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Aims and Scope

The International Journal of Sustainable Future for Human Security (J-SustaiN) originated from the need to provide an inter-disciplinary forum where the most serious problems affecting a sustainable future for human security can be discussed, in recognition of the fact that many future problems cannot be solved by a "siloed" approach. The emphasis on sustainable futures is in response to the general awareness of the need to solve numerous human-related problems resulting from the rapid growth of modern society. The topic of sustainable futures for human security needs to be discussed in an integrated way, in accordance with the principles of sustainability, considering energy and materials supply, economies and trade, technology, cities, agriculture, social and environmental aspects.

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Contents

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Effectiveness of Low Thermal Destruction on Drinking Water Contain Enteropathogenic Bacteria Isolated from Wellspring at Mojo VillageLumajang Regency-Indonesia

N. Nurhayati[a,b]*, Aditya Oktavianto[a], Enny Suswati[c], Dedy Eko Rahmanto[d]

[a]**Faculty of Agricultural Technology – University of Jember, Jl. Kalimantan No. 37 FTP Jember 68121**
[b]**Center for Development of Advanced Science and Technology University of Jember, Jl. Kalimantan 37, Gedung CDAST Jember 68121**
[c]**Faculty of Medicine – University of Jember, Jl. Kalimantan No. 37 FK Jember 68121**
[d]**Department of Engineering – Jember State Polytechnic, Jl. Mastrip PO BOX 164 68101**

Abstract

Thermal processes, such as pasteurization are a component of strategy to ensure microbiological safety and food preservation. The objectives of this study were to evaluate the enteropathogenic bacteria population of Mojo wellspring and determine the effectiveness of low thermal destruction for water drinking contain enteropathogenic bacteria (*Salmonella*sp. and *Eschericia coli*sp) indigenous Mojo wellspring. Population of enterophatogenic bacteria were determined by using *Salmonella Chromogenic Agar* (SCA) and *Enteric Hectoin Agar* (HEA). SCA resulted blue colony for *E. coli* and magentha or violet colony for *Salmonella*. While HEA resulted orange colony for *E. coli* and green for *Salmonella*. Thermal destruction used heating treatment at 70°C, 80°C and 90°C temperature for 1 minute or 2 minutes. Effectiveness of destruction based on the value of the destruction percentage and the coefficient of destruction (k value). The calculation equation was used to determine the destruction percentage of bacteria cell. The equation was percentage of the dead bacteria population divided with the initial bacterial population. The k value obtained using the equation was k = (ln N_o – ln N_t)/t. The result showed that the highest destruction was heating treatment at 90°C temperature for 2 minutes. The percentage of destruction were 83.19% for *E. coli* and 74.78% for *Salmonella*, while k value were respectively 0,89 and 0,69.

Keywords: wellspring; enterophatogenic bacteria; coefficient of destruction; Escherichia coli; Salmonella

* *Corresponding Author: N. Nurhayati*
Tel.: +62331321786   ; Fax.: +62331321784   ;
E-mail: nurhayati.ftp@unej.ac.id; nurhayatiftp@yahoo.com

Abbreviations:

SCA	:	salmonella chromogenic agar
HEA	:	hektoen enteric agar
CFU	:	colony form unit
G	:	Guanine
C	:	Cytosine
DNA	:	Deoxyribonucleic Acid
SNI	:	Standar Nasional Indonesia

1. Introduction

Globally, sustainability of water has been becoming an increasingly important issue in recent decades, and the nexus of water quality and consumption with various economic sectors is important within this. Indonesia has been well-known as an agricultural country for a long time, while more recently various leading agroindustry products have been increasing due to demand from world consumption. On the other hand, wastewater production has increased in parallel with agroindustry production[1].

Availability of clean, healthy and safe water is a vital requirement for human life. One of the villages in need of clean water in Indonesia is Mojo village of Padang District, Lumajang. The village experiences difficulty in supplying sufficient water every dry season. The clean water crisis in Mojo was because the water sources were not used effectively. It caused the community of Mojo village to experience difficulty in obtaining water mainly for cooking and drinking. Mojo village has nine water sources (wellsprings) that are still hampered by the low position from the residents.

The microbiological quality of the water must be free from contamination of pathogenic bacteria especially the sanitation indicator bacteria i.e coliform group. Enteropathogenic bacteria that contaminate water include: *Salmonella* sp, and *Eschericia coli* sp..

