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Procedia Food Science 3 (2015) 465 – 472

Procedia Food Science

The First International Symposium on Food and Agro-biodiversity (ISFA2014)

The Reduction of Aerobic Bacterial Counts of Bovine Milk as Influenced By Heat-Treatments at Pasteurisation Temperatures

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Abstract
A number of experiments were conducted to evaluate the bacterial reduction of milk as influenced by the heat treatments at pasteurisation temperature (72 °C for 15 s) considering the Indonesian Standard for raw milk values. The milk samples were collected from dairy farmers in Semarang, Salatiga and Boyolali, Central Java, Indonesia. The heat treatments were conducted in 45 mL glasses bottles dipped in water bath. Considering the temperature histories of the experiments and the bacterial counts, nine of twenty samples indicated their reductions were less than 2 logs cycle standardised although the initial numbers were surprisingly less than $1 \cdot 10^6$ cfu·mL^{-1}.

Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community

Keywords: aerobic, bacterial counts, bovine milk, heat-treatment, pasteurisation

Introduction

Central Java contributes in supplying raw milk for national consumption. The supply has been increased approximately 2.34 % p.a. by population growth of 5.8% p.a. [1,2]. The most volume of milk are further processed to final products e.g. pasteurised and UHT milks by national dairy industries. Many of them are processed by regional dairy industry into products marketed for regional consumers. Some of them are directly accessed by the consumers as raw milk and cooked at households prior to consumption.

There were some poisoning outbreaks after milk consumption reported [3,4]. A further study indicated that outbreaks were due to high bacterial contents present in milk, including pathogens. Quality standards for raw milk as well as that for ready to consume standard had been set by the National Standardisation Agency of Indonesia. Concerning the bacterial quality, the standards for both the milk are $1 \cdot 10^6$ cfu·mL^{-1} [5] and $1 \cdot 10^4$ cfu·mL^{-1} [6] respectively. In addition, pathogen is obligatory absent in the ready to consume milk. The quality of raw milk is under responsibility of

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Peer-review under responsibility of the organizing committee of Indonesian Food Technologist Community
doi:10.1016/j.profoo.2015.01.051

farmers whereas the later is under either the dairy industries or the household processors which are usually act also as consumers. The later quality could be achieved by heat-treatment as the most popular method in food processing and preservation. Since the heat-treatments are compulsory for milk prior consumption, a serious concern to the efficiency of the treatment to the bacterial quality is necessary.

This work was aimed to evaluate the efficiency of heat-treatments at pasteurisation temperature (72 °C for 15 s) considering the High Temperature Short Time pasteurisation method [7]. The method was applied on several milk samples collected from milk producing regions in Central Java i.e. Semarang, Salatiga and Boyolali. The work is beneficial in supplying an information as a basis for developing a precise heat-treatment resulting in qualified ready-to-consume milk considering the National Standardisation Agency of Indonesia. At end-point of food chain, the qualified milk meets the issue in food safety of local and regional consumer.

Materials and Methods

Samples collection

Milk samples were collected from Salatiga city, Semarang regency and Boyolali regency, all located in Central Java, Indonesia. The conditions of transportation as well as the storage prior further treatments and analysis were set to a temperature limiting the bacterial growth (5 °C).

Heat-treatment

Soon after arrived in the laboratory, the samples were heat-treated at pasteurisation temperature (72 °C for 15 s) in accordance with High Temperature Short Time treatments pasteurisation [7]. The treatments were carried out on the milk containing 45 mL glass bottles dipped in a water bath. The temperature histories during the treatments were recorded by a data logger.

Bacterial enumeration

The bacterial counts were enumerated by mean of spread-plate method on 9 cm Nutrient Agar and incubated for 48 h at 30 °C prior to calculation (Equation 1) [8].

$$\bar{c} = \frac{\sum c}{n_1 \cdot 1 + n_2 \cdot 0.1} \cdot d \qquad \text{Equation 1}$$

c : mean of bacterial counts

$\sum c$: total of bacterial counts observed on all plates used

n_1 : number of plates at the lower dilution step

n_2 : number of plates at the higher dilution step

d : dilution factor

Fat-content analysis

Fat contents were analysed by Gerber method, following the National Standard for Raw Milk Analysis [9]

Analysis data

The heat-treatments efficiency in reducing the bacterial counts were analysis considering the temperature history of the treatments. The "Under Curve Area" (UCA) analysis was carried out to the recorded temperature profiles of the treatments using GNU Octave software package [10].

