

THE CRITICAL CONTROL POINT DETERMINATION BY QUANTITATIVE METHOD OF MILK PASTEURIZING PROCESS IN THE DAIRY COOPERATIVE IN MALANG EAST JAVA, INDONESIA

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(Received 5 September, 2017; accepted 1 November, 2017)

Key words : *E. coli*, Coliforms, Pasteurized milk, Critical control point, TPC.

Abstract—HACCP implementation on pasteurized milk to ensure the food safety has not been fully implemented by Dairy Cooperative in Malang. Microbiological quality evaluation on Critical Control Point (CCP) is needed to detect the pasteurized milk quality and the effectiveness of CCP implementation. The aim of this research was to identify and to describe CCP of the pasteurized milk process in the Dairy Cooperative in Malang, East Java, Indonesia. Milk sample was taken pre- and post-pasteurization and examined for bacteriological counts (*coliforms*, Total Plate Count (TPC), and *E. coli*) using standard plate methods. Quantitative research was done by descriptively to explain the CCP characteristics of the pasteurized milk process with primary and secondary data compilation. Bacteriological assay revealed that number of *coliforms*: 0-349.33 MPN/mL; TPC: 4.22-6.17 log cfu/mL; and *E. coli*: 0-3705 cfu/mL were found in collecting center and transport tank. In the steps of milk pasteurization, counts of *coliforms* were 0-742 MPN/mL; TPC: 2275-6170100 cfu/mL; and *E. coli*: 0-8106.7 cfu/mL. There was no significant difference between milk pasteurizing process and *coliforms* quantity. Milk collecting (A) and flavor mixing (G) gave a significant difference to the *E. coli* amount. CCP of milk pasteurizing process in Dairy Cooperative in Malang, Indonesia were in the milk collecting post (A), heating and homogenizing (E) and flavor mixing (G). These results gave useful information to design a HACCP system in small and medium milk pasteurization industry to ensure the safety of milk product.

INTRODUCTION

Critical control point (CCP) determination is an important step in determining the safety of pasteurized milk product. Even though pasteurization can eliminate most of the vegetative microorganism cells, some species as thermophilic microorganism, thermophilic and some gram negative stem forms are able to survive in pasteurization temperature which affect the final product safety and quality (Lejeune and Rajala-Schults 2009; Kristantiet *al.*, 2016). The pasteurization process, conducted after the milk cools, so when heat stress occurs pasteurization. Heat shock causes holes in the membrane and inactivates the sensitive enzymes and ribosomes, the final result is the reduction of biological activities of the bacteria or their death (Tabatabaie and Mortazavi, 2008).

Indonesian Milk Processing Industries acquire

raw fresh milk from the dairy cooperatives. Some Indonesian dairy cooperatives not only deliver fresh milk to the commercial milk processing company but also process it into pasteurized milk. Milk pasteurization process in dairy cooperative uses the plate heat exchanger (PHE), however HACCP implementation to ensure the food safety has not been fully implemented by cooperatives and small and medium milk industries especially Dairy Cooperatives in Malang.

Microbiological quality evaluation on CCP is needed to detect the pasteurized milk quality and the effectiveness of CCP implementation. The potential hazards that are reasonably likely to cause illness or injury in the absence of their control must be addressed in determining CCPs (Adetunji and Odetokun, 2011). Furthermore, the whole procedures should be overhauled while the following specific corrective actions should be applied at the CCPs to eradicate the pathogenic

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organisms along the processing line (Adetunji and Odetokun, 2013). Lye *et al.* (2013) found *E. coli* O157:H7 in raw milk samples collected from local dairy farms in the state of Selangor, Malaysia, while El-Gedawy *et al.* (2014) identified some zoonotic bacteria (*Staph. aureus*, *St. agalactiae*, *Salmonella* species and *M. bovis*) in milk samples from dairy farms, Sharkia, Egypt. Szabo (2011) reported that critical control point identification is an important element in evaluating the quality system management. The difference in milk pasteurizing process on each industry influences the CCP. One of the indicators to determine the CCP is microorganism test. The milk microorganism quality grouping based on the tidiness is set out as the plate count, while *coliform* test is used for pasteurization control, if as soon after pasteurized no *coliforms* found, then pasteurization has been done efficiently (Olayinka and Omobayonle, 2006; El-Zubeir *et al.*, 2007; Landeiro *et al.*, 2007; Karakok, 2007; Sobukola *et al.*, 2009; Elizondo-Salazar *et al.*, 2010).