Salmonella is the most important bacterial pathogen contained in waste water. It can cause typhoid fever, paratypus and gastroenteritis (inflammation of the stomach/abdomen). Nearly 0.1% of the population show the presence of *Salmonella* in stools. *Salmonella* is a straight rod bacterium, gram-negative, non-spore-forming, peritric flagella. The optimum growth temperature of *Salmonella* sp. is 37°C at pH 6-8. DNA base composition of *Salmonella* sp. was 50-52 mol% G + C, similar to *Eschericia*, *Shigella*, and *Citrobacter*[2].

Eschericia coli is one of the main species of gram-negative bacteria. It is one of the normal inhabitants of the gastrointestinal tract of humans and warm-blooded animals. *E. coli* are rod-shaped bacteria, gram-negative, non-spore-forming, aerobic, facultative adverse lactose to produce acid and gas during 48 hours at 35°C[3]. In general, bacteria found by *Theodor Eschericia*, it can cause problems for all health for humans such as diarrhea, vomiting and other digestive problems. These bacteria ferment glucose and other carbohydrates by converting pyruvate into lactic acid[4]. Most strains ferment lactose. *E. coli* forms indole in large numbers, strongly reducing nitrate. *E. coli* DNA base composition was 48-52 mol% G + C Mol[5].

One way to kill *Salmonella* sp. and *E. coli* is using heating methods such as boiling the water[6]. This technique is used to kill gram-negative bacteria such as *Salmonella* sp, and *Escherichia coli* sp. The objective of this study was to develop the first predictive microbiology model for survival and growth of a low initial dose of

Salmonella and *Eschericia coli* sp isolated from Mojo wellspring in drinking water after treatment by thermal processes.

Treatment using low temperature thermal destruction was considered more applicable and eco-friendly than destruction using chemical compounds. Pasteurization for 1 minute at 75°C yielded average log reductions of 5.48 ± 1.22 for *Salmonella*, 5.71 ± 0.40 for *E. coli* O157:H7 and 5.23 ± 0.61 for *E. faecium*[7]. Further research can examine the use of various temperatures of thermal destruction to aid the exposure assessment phase of quantitative microbial risk assessment. The predictive models will continue to be an essential element of exposure assessment within formal quantitative risk assessment [8].

2. Materials and Methods

2.1 Materials

Water sampling locations include three springs in the village of Padang District Lumajang Mojo - Jirun, Kali Tengah and Sumber Suko Wellspring. Jirun was the primary wellspring to supply seven subsequent springs. Six samples of the water were taken from Mojo village wellspring i.eJirun, Kali Tengah, Sumber Suko, Tandon 1 Jirun, Tandon 2 Jirun, and Tandon 3 Jirun.

Figure 1 Sampling the water from The Wellspring at Mojo Village Lumajang Regency-Indonesia

2.2 Isolation of Enterophatogenic Bacteria

Enterophatogenic bacteria were isolated by using fluorogenic and chromogenic medium i.esalmonella chromogenic agar (SCA) and hektoen enteric agar (HEA). Colonies on SCA media produce a blue color colony of *Eschericia coli sp*. And purple/magenta color colonies of *Salmonella* sp. Colonies on HEA media produce yellow color colonies of *E. coli* sp. and green color colonies of *Salmonella* sp. The methods for isolation of *Salmonella* were undertaken according to the ISO-6579 standard but other methods may be used [9]. Application of SCA and HEA media for enterophatogenic identification can be valid methods to obtain data on populations. Commonly, SCA was considered more valid to determine *Salmonella* and *E. coli*, while HEA was more valid to determine *enterobacteraceae* from human faeces [10]

2.3 Experimental design

Thermal destruction was conducted by heating the water. Steril aquades(100 ml) was inoculated with 1 ml of *Salmonella* sp or *E. coli* sp (10^6CFU/ml) then the water was heated to various temperatures i.e 70°C (B1), 80°C (B2) and 90°C (B3) for one minute (C1) and two minutes (C2). Analysis of enteropathogenic bacteria populations used fluorogenic and chromogenic medium and was undertaken following the bacteriological analytical manual (BAM) method [11]. The consideration of selected heating conditions

(70°C, 80°C and 90°C for 1 minute or 2 minutes) were based on the minimum pasteurization temperature 70°C. These were also practical for community applications using a dispenser (heating drinking water) which can reach 90 °C.

