC level according to SNI is \(3 \times 10^4\) CFU/mL. The lower the value, the better the quality of milk, so that the optimal level setting will be obtained from the results of this study.

Table 1 is the selected factors and level factors [37-40]. There are seven factors and two factor levels for each factor. An example is the storage temperature factor with a factor level of 5°C and 10°C. Previous studies explain the storage at low temperatures in milk aims to restrain the growth of milk-
destroying microbes, so that milk does not quickly break down in a short time. Furthermore, selected factors and levels of factors will be combined to improve the quality of optimal pasteurized milk as specified. The combination is performed according to the appropriate orthogonal array matrix.

The degree of freedom from the factors in this study is 7, so the experiment will be conducted at least 7 experiments. In Taguchi experiments with level 2 there are only orthogonal arrays of L8(2^3), L8(2^7), etc. In this study the number L8(2^3) was chosen which means there were 8 experiments with 7 factors and 2 factor levels [41]. In addition, the replication is performed 3 times because of the number of samples that suggested as many as 20-25 times or the size of the subgroup with a value of 3-5 [42]. The total number of experiments to be performed in this experiment is 24 times.

Analysis of the results of experiments conducted with statistically using analysis of variance (ANOVA) [43]. There are 7 hypotheses were tested for all factors. One of them is for storage temperature factors as follows:

H_0: There is no effect of difference due to factor A (storage temperature) on TPC levels of pasteurized milk.

H_1: There is a difference effect due to factor A (storage temperature) on TPC levels in pasteurized milk.

In addition, this study also calculates the signal to noise ratio (SNR), determines the optimal level setting of each factor, and validates experimental results with confirmation experiments.

| Code | Factors                                      | Factor Levels          |
|------|---------------------------------------------|------------------------|
| A    | Storage temperature                         | 5°C 10°C               |
| B    | Cooling process                             | Acceleration Room temperature |
| C    | Storage time                                | 3 days 2 days          |
| D    | Pasteurization temperature                  | 72°C 75°C              |
| E    | Storage packaging                           | Vacuum standing pouch Plastic bottles |
| F    | Milking method                              | Manual Milking machines |
| G    | Increased temperature of pasteurization media | 90°C 80°C             |

3. Results and discussion

The experimental results are the result of a laboratory test TPC levels that can be seen in Table 2. Experiments performed 8 times with 3 replication, so the total sample is 24 samples. The next stage is to analyze by ANOVA.

ANOVA is a method used to determine the optimal level setting in minimizing variance deviations. ANOVA is a quantitative calculation technique for determining and estimating contributions to factors in all measurement responses. ANOVA calculation for the mean value is conducted in order to find out the factors that significantly influence or not in the process of pasteurized milk production. Eq. 2 is used to get the F ratio value in the ANOVA calculation.

\[
FRatio_A = \frac{MS_A}{MS_e}
\]

Table 3 shows that A, B, C, D, F, and G have a significant influence on TPC levels in pasteurized milk. It is shown from the F-Ratio > F-Table (F_{0.05; 1; 16} = 4.49. In contrast, factor E (storage packaging) has a value of F-Ratio ≤ F-Table (F_{0.05; 1; 16} = 4.49. This shows that factor E has no significant effect on TPC levels in pasteurized milk. For value% ratio (percent contribution) showed that it had a per cent the largest contribution of a factor F, method of milking, with the value of the contribution of 32.238%, while those with levels per cent contribution to its lowest E factor, packaging storage, amounting to 0.062%.
Table 2. Experiment Results for TPC Level in Pasteurized Milk (1 × 10³ CFU/mL).

| Experiment | Factors and Levels | Replications | Mean   |
|------------|--------------------|--------------|--------|
|            | A      | B      | C      | D      | E      | F      | G      | 1     | 2     | 3     |         |
| 1          | 1      | 1      | 1      | 1      | 1      | 1      | 0.973 | 0.98  | 0.90  | 0.951 |
| 2          | 1      | 1      | 1      | 1      | 1      | 2      | 0.74  | 0.75  | 0.76  | 0.750 |
| 3          | 1      | 2      | 2      | 1      | 1      | 2      | 0.6   | 0.65  | 0.675 | 0.642 |
| 4          | 1      | 2      | 2      | 2      | 1      | 1      | 1.15  | 1.2   | 1.21  | 1.187 |
| 5          | 2      | 1      | 2      | 1      | 2      | 1      | 0.75  | 0.75  | 0.75  | 0.750 |
| 6          | 2      | 1      | 2      | 2      | 1      | 2      | 0.83  | 0.81  | 0.80  | 0.813 |
| 7          | 2      | 2      | 1      | 1      | 2      | 2      | 1     | 1     | 1     | 1     |
| 8          | 2      | 2      | 1      | 2      | 1      | 1      | 2     | 1.31  | 1.35  | 1.37  | 1.343 |

