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1 **Conclusion, significance and impact of study:** CCP of milk pasteurizing process
2 in Dairy Cooperative in Malang, Indonesia were in the milk collecting post (A),
3 heating and homogenizing (E) and flavor mixing (G). These results gave useful
4 information to design a HACCP system in small and medium milk pasteurization
5 industry to ensure the safety of milk product.

6 **Keywords:** *E. coli*, *coliforms*, pasteurized milk, critical control point, *TPC*.

7 INTRODUCTION

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

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

1 Microbiological quality evaluation on CCP is needed to detect the pasteurized
2 milk quality and the effectiveness of CCP implementation. The potential hazards that
3 are reasonably likely to cause illness or injury in the absence of their control must be
4 addressed in determining CCPs (Adetunji and Odetokun, 2011). Furthermore, the
5 whole procedures should be overhauled while the following specific corrective
6 actions should be applied at the CCPs to eradicate the pathogenic organisms along
7 the processing line (Adetunji and Odetokun, 2013). Lye *et al.* (2013) found *E. coli*
8 *O157:H7* in raw milk samples collected from local dairy farms in the state of
9 Selangor, Malaysia, while El-Gedawy *et al.* (2014) identified some zoonotic bacteria
10 (*Staph. aureus*, *St. agalactiae*, *Salmonella* species and *M. bovis*) in milk samples
11 from dairy farms, Sharkia, Egypt. Szabo (2011) reported that critical control point
12 identification is an important element in evaluating the quality system management.
13 The difference in milk pasteurizing process on each industry influences the CCP.
14 One of the indicators to determine the CCP is microorganism test. The milk
15 microorganism quality grouping based on the tidiness is set out as the plate count,
16 while *coliform* test is used for pasteurization control, if as soon after pasteurized no
17 *coliforms* found, then pasteurization has been done efficiently (Olayinka and
18 Omobayonle, 2006; El-Zubeir *et al.*, 2007; Landeiro *et al.*, 2007; Karakok, 2007;
19 Sobukola *et al.*, 2009; Elizondo-Salazar *et al.*, 2010).

20 Survey method can be used to measure the CCP. Questioner is used to
21 access management system value and employer skill (Nastasijevic *et al.*, 2016;
22 Tzamalís *et al.*, 2016). Other method to measure *CCP* is the statistic assumption
23 (Bolton and Sheridan, 2002; Gonzales-Miret *et al.*, 2006; Kinsella *et al.*, 2006;
24 Menezes *et al.*, 2010; Garedeew *et al.*, 2012; Escudero-Gilete *et al.*, 2014). The aims
25 of this research were to identify and to describe the CCP of the pasteurized milk

1 process and to identify the microbial quality of the pasteurized milk in each CCP in
2 dairy cooperatives in Malang, East Java, Indonesia.

3 **MATERIALS AND METHODS**

4 **Sampling**

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

11 **Milk samples**

12 Approximately 50 ml of milk samples were collected from all sources at one
13 time. Sampling was done aseptically by 50 ml sterile syringe, then shifted to 100 ml
14 sterile glass tube. Milk samples were immediately refrigerated and transported to the
15 laboratory in a 4°C container storage (Prejit *et al.*, 2007). All milk samples were
16 examined for *coliforms* test, *Total Plate Count (TPC)* test, and *E.coli* test (Benson,
17 2002).

18 **Quantitative analysis of *coliform***

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

23

24 **Quantitative microbial analysis total plate count (TPC)**

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

6 **Quantitative analysis of *Escherichia coli***

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

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

19 **Statistical analysis**

20 Microorganism data analysis in step A, B, C, D, E, F and G used One Way
21 Repeated ANOVA parametric statistical test. All statistical analysis was carried out at
22 0.05 level of error. ANOVA can be done if the data were in assumption so the data
23 were tested by Kolmogorov-Smirnov method test first. To determine the phase
24 difference to the quality of the coliform, TPC, and *E. coli* were tested by repeated

1 measurement ANOVA. Then to determine differences in each stage, Benferroni test
2 was applied. Data analysis was using SPSS version 20.

3 **RESULTS AND DISCUSSION**

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

11 Table 1 shows that the highest TPC was at the A6 collection center and B1
12 transport tank. It happened because B1 transport tank collected milk from A6
13 collection center. Microorganism amount in the A6 collection center was to the result
14 of milk tank's contamination and lack of the farmers' sanitation and hygiene
15 awareness. The microorganism in transport tank increased because milk was not
16 frozen or cooled during the transfer process and other contamination which might
17 happen in the transfer process to the main collection center. The fresh milk
18 microorganism quality of the B2 transport tank was low based on the *E. coli* and
19 *coliforms* amount test. The low rate of the *E. coli* and *coliforms* indicated better
20 sanitation and hygiene in the B2 transport tank compared to B1 and B3 transport
21 tank. According to Indonesian National Standard (2011), the number of TPC in the
22 A6 and B1 exceeded the maximum standard 1×10^6 CFU / mL. Appropriate amount of
23 *coliform* on Indonesian National Standard (2011) is 0 MPN / mL, so A7, B1 and B3
24 are not in accordance with the standards.