2.4. Determination of enterophatogenic bacteria population

The enterophatogenic bacteria population was calculated from two serial dilutions by the BAM method [11]. When counts of duplicate plates fall within and without the 25-250 colony range, use only those counts that fall within this range and calculate using the following equation:

$$N = \{\Sigma C / [(1 \times n_1) + (0.1 \times n_2) \times (d)]\}$$

where N = number of colonies per ml of water, ΣC = sum of all colonies on all plates counted, n_1 = number of plates in first dilution counted, n_2 = number of plates in second dilution counted, d = dilution from which the first counts were obtained.

2.5. Analytical of mathematical models

Mathematical models determined effectiveness of thermal treatments using Adam and Moss`s Equation (2008) [12]. The mathematical models of percentage of destruction apply the following equation:

$$\%Destruction= [(N_o – N_t)/ N_o] \times 100\%$$

Determination of the k value (coefficient of destruction) used the following equation: $k = (\ln N_o – \ln N_t) / t$

Where N_o = bacteria population at 'o' time ($\log_{10}$CFU/ml),N_t= bacteria population at particular time ($\log_{10}$CFU/ml), t = time for thermal destruction (minute), k = coefficient of destruction ($\log_{10}$/min).

2.6. Statistical analysis

The analyses were performed in triplicate, and results were expressed as mean values with standard deviations. Data were analyzed using the statistical analysis package of Microsoft Excel 2007. The differences between the experimental groups and the control containing glucose were evaluated using a Student`s *t*-test.

3. Results and Discussion

3.1 Enteropatogenic Bacteria Population of the Mojo Wellspring

a. Population of enteropathogenic bacteria in the wellspring

Six wellsprings at Mojo village contained enteropathogenic bacteria. Table 1 shows the population of enteropathogenic bacteria of Mojo wellsprings. Each of the wellsprings contain *Salmonella* sp. The highest population was from Tandon 3 Jirun, ie 5.2×10^1 CFU/ml and *E. coli* sp. only in Jirun wellspring, Kali Tengah and Sumber Suko with most colonies in the Jirun wellspring, ie 1.7×10^3 CFU/ml. The bacteria contamination growing in the Jirun wellspring comes from human faecal or freshwater fish especially "waders".

The growth of *Salmonella* sp. in the six water samples can be the source of disease in the Mojo village. Salmonellosis can trigger the disease, such as typhoid fever, paratypus and gastroenteristis if water is incorrectly processed.

Table 1 Population of *Salmonella* and *Eschericia coli* isolated from Wellspring at Mojo Village Lumajang Regency-Indonesia

Wellspring/Water Sources	Population ($\log_{10}$CFU/ml)	
	Salmonella	*E. coli*
Jirun	0.30 ± 0.01	3.32 ± 0.14
Tandon 1 Jirun	0.90 ± 0.02	0.15 ± 0.01
Tandon 2 Jirun	0.15 ± 0.03	0.15 ± 0.06
Tandon 3 Jirun	1.72 ± 0.01	0.24 ± 0.17
Kali Tengah	1.25 ± 0.42	1.25 ± 0.03
Sumber Suko	0.77 ± 0.10	1.11 ± 0.05

Based on the SNI standard, population of *Salmonella* or *E. coli* must be zero in food (ready to eat) including drinking water [13]. While the existence of two types of bacteria is tolerated in the raw materials still in processing stage, if the preparation process could destroy the pathogenic bacteria.

b. Morphology and physiology of enteropathogenic bacteria

On Gram staining, *Salmonella* and *E. coli* are red in color, so that both bacteria are called gram-negative bacteria. *Salmonella* showed rods cell with straight and shorter rods than *E. coli*. *Salmonella* produces catalase enzyme but not more than *E. coli*. *E.coli* can produce gas in lactose broth (LB) media, while *Salmonella* does not [14].