Table 1. Bacterial Counts of Milk Samples Before and After the Heat-Treatments at Pasteurisation Temperatures

Sample	C0	Ct	Δ	Sample	C0	Ct	Δ
Salatiga 1	$1.41 \cdot 10^{5}$	$3.85 \cdot 10^{2}$	$2.74 \cdot 10^{-3}$	Boyolali 21	$5.45 \cdot 10^{4}$	$3.59 \cdot 10^{3}$	$6.58 \cdot 10^{-2}$
Salatiga 2	$3.01 \cdot 10^{5}$	$7.58 \cdot 10^{1}$	$2.51 \cdot 10^{-4}$	Boyolali 22	$8.14 \cdot 10^{4}$	$8.76 \cdot 10^{2}$	$1.08 \cdot 10^{-2}$
Salatiga 3	$2.56 \cdot 10^{5}$	$7.42 \cdot 10^{2}$	$2.90 \cdot 10^{-3}$	Boyolali 23	$3.42 \cdot 10^{4}$	$9.35 \cdot 10^{2}$	$2.73 \cdot 10^{-2}$
Salatiga 4	$1.99 \cdot 10^{7}$	$2.06 \cdot 10^{3}$	$1.04 \cdot 10^{-4}$	Boyolali 24	$4.00 \cdot 10^{5}$	$4.44 \cdot 10^{2}$	$1.11 \cdot 10^{-3}$
Semarang L	$4.85 \cdot 10^{5}$	$1.77 \cdot 10^{3}$	$3.65 \cdot 10^{-3}$	Boyolali 25	$5.45 \cdot 10^{4}$	$1.99 \cdot 10^{5}$	$3.64 \cdot 10^{0}$
Semarang K	$2.18 \cdot 10^{6}$	$6.26 \cdot 10^{3}$	$2.87 \cdot 10^{-3}$	Boyolali 27	$5.52 \cdot 10^{3}$	$1.68 \cdot 10^{3}$	$3.04 \cdot 10^{-1}$
Semarang 8D	$6.36 \cdot 10^{4}$	$1.09 \cdot 10^{2}$	$1.72 \cdot 10^{-3}$	Boyolali 28	$5.70 \cdot 10^{5}$	$4.70 \cdot 10^{2}$	$8.25 \cdot 10^{-4}$
Semarang 6D	$2.97 \cdot 10^{6}$	$6.23 \cdot 10^{2}$	$2.09 \cdot 10^{-4}$	Boyolali 29	$5.45 \cdot 10^{4}$	$3.55 \cdot 10^{3}$	$6.51 \cdot 10^{-2}$
Semarang 9D	$3.17 \cdot 10^{3}$	$8.55 \cdot 10^{1}$	$2.69 \cdot 10^{-2}$	Boyolali 30	$4.54 \cdot 10^{3}$	$6.36 \cdot 10^{3}$	$1.40 \cdot 10^{0}$
Semarang 14D	$9.09 \cdot 10^{3}$	$3.50 \cdot 10^{1}$	$3.85 \cdot 10^{-3}$	Boyolali 31	$6.75 \cdot 10^{2}$	$3.15 \cdot 10^{2}$	$4.66 \cdot 10^{-1}$

C0: initial bacterial-counts, Ct: bacterial-counts after heat-treatments, Δ: ratio of final over initial bacterial-counts

Results & Discussion

The initial bacterial counts and the counts after the heat-treatments at pasteurisation temperatures is presented in Table 1. Most of initial counts of bacteria of the milks samples collected from the farmers were lower than the value standardised ($1 \cdot 0 \text{x} 10^{6}$ cfu/mL) [5]. Such low observed values were interesting when consider the facts that sanitary practices carried out by farmers during milking were apparently low (see Illustration 2). In fact, the farm system as well as its environment potentially contribute to the contamination [11–13]. A deep investigation is therefore required to observe the reason of this low initial bacterial counts.

Table 2. The pH Values of Milk Samples Collected from The Farmers

Sample	pH	Sample	pH
Salatiga 1	4.60	Boyolali 21	6.93
Salatiga 2	4.41	Boyolali 22	6.77
Salatiga 3	5.16	Boyolali 23	6.88
Salatiga 4	4.61	Boyolali 24	6.83
Semarang L	6.76	Boyolali 25	6.72
Semarang K	6.79	Boyolali 27	6.94
Semarang 8D	6.45	Boyolali 28	6.87
Semarang 6D	6.46	Boyolali 29	6.86
Semarang 9D	6.56	Boyolali 30	6.83
Semarang 14D	6.52	Boyolali 31	6.98