1 Lopes and Stamford (1997) mentioned that the pasteurized milk CCP in one of
2 the Brazil's milk companies was (1) the temperature of the fresh milk and pasteurized
3 milk storage tank, (2) pasteurization process, and (3) packing. The microorganism
4 increased significantly in the pasteurized milk storage tank. Foreman (2011), added
5 that the microorganism existing in the pasteurized milk affected by (1) the fresh milk
6 quality and handling, (2) the pasteurization process, (3) the worker and equipment
7 hygiene, (4) milking to ready to process time, (5) preprocess milk cooling, (6)
8 pasteurizing temperature and time. The contamination sources were the cleaning
9 water and insufficient temperature in the tank. Gleeson *et al.*, (2013) argues that
10 steps in processing the milk such as unhygienic water, non standard storing
11 equipment which stimulates the growth of microorganism can add the microorganism
12 amount and type. Microbiological characteristic was one of the main variables to
13 determine the food product quality. Microbiological quality of the milk changed during
14 the process.

15 ANOVA test on Table 2. shows that each pasteurization steps did not have
16 significant difference ($p < 0.05$) to the *coliforms* and *TPC*. However, each
17 pasteurization steps showed significant difference to the *E. coli* amount ($p < 0.05$).
18 Based on Indonesian National Standard (1995) and the international standard set by
19 FDA in the Pasteurized Milk Ordinance (2013), the fresh milk TPC amount before
20 pasteurization was 30,000/mL maximally; the pasteurized milk TPC amount was
21 20,000/mL maximally and the pasteurized milk *coliforms* was maximally 10/mL. TPC
22 and *coliforms* quality to the fresh milk (D) and the pasteurized milk (F) had met the
23 standard requirements. Gandy *et al.* (2008) reported that the pasteurized milk
24 durability was affected by the microorganism amount, pre-pasteurizing
25 contamination, and storage condition. Every 3 °C storage temperatures increase, the

1 shelf life would decrease into half. Prejit *et al.* (2007) stated that high amount of the
2 pathogen microorganism could affect the microbiological quality of the fresh milk and
3 the milk-based on food product safety.

4 **Coliforms**

5 Further test was conducted to determine the differences of the number of
6 *coliforms* in each step which is presented in Table 3. Table 3 shows that all steps did
7 not show any significant different from the 7th process to *coliforms* amount. Therefore
8 it was concluded that there was no microorganism difference in collection center,
9 transport tank, storage tank, transit, homogenize PHE, 2nd PHE and flavour mixing.

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

1 The quality of *coliforms* in fresh milk can be reduced when it was cooled immediately
2 after milking and the process of distribution of milk was done in cold conditions.

3 The decrease of microorganisms from B to C might be caused by the rapid
4 cooling from room temperature becomes 3°C. Cold shock also affects cell division.
5 The temperature downshift results in a growth lag. During the lag phase the organism
6 changes the composition of the cytoplasmic membrane and synthesis sets of specific
7 proteins called cold shock proteins or cold induced proteins. Temperature plays a
8 very important role in the composition, organization and function of biological
9 membranes. Membranes adjust their unsaturated fatty acid composition according to
10 the changes in the environmental temperature (Ulusu and Tezcan, 2001).

11 **Total microorganisms (TPC)**

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

23 Table 2 shows that there was microorganism amount decreased from the step
24 A to step F. However, it increased significantly in step G compared to step E. TPC
25 amount decreased in the collection step due to 4 °C low temperature usage,

1 therefore the microorganism growth could be sustained. Gleeson *et al.* (2013) said
2 that milk microorganism growth depends on early microorganism amount and storage
3 condition. Cooling shock can press the psychrotropic microorganism growth and the
4 milk suggested to be cooled half hour after milking. The most effective method to
5 prevent the thermoduric microorganism to the pasteurized milk is milking hygiene.

6 Gao *et al.* (2004) report that the heat-shock response, which is elicited by a
7 sudden increase in growth temperature, has been widely used as a model system for
8 studying the impact of stress on biological systems. The bacterial heat-shock
9 response is not limited to changes in temperature and is a general stress response,
10 as many of the heat-shock proteins are induced by other environmental changes,
11 such as the addition of ethanol, heavy metals, high osmolarity, pollutants, starvation
12 or interaction with eukaryotic hosts. In several bacterial species heat-shock proteins
13 have been shown to play an important role in pathogenesis and survival within
14 macrophages (Ron *et al.*, 2000).

15 ***Escherichia coli***

16 Further test was done to find the significant difference between steps of the
17 *E. coli* amount. From Table 3 it is known that the collection center step to the flavor
18 mixing steps was significantly different to the *E. coli* amount with the microorganism
19 average in the collection center accounted for 844,57 cfu/mL and the
20 microorganism average in the flavor mixing steps was 160 cfu/mL. The *E. coli*
21 amount decreased significantly from the collection center to the flavor mixing. While
22 to the other 6 steps which were: transport tank, storage tank, transit, homogenize
23 PHE, and 2nd PHE there was no significant difference. The *E. coli* amount at the
24 collection center increased therefore the transport tank could not eliminate the

1 acceptable danger potential. The entire process of development of the amount of *E.*
2 *coli* at each stage is presented in Table 2.