Figure 2 showed there was *Salmonella* sp marked with a purple colony. In addition, also found a blue colony that indicated the colonies was *E. coli* sp.
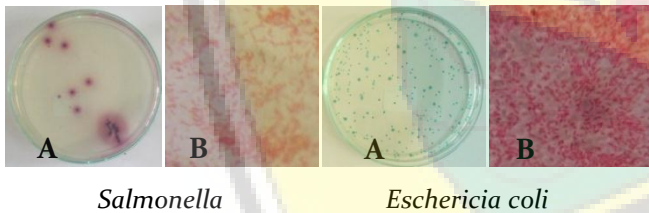
Figure 2 Appearance of *Salmonella* (violet color) and *Eschericia coli* (blue color) on: SCA media (A), microscopy appearance at 1000x magnification (B)

c. Thermal destruction of enteropathogenic bacteria isolated from Mojo village

Thermal destruction of *Salmonella* sp dan *Eschericia coli* sp were conducted at 70°C, 80°C or 90°C for 1 and 2 minutes. The result showed that the treatments were capable of killing enterophatogenic bacteria (Figure 3.). According to Hariyono [13] *Salmonella* sp. and *E. coli* were unable to grow at temperatures more than 56°C for 30 minutes and *Eschericia coli* sp [15]. In the current treatment, the temperature was higher (equal to, or more than, 70°C), but the time was shorter, at just 1 minute or 2 minutes.
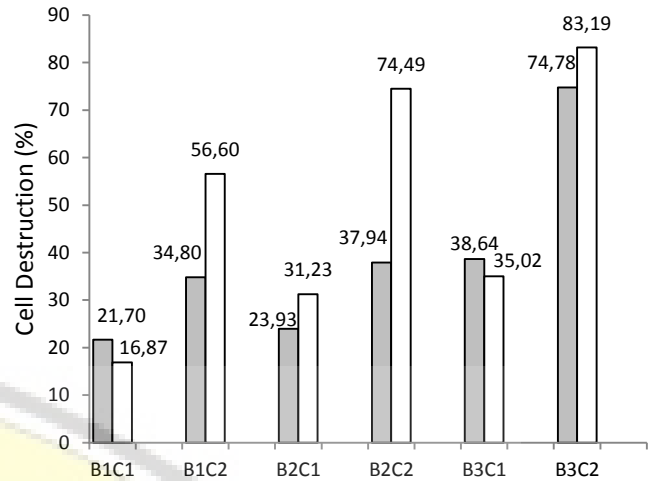
Figure 3 Percentage of destruction of *Salmonella* sp. (⬜), *Escherichia coli* sp. (⬜) at heating temperature 70°C(B1), 80°C(B2), 90°C(B3) for 1 minute(C1) and 2 minutes(C2)

Figure 3 showed the highest percentage destruction was at B3C2 treatment both of *Salmonella* and *Escherichia coli*, which were respectively 74.78% and 83.19%. The lowest percentage destruction was at B1C1, ie respectively 21.70% for *Salmonella* and 16.87% for *Escherichia coli*. Increasing temperature or the heating time increased percentage of destruction. All treatments increased the percentage ofdestruction if the heating temperature was 2 minutes (C2).

The percentage destruction of *E. coli* was greater after heating at 90°C for 2 minutes (83.19%) than *Salmonella* (74.78%), showing a reversal of relative performance. After heating for 1 minute, most of the bacteria were still able to survive, in contrast to *Salmonella* that had suffered greater destruction than *E. coli* in the 1 minute heating treatment.

Differences of gram-negative bacteria resistance to warming could be due to differences in the peptidoglycan components of the cell such as lipoprotein, outer membrane and lipopolysaccharide (LPS). Although the peptidoglycan is thinner, gram-negative bacteria can with stand the heat for some time because of the complex ties of the constituent components. Lipoprotein serves as a stabilizer in the outer membrane and outer membrane attachments. LPS is bound to the outer membrane with a hydrophobic bond [12].

The lipid component of *Escherichia coli* cell walls was greater than *Salmonella*. *E. coli* and was more resistant to heat than *Salmonella*,because the heat will first interact with the lipid constituent of the cell wall [15].