Table 3: The Fat Contents of Milk Samples Collected from the Farmers

Sample	Fat [%]	Sample	Fat [%]
Salatiga 1	3.40	Boyolali 21	2.40
Salatiga 2	3.45	Boyolali 22	2.55
Salatiga 3	3.20	Boyolali 23	2.40
Salatiga 4	3.45	Boyolali 24	2.80
Semarang L	3.45	Boyolali 25	2.75
Semarang K	2.95	Boyolali 27	2.65
Semarang 8D	3.50	Boyolali 28	2.45
Semarang 6D	3.75	Boyolali 29	2.05
Semarang 9D	3.70	Boyolali 30	3.05
Semarang 14D	3.50	Boyolali 31	3.25

Table 4. Under Curves Area (UCA) Generated Using Octave Software Package of Temperature Profiles of the Heat-Treatments Applied on Milk Samples

Sample	UCA [°C·s]	Sample	UCA [°C·s]
Salatiga 1	54936	Boyolali 21	38921
Salatiga 2	54936	Boyolali 22	22154
Salatiga 3	54936	Boyolali 23	26257
Salatiga 4	30162	Boyolali 24	21084
Semarang L	30162	Boyolali 25	21920
Semarang K	30162	Boyolali 27	25467
Semarang 8D	29426	Boyolali 28	24532
Semarang 6D	29426	Boyolali 29	26046
Semarang 9D	29426	Boyolali 30	25265
Semarang 14D	29426	Boyolali 31	25322

Milk acidity as presented in pH values indicate acid contents as influenced by bacterial activities in converting lactose to acid [7]. Most of the pH values were close to the standard value 6.3 – 6.8 [5] but tended to higher in samples of Boyolali 21, Boyolali 23, Boyolali 27, Boyolali 28, Boyolali 29, Boyolali 31 (see Table 2). Furthermore, the observed pH values of milk samples were did not perfectly correspond to the initial bacterial-counts in cases of Salatiga 1, Salatiga 2 and Salatiga 3 samples (see Table 1 and Table 2).

The reduction in bacterial counts varied among samples. Half of them achieved 2 log cycles or more and the rest lower (samples of Semarang 9D, Boyolali 21, Boyolali 22, Boyolali 23, Boyolali 25, Boyolali 27, Boyolali 29, Boyolali 30 and Boyolali 31, see Table 1).

Illustration 1. A milking practise by a farmer did not consider the sanitary principal

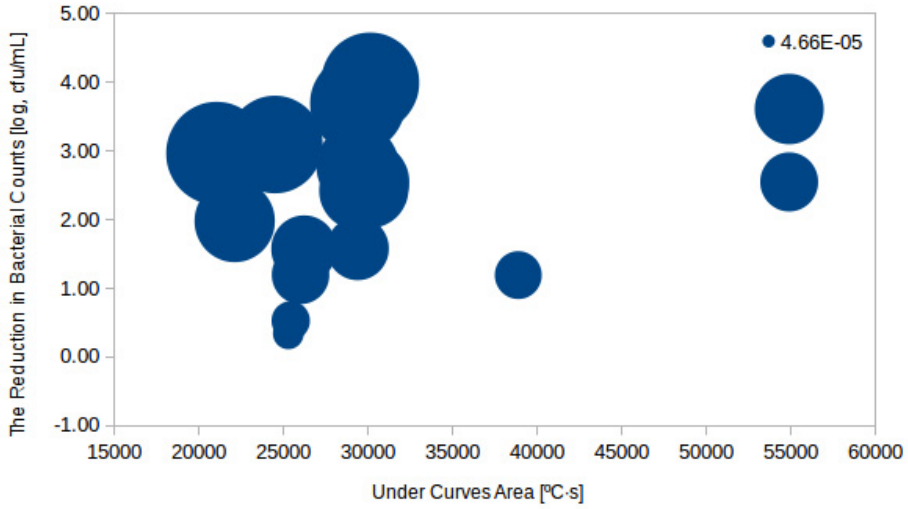

Illustration 2.The Distribution of Bacterial-Counts Reduction over Under Curves Area of Heat-Treatments Applied on Milk Samples. Bubbles' sizes indicate the effect of heat treatments on the reductions [log, cfu·mL-1·°C-1·s-1]

The heat is the main factor influencing the reduction in bacterial-counts [7]. The heat applied on the samples seemed to be uneven (see Table 4). Such uneven heat-treatments happen in daily practices

considering the fact that low controlled heating instruments are usually used e.g. cooking pan on gas stove and therefore potentially contribute to unevenly safe of ready to consume milk produced. An analysis of Under Curve Area indicated that uneven heats. The distribution as well as the effect of the heats on the reduction in bacterial counts varied (see Illustration 1).