Table 3. ANOVA Results for Means of TPC Levels in Pasteurized Milk

| Source    | SS      | DF | MS    | F Ratio | SS'   | Rho% | F tabel 0.05 (1:16) |
|-----------|---------|----|-------|---------|-------|------|-------------------|
| A         | 0.053   | 1  | 0.053 | 74.658  | 0.053 | 4.310% | 4.49              |
| B         | 0.309   | 1  | 0.309 | 431.676 | 0.308 | 25.199% | 4.49              |
| C         | 0.160   | 1  | 0.160 | 223.361 | 0.159 | 13.011% | 4.49              |
| D         | 0.211   | 1  | 0.211 | 295.473 | 0.211 | 17.230% | 4.49              |
| E         | 0.001   | 1  | 0.001 | 2.059   | 0.001 | 0.062%  | 4.49              |
| F         | 0.395   | 1  | 0.395 | 551.974 | 0.394 | 32.238% | 4.49              |
| G         | 0.081   | 1  | 0.081 | 113.866 | 0.081 | 6.604%  | 4.49              |
| Error     | 0.011442| 16 | 0.000715| 1     | 0.016 | 1.346% |                   |
| SST       | 1.222   | 23 |       | 1.222  | 100%  |        |                   |
| SS Mean   | 20.735  | 1  |       |        |       |        |                   |
| SS Total  | 21.958  | 24 |       |        |       |        |                   |

Seven factors show the results that factors A, E, and G have the lowest F-ratio value compared to other factors, so that pooling is done for these factors. If the percent contribution of error is low (less than 50%) it can be concluded that there is no significant factor that is missing from an experiment. However, if the percent contribution of error is high (50% or more) it can be concluded that several factors, including significant has been lost and there is also a calculation can be concluded that having an error in the experiment [44]. Based on Table 4 the results obtained value that there are no significant factors missing from the experiment because the value of the percentage of error contributions is low with the results of the calculation of pooling that is equal to 14.382%.

Table 4. ANOVA Pooling Means for TPC Levels in Pasteurized Milk

| Source | Pooled SS | DF | MS    | F Ratio | SS'   | Rho% | F tabel |
|--------|-----------|----|-------|---------|-------|------|---------|
| A      | 0.053     | 1  | -     | -       | -     | -    | -       |
| B      | 0.309     | 1  | 0.309 | 39.702  | 0.301 | 24.622% | 4.38   |
| C      | 0.160     | 1  | 0.160 | 20.543  | 0.152 | 12.433% | 4.38   |
| D      | 0.211     | 1  | 0.211 | 27.175  | 0.204 | 16.652% | 4.38   |
| E      | 0.001     | 1  | -     | -       | -     | -    | -       |
| F      | 0.395     | 1  | 0.395 | 50.766  | 0.387 | 31.661% | 4.38   |
| G      | 0.081     | 1  | -     | -       | -     | -    | -       |
| Error  | 0.011442  | 16 | -     | -       | -     | -    | -       |
| Pooled e | 1.222  | 19 | 0.00777| 1     | 0.179 | 14.632% |       |
| SST    | 20.735    | 23 |       | 1.222  | 100%  |      |         |
| SS Mean| 21.958    | 1  |       |        |       |      |         |
| SS Total| 0.053  | 24 |       |        |       |      |         |
In the above hypothesis, it can be seen that the factors B, C, D and F have a value of F ratio ≥ F-Table F 0.05 (1; 19) = 4.38. So it can be said that B, C, D, and F have an influence on the TPC levels of pasteurized milk. The pooling up results also showed, that the percent contribution to B, C, D and F are 24.622%, 12.433%, 16.652% and 31.666%, respectively. So it can be concluded that the four factors affect the TPC levels of pasteurized milk.