3.2. Coefficient of destruction (k value)

Coefficient of destruction (k value) was determined as the number of cell deaths per minute for thermal destruction. The greater k value indicated that the bacterial cells were more sensitive to the heating process. The k value also showed when the heating treatments were enough to kill germs either *E. coli* or *Salmonella*. Figure 4 shows the coefficient of destruction (k value) for *Salmonella* sp. and *Escherichia coli* sp that were isolated from Jirun wellspring Mojo village.
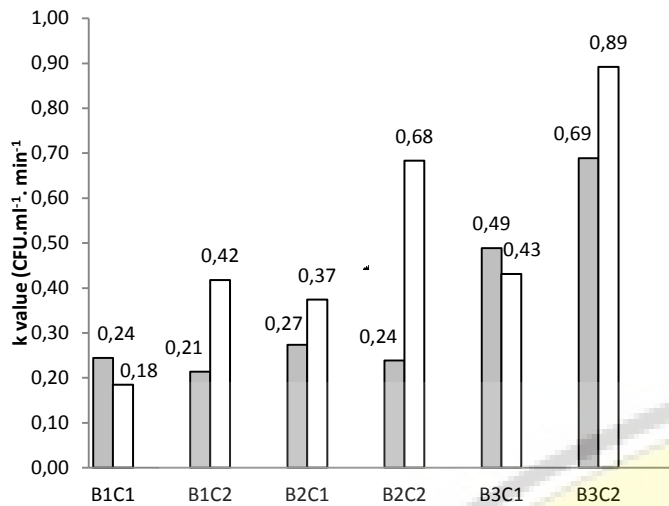
Figure 4 Coefficients of destruction of *Salmonella* sp. (▨) and *Escherichia coli* sp. (☐) at heating temperature: 70 °C(B1), 80 °C(B2), 90 °C(B3) for 1minute (C1) and 2 minutes (C2)

Coefficient of destruction increased significantly at high temperatures (90°C) and 2 minutes of heating treatment. The coefficient of destruction *for* both were highest at B3C2 treatment (k value = 0.69 and 0.89, for *Salmonella* and *E. coli* respectively). The k value difference of *Salmonella* and *E. coli* was caused by differences in the composition of the cell wall constituent components. The k value is also high (≤ 1) indicates the number of destroyed bacteria in 10 CFU/ml or $\log_{10}$ units/min.

4. Mojo Wellspring Sustainability

Ground water and surface water have begun to decrease in carrying capacity. This is indicated by the rapid decline of wellspring deficit in the dry season. The other condition is that they are easily flooded by a slight increase of rainfall. Carrying capacity of the soil to absorb and transmit rain water has begun to decrease.

The efforts to safeguard the sustainability of the wellsprings can be undertaken by carrying out reforestation around the wellspring. Reforestation can be conducted by planting Perennials (hardwoods) like sengon (*Albizia chinensis*), trembesi (*Samanea saman*). Perennials can store more soil water than shrubs. Ensuring that the area around the wellspring is free from factories or settlements that could contaminate the wellsprings is also important. Potential wastewater, especially from home waste water could be a major problem as well.

Effective waste water treatment can reduce and remove microbial contaminant loads to safe levels. Encouraging proper sanitation and wastewater treatment facilities in the Mojo village will resultnot only in improvements in human health but it would also indirectly result in improvements in ecosystem health.

Human intervention can result in significant effects on waterquality. Some of these effects are the result of hydrological changes, such as the building of dams, draining of wetlands and diversion of flow. More obvious are the polluting activities such as the discharge of domestic and industrial waste, and the spreading of chemicals on agricultural land in the drainage basin.

Wellspring pollution by human faeces is attributable to only one source, but the reasons for this type of pollution, its impacts on water quality and the necessary remedial or preventive measures are varied. Faecal pollution may occur because there are no community facilities for waste disposal, because collection and treatment facilities are inadequate or improperly operated,or

because on-site sanitation facilities (such as latrines) drain directly into aquifers. All of these facets are illustrated in Fig. 5.

Figure 5 Wellsprings Sustainability at Mojo Village Lumajang Regency-Indonesia

5. Conclusion

The water sampled from three wellsprings at Mojo Village Lumajang Regency (Jirun, Kali Tengah and Sumber Suko) contained bacteria that can cause gastrointestinal disorders (enteropathogenic) i.e *Salmonella* sp. and *Escherichia coli* sp. Thermal destruction using heating at 90 ° C for 2 minutes was more effective (thermal adequate) to destroy *Salmonella* (74.49%) and *E. coli* (83.19%). The coefficient of destruction (k value) of The heating process at 90°C for 2 minutes were 0.89 for *E. coli* and 0.69 for *Salmonella*. It can be recommended that the drinking water must be heated at the minimum temperature and time (90°C for 2 minutes) or boiled before consuming.

6. Future Prospect

Utilization of wellsprings at Mojo Village as drinking water must heated at maximum temperature of pasteurization or boiling process. In addition, other necessary destruction technologies such as filtration membranes to filter out the bacteria cells especially pathogenic bacteria or other microbes should be considered. We suggest that the wellsprings intended for drinking water must avoid contamination from biota such as fish, frogs etc. It also has to be treated with both thermal and non-thermal processes.

Acknowledgements

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