

PAPER • OPEN ACCESS

## Inactivation of Bacteria *S. aureus* ATCC 25923 and *S. Typhimurium* ATCC 14 028 Influence of UV-HPEF

To cite this article: A Bakri *et al* 2018 *J. Phys.: Conf. Ser.* **953** 012122

View the [article online](#) for updates and enhancements.



**IOP | ebooks™**

Bringing together innovative digital publishing with leading authors from the global scientific community.

Start exploring the collection—download the first chapter of every title for free.

## Inactivation of Bacteria *S. aureus* ATCC 25923 and *S. Thyphimurium* ATCC 14 028 Influence of UV-HPEF

A Bakri<sup>1</sup>, B Hariono<sup>2</sup>, M M D Utami<sup>3</sup>, Sutrisno<sup>4</sup>

<sup>1,2,3</sup> Politeknik Negeri Jember, <sup>4</sup>Institut Pertanian Bogor

abibakri@gmail.com<sup>1</sup>, budihariono1966@gmail.com<sup>2</sup>, merry.mdu@gmail.com<sup>3</sup>

**Abstract.** The research was objected to study the performance of the UV unit - HPEF in inactivating bacteria population of Gram-positive (*S aureus* ATCC 25923) and Gram-negative (*S Thyphimurium* ATCC 14028) inoculated in sterilized goat's milk. UV pasteurization instrument employed three reactors constructed in series UV-C system at 10 W, 253.7 nm wavelength made in Kada (USA) Inc. with 1.8 J/cm<sup>2</sup> dose per reactor. HPEF instrument used high pulsed electric field at 31.67 kV/cm, 15 Hz and goat's milk rate at 4:32 ± 0.71 cc/second. Pathogenic bacteria was observed According to Indonesian National Standard 01-2782-1998. Inactivation rate of pathogenic bacteria ie *S Thyphimurium* ATCC 14028 and *S. aureus* ATCC 25923 was 0.28 and 0.19 log cycle or 6.35 and 4.34 log cfu/ml/hour, respectively; D value was 0.16 and 0.23 hour with k value was 14.62 and 10 hour<sup>-1</sup> respectively.

### 1. Introduction

Factor of food security by maintaining the sensory and nutritional quality of food is the basis of the application of a combination of food preservation method called hurdle technology [1]. Consumer awareness of natural food products, products in fresh condition with minimal processing and food ingredients maintained continues to increase, although the product is economically more expensive. It encourages the development of alternative food processing technologies such as UV technology [2] and technology HPEF [3].

UV light has been widely used in the food, though it is still very limited information about the study of UV light applications for foodstuffs were reported [4]. The chamber treatment should be designed to pass liquid food ingredients with a thin layer to medium to increase the rate of microbial inactivation. The level of microbial inactivation depends on the UV dose applied although also a slight increase in temperature due to exposure to UV rays [5].

Treatment HPEF in food produce sensory changes and minimal food ingredients [3], due to foodstuffs subjected to a high voltage electrical pulse field in a short time. The level of microbial inactivation by HPEF method depends on the electric field strength, pulse width and number of pulses [6].

Milk can be one of the means for the development of pathogenic bacteria. In the United States reported that about 76 million cases of food poisoning that caused 325 thousand people are hospitalized, and 5200 deaths [7]. CDC (Centers for Disease Control and Prevention) reports there were 17,248 and 1,413 cases of poisoning caused by *S. aureus* during the periods 1973-1987 and 1983-1987, or about 14% and 1.6% of the total cases were caused by bacterial pathogens [8]. *S. aureus* is found in milk and dairy products as one of the causes.

*Salmonella* contamination in fresh milk in the USA and UK respectively 4.7% and 12.06% [9]. *Salmonella* contamination in fresh milk usually occurs because the milking process that is not



clean and poor sanitation. Outbreak of food poisoning due to *Salmonella* (salmonellosis) still occurs in many countries.

This study aimed to compare the rate of inactivation of pathogenic Gram-positive bacteria (*S. aureus* ATCC 25923) and Gram-negative bacteria (*S. Typhimurium* ATCC 14208) in goat milk inoculated with UV conditions (length 23.5 cm, power 10 W, a dose of 5.4 J/cm<sup>2</sup>) and conditions HPEF (electric field strength 31.67 kV/cm, distance of electrodes 3 mm, a frequency of 15 Hz) and determine the curve of death of bacteria used as the basis for determining the resistance of microbes which is indicated by the value of D (time required to reduce the number of bacteria 1 log cycle) and the value of k (the rate of bacterial sensitivity to UV light and shock electric field high voltage).