Furthermore, the signal to noise (SNR) ratio analysis is performed by involving many factors to compare the mean (signal) with the standard deviation (noise). By using this SNR calculation, it is known what gives effect to the value of variance in experiments. The SNR conducted in this study is smaller the better which means the smaller the better the value. Eq. 3 is the formula for calculating SNR.

$$\eta = -10 \log_{10} [\sigma^2 + \bar{y}^2]$$

(3)

The ANOVA for SNR values as shown in Table 6. The ANOVA results for SNR show that the most influential factor with the biggest contribution is the F factor and followed by Factors C, B, and D. Furthermore, the calculation of the contribution percentage shows that percent contribution of pooled error is 25.465%. That means there is no significant factor missing from the experiment and means important factors in the experiment involved in designing robust design.

Two calculations have been made as a goal to determine the optimal level setting. Based on the calculation of the mean value and the SNR, the data showed equally good results. Factor B, D, and F provide influence and a major contribution from this experiment. This is because they have an average value and variance that can be seen from the average ANOVA table where the F Ratio value is greater than the F table value and has a high percent contribution value in the SNR calculation. In contrast to factors A, C, G. They make small influences and contributions in experiments. The E factor does not even have a very small effect or contribution.

The combination of levels selected is based on the response table of the mean value and the SNR where selected gives the smallest value. Results are due in this experiment using a characteristic value smaller the better, so we get a combination of factors and factor levels accordingly. The selected level combinations are A1, B1, C2, D1, E2, F2, and G2, which are 5°C storage temperature; cooling process with acceleration; storage time 2 days; pasteurization temperature of 72°C; plastic bottle storage packaging; milking method using Milking machine; and increased media pasteurization temperature 80 °C, respectively.

| Table 5. SNR Values for TPC Level in Pasteurized Milk |
|-----------------------------------------------|----------------|----------------|-----------------|------------------------|----------------|
| Experiment | Replications (1 × 10³ CFU/mL) | σ² | \(\bar{y}^2\) | \(\sigma^2 + \bar{y}^2\) | SNR |
|-------------|-------------------------------|----|-------------|-----------------|-----|
| 1           | 0.973                         | 0.98 | 0.90 | 0.001 | 0.904 | 0.906 | 0.430 |
| 2           | 0.74                          | 0.75 | 0.76 | 0    | 0.563 | 0.563 | 2.498 |
| 3           | 0.6                           | 0.65 | 0.675 | 0.001 | 0.412 | 0.413 | 3.844 |
| 4           | 1.15                          | 1.2 | 1.21 | 0.001 | 1.408 | 1.409 | -1.489 |
| 5           | 0.75                          | 0.75 | 0.75 | 0    | 0.563 | 0.563 | 2.499 |
| 6           | 0.83                          | 0.81 | 0.80 | 0    | 0.662 | 0.662 | 1.794 |
| 7           | 1                             | 1 | 1 | 0    | 1 | 1 | 0 |
| 8           | 1.31                          | 1.35 | 1.37 | 0.001 | 1.805 | 1.805 | -2.565 |
Table 6. ANOVA Results for SNR Values

| Source | SS  | df | MS  | F Ratio | SS' | Rho%   | F Table |
|--------|-----|----|-----|---------|-----|--------|---------|
| B      | 6.903 | 1  | 6.903 | 5.567   | 5.663 | 16.615% | 10.12   |
| C      | 7.402 | 1  | 7.402 | 5.970   | 6.162 | 18.082% | 10.12   |
| D      | 5.337 | 1  | 5.337 | 4.305   | 4.098 | 12.023% | 10.12   |
| F      | 10.719 | 1 | 10.719 | 8.646   | 9.480 | 27.815% | 10.12   |
| Pooled | e    | 3  | 1.24 |         | 8.679 | 25.465% |         |
| SST    | 34.081 | 7 |     |         | 34.081 | 100%    |         |
| SSMean | 6.143 | 1  |      |         |       |        |         |
| SSTotal| 40.224 | 8 |     |         |       |        |         |

Taguchi experiments that have been conducted must be validated using the optimal factor settings and factor levels that have been produced previously. This test is called a confirmation experiment [45]. The optimal level setting is used for 10 confirmation replication trials. The value of the confidence interval for the quality test is calculated from the results of the mean and SNR predictions as well as from the results of the mean confirmation and the SNR. This is useful to prove that confirmation experiments that use optimal level settings are acceptable or not. The results of the comparison of confidence intervals for the two tests are shown in Table 7. The results of the comparison of the confidence interval of the Taguchi experiment and the confirmation experiment can be seen in Figures 1 and 2.