Survey method can be used to measure the CCP. Questioner is used to access management system value and employer skill (Nastasijevic *et al.*, 2016; Tzamalidis *et al.*, 2016). Other method to measure CCP is the statistic assumption (Bolton and Sheridan, 2002; Gonzales-Miret *et al.*, 2006; Kinsella *et al.*, 2006; Menezes *et al.*, 2010; Garedew *et al.*, 2012; Escudero-Gilete *et al.*, 2014). The aims of this research were to identify and to describe the CCP of the pasteurized milk process and to identify the microbial quality of the pasteurized milk in each CCP in dairy cooperatives in Malang, East Java, Indonesia.

MATERIALS AND METHODS

Sampling

Milk sample was taken pre- and post-pasteurization as well as from Collecting Center until Mix Flavor Tank, in the Dairy Cooperative in Malang, Indonesia. A total of 9 milk samples from collecting centers, 3 milk samples from transport tanks, 1 milk sample from storage tank, transit tank, after pasteurization with homogenization tank, after 2nd pasteurization tank and after mix flavor tank, respectively. Each step was repeated 3 times. The farms used manual milking.

Milk samples

Approximately 50 mL of milk samples were

collected from all sources at one time. Sampling was done aseptically by 50 mL sterile syringe, and then shifted to 100 mL sterile glass tube. Milk samples were immediately refrigerated and transported to the laboratory in 4°C container storage (Prejit *et al.*, 2007). All milk samples were examined for *coliforms* test, *Total Plate Count* (TPC) test, and *E. coli* test (Benson, 2002).

Quantitative analysis of *coliform*

The analysis consists of three stages: (1) presumptive test used *lactose broth* by Oxoid, (2) confirmed test used EMBA media (Eosin Methylene Blue Agar) by Oxoid and (3) completed test. The analysis was performed based on Benson (2002) and Jayarao *et al.*, (2004).

Quantitative microbial analysis total plate count (TPC)

The analysis was conducted according to Benson (2002). One milliliter of 10^{-3} to 10^{-5} dilution of the stock culture was put into petridish (duplicate). PCA media (Plate Count Agar by Oxoid) which has been cooled to $45^{\circ}\text{C} \pm 1^{\circ}\text{C}$ was then poured into each petridish in about 15-20 mL, and kept until solidified. Total bacteria was counted after incubation at $34-36^{\circ}\text{C}$ for 24-48 hours.

Quantitative analysis of *Escherichia coli*

One milliliter of 10^{-3} to 10^{-5} dilutions was transferred to a sterile petridish (triplicate). VRBA (Violet Red Bile Agar by Oxoid) was added into that petridish. Isolation was conducted using pour plate method (Benson, 2002). Twenty milliliters of sample was poured and then homogenized. Once the agar solidified, petridishes were incubated upside down at 37°C for 24 hours. Colonies of *E. coli* were seen in a greenish color under the light exposure.

The research method was explorative research aiming to explore data to create a deep understanding of the research while the result would be tested in the next quantitative research. Quantitative research was done by descriptively to explain the CCP characteristics of the pasteurized milk process with primary and secondary data compilation. Compiled data were analyzed statistically. Milk pasteurization process described as follow in Fig. 1.