## 2. Materials Research

The materials used are cultures of *S. aureus* ATCC 25923 and *S. Typhimurium* ATCC 14208 obtained from culture collections Faculty of Animal Science, Bogor Agricultural University. Other ingredients are goat's milk obtained from the farm people in Ciampea Bogor, media Plate Count Agar (PCA), media Buffer Peptone Water (BPW), media Sodium Agar, media eosin Metylen Blue Agar (EMBA), media Beird Park Agar (BPA), telluride, egg yolk (egg yolk), 0.85% physiological saline, crystal violet, Gram iodine, Lugol, safranin, and alcohol 70%, 90% alcohol.

Materials for observation of morphological changes bacterial preparation with SEM is cacodilat buffer, glutaraldehyde, tannic acid and OSO<sub>4</sub>, ethanol, acetone, resin, film obtained from the Laboratory of Zoology LIPI Cibinong, Bogor.

## 3. Research methods

### 3.1. Preparation of Test Bacteria

Preparation of test bacteria *S. aureus* ATCC 25923 and *S. Typhimurium* ATCC 14208 is performed by examination of bacterial purity through microscopic observation of bacterial preparations with the aid of Gram staining, to determine cell uniformity and free of contaminants. Catalase testing performed by the addition of H<sub>2</sub>O<sub>2</sub> against bacterial preparations. Refresher is done to get bacteria aged 24 hours.

### 3.2. Bacterial Refresher Test

1 ml bacterial stock was grown in media Nutrient Broth (NB), cultured into tubes containing 9 ml of sterile NB media, incubated for 24 hours at 37°C for Standardization population of bacteria by measuring the value of optical density (OD) using a spectrophotometer with a wavelength of 620 nm, to obtain a test population of 10<sup>5</sup> cfu/ml, equivalent to OD of 0.250.

### 3.3. Recontamination on Goat Milk

1000 ml goat milk is sterilized by autoclaving at 115°C for 3 minutes and ready to recontamination with test bacteria which have been cultured beforehand with a concentration of 10<sup>5</sup> cfu/ml.

### 3.4. Calculation of Total Bacteria *S. aureus* (DSN 1998)

Milk samples were taken from the treatment chamber of 1 ml with a micro pipette and put into a test tube which already contains 9 ml of sterile BPW as a tenth dilution (P<sup>1</sup>). The result of this dilution pipette 1 ml for further diluted into 9 ml of sterile BPW as a hundredth dilutions (P<sup>2</sup>). Dilution is done to P<sup>5</sup>. Fertilization is carried out on the dilution P<sup>3</sup> to P<sup>5</sup> in duplicate. 1 ml of each dilution P<sup>5</sup> to P<sup>3</sup> fertilized into a sterile petri dish that already contains media BPA - Egg Yolk Telluride as much as 12-15 ml which has been solidified. The samples were dispersed using a sterile hockey stick. After drying the samples, petri dishes were incubated at 37°C in the inverted position for 24 hours. The same is done on the control sample, i.e. milk inoculated with pathogenic bacteria *S. aureus* ATCC 25923 without treatment UV-HPEF.

### 3.5. Calculation of Total Bacteria *S. Typhimurium* (DSN 1998)

1 ml milk samples were taken using a sterile Pasteur pipette and put into 9 ml Buffer Peptone Water (BPW) as a one-tenth dilution ( $P^{-1}$ ). Decimal dilutions then performed with 1 ml pipette from the tube  $P^{-1}$  for further diluted into 9 ml BPW to obtain a hundredth dilutions ( $P^{-2}$ ). The dilution thus continue to obtain  $P^{-5}$ . Fertilization is carried out on dilution  $P^{-3}$ ,  $P^{-4}$  and  $P^{-5}$  in triple. 1 ml of each dilution  $P^{-3}$ ,  $P^{-4}$  and  $P^{-5}$  pipetted into sterile petri dish and medium fertilized with Salmonella and Shigella Agar (SSA) sterilized at a temperature 37-40°C as much as 12-15 ml. The mixture is homogenized by the way the petri dish is moved to form an eight figure. After that hardens, petri dishes were incubated upside down at 37+1°C for 24 hours.

Analysis of calculation of the number of test bacteria using Standard Plate Count (SPC) which refers to the Bacteriological Analytical Manual (BAM).

## 4. Results and Discussion

### 4.1. Characteristics of Bacteria Test *S. aureus* ATCC 25923

The results of microscopic examination of *S. aureus* bacterium through Gram staining. Characteristics of test bacteria *S. aureus* showed round cell shape, uniform with a single fabric, a pair and a collection of irregular shape like grapes [10]. *S. aureus* bacteria 0.5-1  $\mu\text{m}$  in size, facultative anaerobic, immobilized, non-encapsulated and spore-forming, belonging to mesophyll repellent because it can live at 7-48°C and produce enterotoxin optimally at 37-40°C.