Table 7. Pasteurization Milk Quality Test on TPC levels ($1 \times 10^3$ CFU/mL)

| Quality test          | Prediction values | Optimum values    |
|-----------------------|-------------------|-------------------|
| Taguchi experiment    | Mean              | 0.512             | 0.426 ± 0.596    |
|                       | SNR               | 4.472             | 1.942 ± 7.542    |
| Confirmation experiment | Mean             | 0.636             | 0.532 ± 0.740    |
|                       | SNR               | 3.912             | 0.896 ± 6.928    |

Figure 1. The comparison of confidence interval for the mean of TPC levels in pasteurized milk ($1 \times 10^3$ CFU/mL).
Based on Figures 1 and 2 it can be concluded that the experiment was accepted. This can be seen from the value of the confidence intervals, which intersect between the predicted experiments with confirmation for the mean value and SNR [46]. Therefore, factors and factor levels can be used as a reference in improving the quality of pasteurized milk on TPC levels.

Several studies on quality testing of pasteurized milk have actually been performed. However, the results of those studies have not determined the parameters to obtain optimal conditions, particularly the minimum levels of TPC [47-49]. Based on the optimal level setting results it is known that milking method, cooling process, pasteurization temperature and proper storage time are the most influential factors. Contrary to Klungel et al (2000), the milking method with a dairy machine is considered optimal, because it can minimize bacteria at the raw material level compared to manual using hands [50]. While the process of cooling with acceleration is considered better than room temperature, because cooling with room temperature takes longer and allows the proliferation of bacteria. The recommended pasteurization temperature factor is 72°C, because based on the results of experiments with different temperatures it is known that the higher the pasteurization temperature used, it can cause discoloration to become more turbid, and change the aroma and taste in pasteurized milk. The last factor that affects is the storage process. 2-day storage process is the optimal time for checking cow's milk that is stored in the refrigerator. Although pasteurized milk has a longer shelf life, but the longer storage time causes the total bacteria also tends to be higher.

After knowing the optimal level setting, it can be concluded that this research can be applied to improve the quality of pasteurized cow's milk. The level of TPC produced is also better than the SNI requirements. The mean confirmation experiment was $0.636 \times 10^3$ CFU/mL. This value is quite low when compared to SNI requirements, namely the total value of TPC $3x10^4$ CFU / mL. It is known that the lower the TPC value, the better the milk quality will be. In addition, the results of this experiment can also be a reference for cooperatives engaged in dairy production. It is endeavored to improve the quality of milk production and increase productivity and profitability for cooperatives to produce quality milk products that are fit for consumption.

4. Conclusions
Food quality and safety are important considerations for consumers, including dairy products such as pasteurized milk. The condition of milk that is still fresh and comes from healthy cows, and good processing does not guarantee milk is safe for consumption. One of the quality parameters for this product is microbiological quality, which can be measured from TPC levels. High levels of TPC found
in pasteurized milk cause problems in dairy producers' cooperatives. This study aims to determine the factors that influence TPC levels in pasteurized milk using one of the off-line quality controls, the Taguchi method.

The results showed that there are four factors of the seven factors that influence the improvement of the quality of pasteurized milk based TPC levels. These factors are the cooling process, storage time, pasteurization temperature and milking method. The pooling results also explained that there was no significant effect factor missing due to the contribution of error in the pooling result of 14.362%. The calculation of the mean value and the SNR for optimal factor level settings showed equally good results. Optimal level settings that have been selected are A1, B1, C2, D1, E2, F2, and G2, which are 5°C storage temperature; cooling process with acceleration; storage time 2 days; pasteurization temperature of 72°C; plastic bottle storage packaging; milking method using a dairy machine; and an increase in the temperature of pasteurization media to 80°C, respectively. Confirmation experiments have also been conducted and show the results of TPC levels produced are better than SNI with an average of 0.636 × 10^3 CFU/mL.

In addition to the TPC quality standards, there are other standards such as *Staphylococcus aureus* and *Enterobacteriaceae* that can be investigated for future studies. This research is expected to be a reference for pasteurized milk producers in improving quality and productivity, creating food security, and several other development goals.

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