Statistical analysis

Microorganism data analysis in step A, B, C, D, E, F and G used One Way Repeated ANOVA parametric

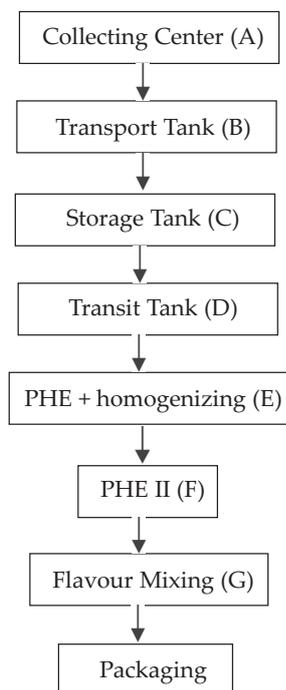


Fig. 1. Milk Pasteurization Process in the Dairy Cooperative in Malang, Indonesia.

statistical test. All statistical analysis was carried out at 0.05 level of error. ANOVA can be done if the data were in assumption so the data were tested by Kolmogorov-Smirnov method test first. To determine the phase difference to the quality of the coliform, TPC, and *E.coli* were tested by repeated measurement ANOVA. Then to determine differences in each stage, Benferronitest was applied. Data analysis was using SPSS version20.

RESULTS AND DISCUSSION

The main raw material in milk pasteurization was fresh milk from farmer members of the Dairy Cooperative. The fresh milk was collected in the working area of the Dairy Cooperative in Malang. The Dairy Cooperative in Malang had 9 collection centers and 3 transport tanks. The diversity of the milk source, milk collection center condition and the transport caused different contributions to the microorganism fresh milk quality. TPC, *E.coli* and *coliforms* test result in the collection center and transport tank was shown in the Table 1.

Table 1 show that the highest TPC was at the A6 collection center and B1 transport tank. It happened because B1 transport tank collected milk from A6

Table 1. Cell number of *Coliform*, TPC and *E. coli* in different location

Location	<i>Coliforms</i> (MPN/ml)	TPC (log cfu/ml)	<i>E. coli</i> (cfu/ml)
A1	0.00	4.25	0.00
A2	5.83	4.90	118.33
A3	0.00	4.70	0.00
A4	0.83	4.22	0.00
A5	2.50	5.77	25.00
A6	6.67	6.07	113.33
A7	10.17	5.80	152.33
A8	0.00	5.68	0.00
A9	1.50	4.62	13.33
B1	349.33	6.17	3,705.00
B2	5.83	4.88	86.67
B3	15.67	5.02	261.67

Description: Collecting Center (A) dan Transport Tank (B)

collection center. Microorganism amount in the A6 collection center was to the result of milk tank's contamination and lack of the farmers' sanitation and hygiene awareness. The microorganism in transport tank increased because milk was not frozen or cooled during the transfer process and other contamination which might happen in the transfer process to the main collection center. The fresh milk microorganism quality of the B2 transport tank was low based on the *E. coli* and *coliforms* amount test. The low rate of the *E. coli* and *coliforms* indicated better sanitation and hygiene in the B2 transport tank compared to B1 and B3 transport tank. According to Indonesian National Standard (2011), the number of TPC in the A6 and B1 exceeded the maximum standard 1×10^6 CFU/mL. Appropriate amount of *coliform* on Indonesian National Standard (2011) is 0 MPN/mL, so A7, B1 and B3 are not in accordance with the standards.

Lopes and Stamford (1997) mentioned that the pasteurized milk CCP in one of the Brazil's milk companies was (1) the temperature of the fresh milk and pasteurized milk storage tank, (2) pasteurization process, and (3) packing. The microorganism increased significantly in the pasteurized milk storage tank. Foreman (2011), added that the microorganism existing in the pasteurized milk affected by (1) the fresh milk quality and handling, (2) the pasteurization process, (3) the worker and equipment hygiene, (4) milking to ready to process time, (5) preprocess milk cooling, (6) pasteurizing temperature and time. The contamination sources were the cleaning water and insufficient temperature in the tank. Gleeson *et al.*,

(2013) argues that steps in processing the milk such as unhygienic water, non standard storing equipment which stimulates the growth of microorganism can add the microorganism amount and type. Microbiological characteristic was one of the main variables to determine the food product quality. Microbiological quality of the milk changed during the process.