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

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

19 Table 3 show that there were significant differences to the step collection
20 center, heating to homogenizing and flavor mixing step. Therefore it was found that
21 the CCP to the pasteurized milk process were at the collection center, first PHE and
22 flavor mixing.

23 Collection center was the CCP because the fresh milk from the farmer was
24 collected in the collection center. There were 9 collection centers in the working area
25 of the dairy cooperative, the milk distribution from the farmer to the collection center

1 only by milk tank without cooling facilities. The next CCP was heating and
2 homogenizing. The temperature in this step was not sufficient to kill all
3 microorganisms and the second heating also could not kill all microorganisms in the
4 pasteurized milk. The flavor addition was the last CCP. Even though this step used
5 the cool temperature, the contamination control was not done.

6 Aggad *et al.* (2010) says high contamination in the pasteurized milk might be
7 caused by poor hygiene in the process of: (1) milking, (2) collecting, (3) milk
8 transportation, and (4) milk mixing with other milk. In other word, factors that
9 influence the microorganisms amount in the milk were: fresh milk microorganisms
10 amount, milk handling, re-contamination during pasteurization, and pasteurization
11 temperature. CCP on the processing of pasteurized milk in the area of Ethiopia were:
12 (1) the handling of the teats when milking, (2) sanitation buckets for milking and
13 container during transport in the shelter, (3) transportation from shelters to
14 processing area (Garedew *et al.*, 2012). Kalupahana and Silva-Fletcher (2016)
15 developed the HACCP plan for clean milk production and identified 13 critical control
16 points from dairy cow farm until milk reaching a processing plant. While Belli *et al.*
17 (2013), has identified potential hazards and evaluation microorganism contamination
18 of fresh milk and dairy products in small-scale milk processing units in Cameroon.
19 The research result showed that microorganism contamination was caused by
20 milking contamination and handling process (processing, packing, and storing),
21 therefore it requires the training to the farmer and processing staff to increase the
22 milk hygiene and milk production chain.

23 **CONCLUSION**

24 In conclusion, there was no significant difference between milk pasteurizing
25 process to the *coliform*'s quantity. However, there was a high significant ($p < 0.05$)

1 difference between heating and homogenizing process (E) and flavor mixing (G) to
2 the TPC amount. There was significant different ($p < 0.05$) between milk collecting (A)
3 and flavor mixing (G) to the *E. coli* amount. The CCP in the milk pasteurization
4 process in the Dairy Cooperative in Malang were: 1) milk collecting process from the
5 farmers, 2) heating and homogenizing, and 3) flavour mixing tank.

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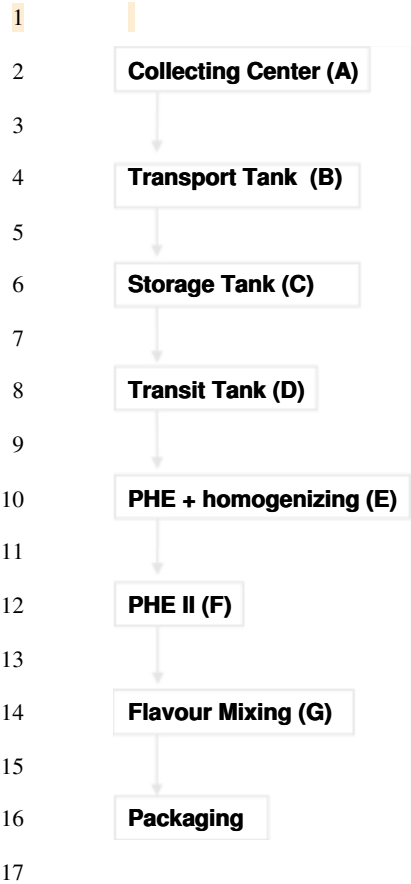


Figure 1. Milk Pasteurization Process in the Dairy Cooperative in Malang, Indonesia.

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

Location	<i>Coliforms</i> (MPN/ml)	<i>TPC</i> (cfu/ml)	(log <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

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

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1 **Table 2. Description of Microbiology Variables in Each Stage**

Stages	Type of Microorganisms		
	<i>Coliforms</i> (MPN/mL)	<i>TPC</i> (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

2 Collecting milk(A), Transport Tank (B), Storage Tank (C), Transit Tank (D), PHE +

3 Homogenize (E), PHE II (F), mix flavor (G).

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1 **Table 3. Effect of Stage Process for Total Microorganisms**

Stages	Microorganism	Significance						
		A	B	C	D	E	F	G
A	<i>TPC</i>	-	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	<i>TPC</i>	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	<i>TPC</i>	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	<i>TPC</i>	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
	<i>TPC</i>	0.201	0.173	0.353	0.289	-	0.092	0.003*
E	<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
	<i>TPC</i>	0.201	0.172	0.339	0.152	0.092	-	0.291
F	<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
	<i>TPC</i>	0.201	0.173	0.326	0.208	0.003*	0.291	-
G	<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	-

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

5

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