*S. aureus* belonging to Gram-positive bacteria because it produces a purple color when done Gram stain. Gram positive bacteria have cell walls mostly composed of peptidoglycan layer (90%) and other coatings such as teic acid. This thick layer of peptidoglycan retains the complexes of the violet base bases of violet crystals and Iodine (Lugol) solution when it is washed cell preparations with alcohol. The theitic acid in the wall of negatively charged cells will react with the alcohol, thus causing dehydration in the cell wall. Dehydration causes the pores to shrink (decrease) and decrease the permeability of the cell wall, so that the violet crystal complex cannot get out of the cell, causing the cell to remain purple. This condition causes further staining with safranin has no effect on cells [11].

Catalase test results showed that *S. aureus* is catalase positive characterized by gas bubbles produced  $\text{O}_2$  in the bacterial preparations were etched with  $\text{H}_2\text{O}_2$ . These bacteria produce the enzyme catalase to break down the  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$  and  $\text{O}_2$ . Bakteri *S. aureus* have the biochemical characteristics of catalase positive. Component  $\text{H}_2\text{O}_2$  This is one result of aerobic respiratory metabolism of bacteria that can inhibit the growth of bacteria because it is toxic for the bacteria itself so that these components should be split into  $\text{H}_2\text{O}$  and  $\text{O}_2$  [10].

### 4.2. Characteristics of Bacteria Test *S. Typhimurium* ATCC 14 208

Test bacteria *S Typhimurium* was first examined for its characteristics by microscopic observation of bacterial preparations with the aid of Gram staining for the determination of cell uniformity and the absence of contaminants. Morphological characteristics *S. Typhimurium* ATCC 14028 shows a uniform shape stem cells with a single composition or short-chain and relatively bacteria Gram-negative because it produces a red color when done Gram stain.

According to [11], Gram negative bacteria is a bacterium that has a thin layer of peptidoglycan (5-20%) on the cell wall so that when the Gram staining test is in the washing stage with alcohol will cause fat extracted from cell walls and pores Will enlarge so that the violet and iodine crystals complex out of the bacterial cell wall. Subsequent staining with safranin causes red cell bacteria to absorb safranin. [12] Explains that the Salmonella bacterium has the characteristics of rod-shaped Gram-negative, spore-forming, aerobic/facultative anaerobes.

*Salmonella* colonies grown on selective media Shigella and Salmonella In order to have specific characteristics. Bacteria that do not ferment lactose such as Salmonella colonies grow with the characteristics of a colorless, produce  $\text{H}_2\text{S}$ , making the center of the colony becomes black. Tiosulfate

in combination with iron (Fe) as an indicator of sulfide formation is indicated by the blackening of the central part of the colony [13].

Catalase test results found that the bacterium *S. Typhimurium* is positive catalase as indicated by the resulting O<sub>2</sub> gas bubbles form O<sub>2</sub> in growth media drops of H<sub>2</sub>O<sub>2</sub>. It this is in line with the explanation [14] which states that Salmonella bacteria have the biochemical characteristics of catalase positive. These bacteria produce the enzyme catalase to break down the H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub>.

#### 4.3. Inactivation Rate of Pathogenic Bacteria

*S. Typhimurium* is a rod-shaped Gram-negative bacteria, spore-forming, motile with flagella peritrikat measuring 0.7-1.5 μm. *S. Typhimurium* showed normal cell surface smooth, slightly rounded elongated with a diameter of 1.2 - 2 μm [15]; and measuring about 0.7-1.5 x 2.0-5.0 μm [12].

*S. aureus* is a Gram-positive bacterium that is very important in the process of the food product as it can be toxic in humans. These microbes are harmful to health, with a concentration of more than 10<sup>5</sup> cfu/ml can cause disease. *S. aureus* is very resistant, can grow at high salt concentration, water activity (a<sub>w</sub>) is low and relatively low pH [15]. *S. aureus* normal cells are round with a diameter of 0.5-2.0 μm [16], clustered like grapes with smooth surface. *S. aureus* cell wall vary depending on the strain and age cells. Generally young thin wall cells with a thickness of 15 nm and in the old culture ranged 80 nm. According [17] *Staphylococcus* grown at 37°C for 24 hours in broth having diameters ranging from 0.7-0.9 μm with a wall thickness ranging from 50 nm.

Cellular damage mechanisms vary depending on the type of constituent component. [18] Distinguishes the mechanism of the antimicrobial component as follows: 1) influences the cell wall, 2) affects cell membrane and nutrient transport mechanism, 3) affects the enzyme, and 4) influences protein synthesis and lactic acid,

The rate of inactivation, k value of D and *S. Typhimurium* pathogenic bacteria and *S. aureus* treated with UV-HPEF methods listed in Table 1.