ANOVA test on Table 2. Shows that each pasteurization steps did not have significant difference ($p < 0.05$) to the *coliforms* and TPC. However, each pasteurization steps showed significant difference to the *E. coli* amount ($p < 0.05$). Based on Indonesian National Standard (1995) and the international standard set by FDA in the Pasteurized Milk Ordinance (2013), the fresh milk TPC amount before pasteurization was 30,000/mL maximally; the pasteurized milk TPC amount was 20,000/mL maximally and the pasteurized milk *coliforms* was maximally 10/mL. TPC and *coliforms* quality to the fresh milk (D) and the pasteurized milk (F) had met the standard requirements. Gandy *et al.* (2008) reported that the pasteurized milk durability was affected by the microorganism amount, pre-pasteurizing contamination, and storage condition. Every 3°C storage temperatures increase, the shelf life would decrease into half. Prejit *et al.* (2007) stated that high amount of the pathogen microorganism could affect the microbiological quality of the fresh milk and the milk-based on food product safety.

Coliforms

Further test was conducted to determine the differences of the number of *coliforms* in each step which is presented in Table 3. Table 3 shows that all steps did not show any significant different from the 7th process to *coliforms* amount. Therefore it was

concluded that there was no microorganism difference in collection center, transport tank, storage tank, transit, homogenize PHE, 2nd PHE and flavour mixing.

Table 2 shows that there was microorganism amount increased from the collection center to the transport tank step, however the increasing was not significant statistically. After the *coliforms* increased, the amount of microorganism reduced up to step 6, 2nd PHE, and the microorganism amount increased again in the mix flavor step. Increasing amount of microorganisms was caused by contamination of the instrument or hygienic working on flavor mixing stages. A study conducted by Agarwal *et al.* (2012) concerning microbiological profile of milk on household showed that *coliform*, yeast and mold were found on all vendors and pasteurized milk. According to Salman and Hamad (2011; 2013), international standard amount of *coliforms* in fresh milk is 100 cell/mL. *Coliforms* in milk are generally of a group of *E. coli* (32%), *Enterobacter* (29.2%), *Klebsiella* (19.4%), *Serratia* (11.1%) and *Citrobacter* (1.0%) and some *enterobacteriaceae*. Elmagli and ElZubeir (2006), added that the factors that influence the presence of *coliforms* in milk were the lack of sanitation and hygienic milk, either at home or in shelters. This happened due to the limited knowledge of sanitary production, the use of hygienic equipment and water. The quality of *coliforms* in fresh milk can be reduced when it was cooled immediately after milking and the process of distribution of milk was done in cold conditions.

The decrease of microorganisms from B to C might be caused by the rapid cooling from room temperature becomes 3°C. Cold shock also affects cell division. The temperature downshift results in a growth lag. During the lag phase the organism changes the composition of the cytoplasmic

Table 2. Description of Microbiology Variables in Each Stage

Stages	Type of Microorganisms		
	<i>Coliforms</i> (MPN/mL)	TPC (cfu/mL)	<i>E. coli</i> (cfu/mL)
A	56 ± 13.23	6170100 ± 5680470	844.57 ± 265.61
B	742 ± 353.80	3494400 ± 2899560	8106.7 ± 1174.11
C	51 ± 73.30	186300 ± 254352	666.67 ± 1019.3
D	19.33 ± 33.49	20683 ± 137201	110 ± 190.53
E	0	9016.67 ± 6177.05	0
F	0	2275 ± 2447.3	0
G	21 ± 36.37	5383.33 ± 6179.8	160 ± 277.13

Collecting milk(A), Transport Tank (B), Storage Tank (C), Transit Tank (D), PHE + Homogenize (E), PHE II (F), mix flavor (G).

membrane and synthesis sets of specific proteins called cold shock proteins or cold induced proteins. Temperature plays a very important role in the composition, organization and function of biological membranes. Membranes adjust their unsaturated fatty acid composition according to the changes in the environmental temperature (Ulusu and Tezcan, 2001).