Other factors which probably contributed in the uneven reduction in bacterial counts were some environmental variables including pH [14,15], water activity [16,17] and fatty acids [18,19] supplied by fat contents (see Table 3) that are potential to protect the organisms against the heat-treatments. Half of collected milk samples contained fat lower than 3.0 % standardised [5]. The occurrence of spore forming strain also possibly influenced such a variation. Those spores germinated at pasteurisation temperature so that they were observed during bacterial enumeration [20–27].

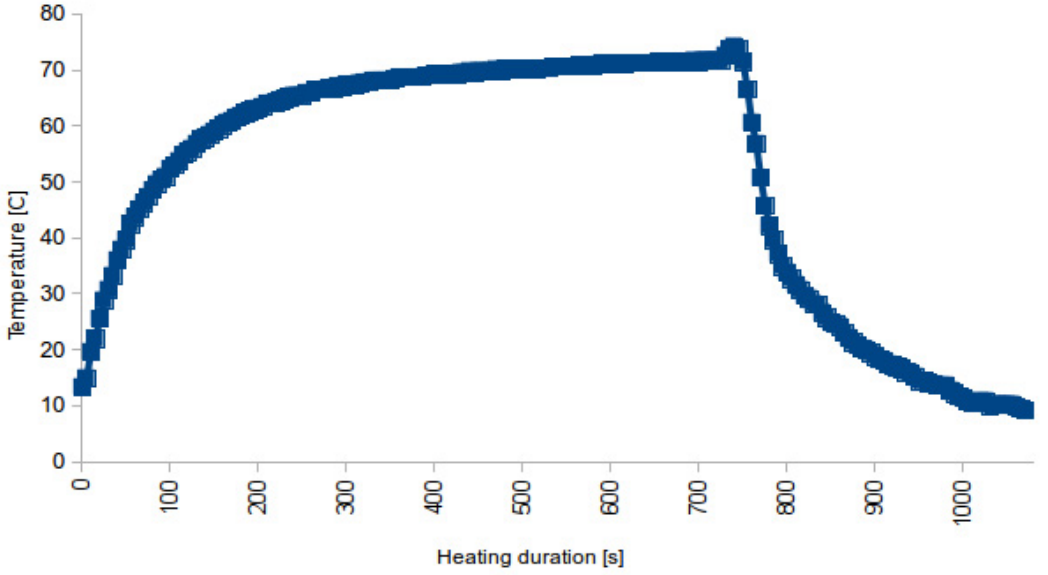

Illustration 3. A Sample of Temperature History of a Heat-Treatment Applied on a Milk Sample

Conclusion

Initial bacterial counts were apparently to low, under the limit by National Standardisation Agency of Indonesia. The reductions in bacterial counts as influenced by the heat treatments at pasteurisation temperature were uneven due to uneven heat-treatments, milk properties and heat-resistant strains which potentially presented in milk samples.

Acknowledgement

The authors would like to thank Diponegoro University for the project financed under the National Non-Tax Budgets (PNBP). The authors also thank Anugrah Tamam Basroni, Fransisca Pramiarti Pasca and Adam Syafri for assisting the laboratory works.