Total microorganisms (TPC)

Pasteurization process there was not significant effect to the microorganism amount by TPC test. The further test was done to find out the differences in each step. The further step test by Benferroni test result was shown in Table 3. Table 3 shows that step E with step G showed highly significant difference to the microorganism amount. Step E average microorganism amount was 9016.67 cfu/mL and the step G average microorganism amount was 5383.33cfu/mL. The amount of the microorganism decreased significantly from step E to step G, while 5 other steps ie A, B, C, D and E did not show a highly significant difference to the microorganism amount. Step E was heating and homogenizing. Steps E was expected to eliminate the accepted danger potential. The step G i.e. flavour mixing tank was one of the CCP because the next step could not guarantee the danger potential.

Table 2 shows that there was microorganism amount decreased from the step A to step F. However, it increased significantly in step G compared to step E. TPC amount decreased in the collection step due to 4°C low temperature usage, therefore the microorganism growth could be sustained. Gleeson *et al.* (2013) said that milk microorganism growth depends on early microorganism amount and storage condition. Cooling shock can press the psychotropic microorganism growth and the milk suggested being cooled half hour after milking. The most effective method to prevent the thermoduric microorganism to the pasteurized milk is milking hygiene.

Gao *et al.* (2004) report that the heat-shock response, which is elicited by a sudden increase in growth temperature, has been widely used as a model system for studying the impact of stress on biological systems. The bacterial heat-shock response is not limited to changes in temperature and is a general stress response, as many of the heat-shock proteins are induced by other environmental changes, such as the addition of ethanol, heavy

metals, high osmolarity, pollutants, starvation or interaction with eukaryotic hosts. In several bacterial species heat-shock proteins have been shown to play an important role in pathogenesis and survival within macrophages (Ron *et al.*, 2000).

Escherichia coli

Further test was done to find the significant difference between steps of the *E. coli* amount. From Table 3 it is known that the collection center step to the flavor mixing steps was significantly different to the *E. coli* amount with the microorganism average in the collection center accounted for 844,57 cfu/mL and the microorganism average in the flavor mixing steps was 160 cfu/mL. The *E. coli* amount decreased significantly from the collection center to the flavor mixing. While to the other 6 steps which were: transport tank, storage tank, transit, homogenize PHE, and 2ndPHE there was no significant difference. The *E. coli* amount at the collection center increased therefore the transport tank could not eliminate the acceptable danger potential. The entire process of development of the amount of *E. coli* at each stage is presented in Table 2.

Table 2 shows that *E. coli* amount increased from the collection center step to transport tank, however it was not significant. Meanwhile on the storage tank, which was the next step, the microorganism amount decreased until step 6th i.e 2nd PHE, and there was increasing in the flavor mixing step.

E. coli and *coliforms* were not found after pasteurization; however they were found after cooling and flavor mixing. Agarwal *et al.* (2012) says, *E. coli* and *coliforms* can be found 24 hours after room temperature storage. Microbiological quality was affected by cattle contamination, farmers, equipments, mastitis milk contamination and storage condition. *E. coli* and most of *coliform* group were categorized as mesofil bacteria that cannot survive in pasteurized temperature. It can be concluded that heating temperature in pasteurization process will eliminate *E. coli* and *coliform*. Cold-shock process after pasteurization may prevent the growth of spores. According to Giuliodori *et al.* (2004) upon temperature downshift below the lower threshold of balanced growth ($\leq 20^{\circ}\text{C}$), the *E. coli* translational apparatus undergoes modifications allowing the selective translation of the transcripts of cold-shock induced genes, while bulk protein synthesis is drastically reduced.

Table 3 show that there were significant

Table 3. Effect of Stage Process for Total Microorganisms

Stages	Microorganism	Significance						
		A	B	C	D	E	F	G
A	TPC	-	0.271	0.198	0.201	0.201	0.201	0.201
	<i>E.coli</i>	-	0.240	1.000	0.078	0.660	0.660	0.012*
	<i>Coliform</i>	-	1.000	1.000	1.000	0.380	0.380	1.000
B	TPC	0.271	-	0.172	0.173	0.173	0.172	0.173
	<i>E.coli</i>	0.240	-	0.438	0.180	0.145	0.145	0.198
	<i>Coliform</i>	1.000	-	1.000	1.000	1.000	1.000	1.000
C	TPC	0.198	0.172	-	0.358	0.353	0.339	0.346
	<i>E.coli</i>	1.000	0.438	-	1.000	1.000	1.000	1.000
	<i>Coliform</i>	1.000	1.000	-	1.000	1.000	1.000	1.000
D	TPC	0.201	0.173	0.358	-	0.289	0.152	0.208
	<i>E.coli</i>	0.078	0.180	1.000	-	1.000	1.000	1.000
	<i>Coliform</i>	1.000	1.000	1.000	-	1.000	1.000	1.000
E	TPC	0.201	0.173	0.353	0.289	-	0.092	0.003*
	<i>E.coli</i>	0.660	0.145	1.000	1.000	-	1.000	1.000
	<i>Coliform</i>	0.380	1.000	1.000	1.000	-	1.000	1.000
F	TPC	0.201	0.172	0.339	0.152	0.092	-	0.291
	<i>E.coli</i>	0.660	0.145	1.000	1.000	1.000	-	1.000
	<i>Coliform</i>	0.380	1.000	1.000	1.000	1.000	-	1.000
G	TPC	0.201	0.173	0.326	0.208	0.003*	0.291	-
	<i>E.coli</i>	0.012*	0.198	1.000	1.000	1.000	1.000	-
	<i>Coliform</i>	1.000	1.000	1.000	1.000	1.000	1.000	-

Collecting milk (A), Transport Tank (B), Storage Tank (C), Transit Tank (D), PHE + Homogenize (E), PHE II (F), mix flavor (G). Sign* indicates that there are significant differences between each step in the process of milk pasteurization to the number of microorganisms.

differences to the step collection center, heating to homogenizing and flavor mixing step. Therefore it was found that the CCP to the pasteurized milk process were at the collection center, first PHE and flavor mixing.

Collection center was the CCP because the fresh milk from the farmer was collected in the collection center. There were 9 collection centers in the working area of the dairy cooperative, the milk distribution from the farmer to the collection center only by milk tank without cooling facilities. The next CCP was heating and homogenizing. The temperature in this step was not sufficient to kill all microorganisms and the second heating also could not kill all microorganisms in the pasteurized milk. The flavor addition was the last CCP. Even though this step used the cool temperature, the contamination control was not done.

Aggad *et al.* (2010) says high contamination in the pasteurized milk might be caused by poor hygiene in the process of: (1) milking, (2) collecting, (3) milk transportation, and (4) milk mixing with other milk. In other word, factors that influence the

microorganism's amount in the milk were: fresh milk microorganism's amount, milk handling, re-contamination during pasteurization, and pasteurization temperature. CCP on the processing of pasteurized milk in the area of Ethiopia were: (1) the handling of the teats when milking, (2) sanitation buckets for milking and container during transport in the shelter, (3) transportation from shelters to processing area (Garedew *et al.*, 2012). Kalupahana and Silva-Fletcher (2016) developed the HACCP plan for clean milk production and identified 13 critical control points from dairy cow farm until milk reaching a processing plant. While Belli *et al.* (2013), has identified potential hazards and devaluation microorganism contamination of fresh milk and dairy products in small-scale milk processing units in Cameroon. The research result showed that microorganism contamination was caused by milking contamination and handling process (processing, packing, and storing), therefore it requires the training to the farmer and processing staff to increase the milk hygiene and milk production chain.

CONCLUSION

In conclusion, there was no significant difference between milk pasteurizing process to the *coliform's* quantity. However, there was a high significant ($p < 0.05$) difference between heating and homogenizing process (E) and flavor mixing (G) to the TPC amount. There was significant different ($p < 0.05$) between milk collecting (A) and flavor mixing (G) to the *E.coli* amount. The CCP in the milk pasteurization process in the Dairy Cooperative in Malang were: 1) milk collecting process from the farmers, 2) heating and homogenizing, and 3) flavour mixing tank.

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