References

[1] The Central Java Office for Livestock and Veterinary, Monitoring and Evaluation of Livestock Development and Veterinary in Central Java, Semarang, 2012.
[2] The Central Java Office for Livestock and Veterinary, Monitoring and Evaluation of Livestock Development and Veterinary in Central Java, Semarang, 2013.
[3] SUARA MERDEKA – (National Newspaper), Some Students of an Elementary School were Indicated being poisoned by Milk (in Indonesian). http://suaramerdeka.com/harian/0511/30/nas17.htm (accessed October 09, 2014).
[4] Fokus Indosiar (Indonesian News), Being Poisoned by Milk, Tens of Students of an Elementary School became Victims (in Indonesian) http://www.indosiar.com/fokus/keracunan-susu-puluhan-siswa-sd-jadi-korban_74674.html (accessed October 09, 2014).
[5] National Standardisation Agency of Indonesia, National Standard of Indonesia for Raw Milk, Indonesia, 2011.
[6] National Standardisation Agency of Indonesia, National Standard of Indonesia for Pasteurised Milk, 1995.
[7] G. Bylund, Dairy Processing Handbook, Tetra Pak Processing Systems AB, Lund, Sweden, 2003.
[8] VDLUFA, Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten Methodenbuch, (1985).
[9] National Standardization Agency of Indonesia, National Standard Method for Raw Milk Analysis, Indonesia, 1998.
[10] J.W. Eaton, GNU Octave, (n.d.). http://www.gnu.org/software/octave/ (accessed October 09, 2014).
[11] A. Coorevits, V. De Jonghe, J. Vandroemme, R. Reekmans, J. Heyrman, W. Messens, et al., Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms, Syst. Appl. Microbiol. 31 (2008) 126–140. doi:10.1016/j.syapm.2008.03.002.
[12] M.C. te Giffel, A. Wagendorp, A. Herrewegh, F. Driehuis, Bacterial spores in silage and raw milk, Antonie Van Leeuwenhoek. 81 (2002) 625–630. doi:10.1023/A:1020578110353.
[13] P. Scheldeman, A. Pil, L. Herman, P. De Vos, M. Heyndrickx, Incidence and Diversity of Potentially Highly Heat-Resistant Spores Isolated at Dairy Farms, Appl. Environ. Microbiol. 71 (2005) 1480–1494. doi:10.1128/AEM.71.3.1480.
[14] O. Couvert, I. Leguerinel, P. Mafart, Modelling the overall effect of pH on the apparent heat resistance of Bacillus cereus spores, Int. J. Food Microbiol. 49 (1999) 57–62.
[15] M.A. Casadei, R. Ingram, R.J. Skinner, J.E. Gaze, Heat resistance of Paenibacillus polymyxa in relation to pH and acidulants, J. Appl. Microbiol. 89 (2000) 801–806.
[16] L. Coroller, I. Legue, P. Mafart, Effect of Water Activities of Heating and Recovery Media on Apparent Heat Resistance of Bacillus cereus Spores, Appl. Environ. Microbiol. 67 (2001) 317–322. doi:10.1128/AEM.67.1.317.
[17] M. Lopez, M. Mazas, S. Martınez, A. Bernardo, R. Martin, Thermal inactivation of Bacillus cereus spores affected by the solutes used to control water activity of the heating medium,

Int. J. Food Microbiol. 53 (1999) 61–67.
[18] L.H. Ababouch, L. Grimit, R. Eddafry, F.F. Busta, Thermal inactivation kinetics of Bacillus subtilis spores suspended in buffer and in oils, J. Appl. Bacteriol. 78 (1995) 669–676. doi:10.1111/j.1365-2672.1995.tb03114.x.
[19] B.M. Lekogo, L. Coroller, A.G. Mathot, P. Mafart, I. Leguerinel, Modelling the influence of palmitic, palmitoleic, stearic and oleic acids on apparent heat resistance of spores of Bacillus cereus NTCC 11145 and Clostridium sporogenes, Int. J. Food Microbiol. 141 (2010) 242–247. doi:10.1016/j.ijfoodmicro.2010.05.023.
[20] H. Terano, K. Takahashi, Y. Sakakibara, Characterization of Spore Germination of a Thermoacidophilic Spore-Forming Bacterium, Alicylobacillus acidoterrestris, Biosci. Biotechnol. Biochem. 69 (2005) 1217–1220.
[21] R.G.K. Leuschner, P.J. Lillford, Thermal properties of bacterial spores and biopolymers, Int. J. Food Microbiol. 80 (2003) 131–143.
[22] P.A. Hoskisson, G. Hobbs, G.P. Sharples, Response of Micromonospora echinospora (NCIMB 12744) spores to heat treatment with evidence of a heat activation phenomenon, Lett. Appl. Microbiol. 30 (2000) 114–117.
[23] T.R. Gurney, L.B. Quesnel, Thermal Activation and Dry-heat Inactivation of Spores of Bacillus subtilis MD2 and Bacillus subtilis var. niger, J. Appl. Bacteriol. 48 (1980) 231–247.
[24] H.R. Curran, F.R. Evans, Heat Activation Inducing Germination in the Spores of Thermotolerant and Thermophilic Aerobic Bacteria, J. Bacteriol. 49 (1944) 335–346.
[25] N. Finley, M.L. Fields, Heat Activation and Heat-induced Dormancy of Bacillus stearothermophilus Spores, 10 (1961) 231–236.
[26] M.T. Hyatt, H.S. Levinson, Water Vapor, Aqueous Ethyl Alcohol, and Heat Activation of Bacillus megaterium, J. Bacteriol. 95 (1968) 2090–2101.
[27] F.F. Busta, Z.J. Ordal, Heat-Activation Kinetics of Endospores Bacillus subtilis ", in: Twentythird Annu. Meet. Inst. Food Technol., Detroit, Michigan, 1963: pp. 345–353.

Presented at ISFA (September 16-17, 2014-Semarang, Indonesia) as Paper #119, "Managing Biosafety and Biodivesity of Food from Local to Global Industries