**Table 1.** The rate of inactivation ,the value of D and k pathogens with UV-treatment method HPEF

Type of bacteria	Inactivation rate (log cycle)	Inactivation rate (log cfu / ml / hour)	D value (hours)	The value of k (h <sup>-1</sup> )
<i>S. Typhimurium</i>	0.28	6.35	0.16	14.62
<i>S. aureus</i>	0.19	4.34	0.23	10.00

*S. aureus* is a Gram-positive bacterium has a degree of crosslinking between adjacent peptide is very high (100%) with a cell wall that contains the amino acid alanine hydrophobic (Franklin and Snow 1989). While Gram negative cell wall bacteria have hydrophilic side i.e. carboxyl, amino and hydroxyl [19]. Gram negative bacterial cell wall is layered with peptidoglycan layer only 5-20% of cell wall, other layer consists of protein, lipopolysaccharide and lipoprotein [11].

The fundamental difference between Gram positive and negative bacteria is in its cell wall components. The iodine compound is trapped between the cell wall and the cytoplasmic membrane of a Gram-positive organism, whereas the removal of lipid substances from the cell wall of Gram-negative organisms by alcohol washing allows the cell to disappear. Gram-positive bacteria have a single membrane coated with thick peptidohlycan (25-50 nm) whereas Gram-negative bacteria have a thin layer of peptidoglycogen (1-3 nm).

## 5. Conclusion

The conclusions obtained from the research influence the inactivation of pathogenic bacteria *S. aureus* ATCC 25923, *S. Typhimurium* ATCC 14028 with UV-HPEF method to damage bacterial cells:

- a. The rate of inactivation of pathogens *S. Typhimurium* ATCC 14028 and *S. aureus* ATCC 25923 in a row is 0.28 and 0.19 log cycle, the value D respectively 0.16 and 0.23 hours with a value of k successive 14.62 and 10 h<sup>-1</sup>.
- b. Based on the rate of inactivation of the bacteria *S. Typhimurium* is more sensitive to the treatment of the combination of UV-HPEF pasteurization when compared with *S. aureus* or Gram-positive bacteria (*S. aureus*) are more resistant to physical treatment (UV-HPEF) when compared to Gram-negative bacteria.

## References

- [1] Leistner L. 2000. Review: Basic aspects of food preservation by hurdle technology. *Intl J Food Microbiol* 55:181-186.
- [2] Guerrero-Beltrán JA, Barbosa-Cánovas GV. 2004. Advantages and limitations on processing foods by UV Light. *Intl J Food Sci Technol* 10: 137-147.
- [3] Ho S, Mittal GS. 2000. High voltage pulsed electrical field for liquid food pasteurization. *Intl J Food Rev* 16: 395-434.
- [4] Koutchma TN, Larry JF, Carmen IM. 2009. *Ultraviolet Light In Food Technology: Principles and Application*. Boca Raton USA: CRC Press.
- [5] Binstsis T, Litopoulou-Tzanetaki E, Robinson R. 2000. Existing and potential applications of ultraviolet light in the food industry a critical review. *J Sci Food Agric* 80: 637-645.
- [6] Barbosa-Cánovas GV, Góngora-Nieto MM, Swanson BG. 1998. Nonthermal electrical methods in food preservation. *Int J Food Sci Technol* 4: 363-370.
- [7] Mead PS et al. 1999. Food-related illnesses and death in the United States. *Emerg Infect Dis* 5: 607-625.
- [8] Olsen SJ, MacKinon LC, Goulding JS, Bean NH, Slutsker L. 2000. Surveillance for foodborne disease outbreaks - United States. 1992-1997. *Morb Mortal Week Rep* 49: 1-66.
- [9] Anon. 1998. Surveillance of the microbiological status of raw milk milk on retail sale. *Microbiological food safety surveillance*. London: Department of Health.
- [10] Ray B, Bhunia A. 2007. *Fundamental Food Microbiology*. Ed 4th. Boca Raton. New York: CRC Pr.
- [11] Fardiaz S. 1992. *Food Microbiology*. Jakarta: Gramedia Pustaka Utama.
- [12] Bell C and A Kyriakides. 2003. *Salmonella*. Inside Blackburn C and McClure PJ, editor. *Foodborne Pathogens (Hazards, Risk Analysis and Control)*. Cambridge: Woodhead Publishing Limited. Chapter XI.
- [13] Suliantari. 2009. The antibacterial activity and mechanism of inhibition of extracts of green betel (*Piper betle* Linn) against food pathogenic bacteria [dissertation]. Bogor: Graduate Program, Bogor Agricultural University.
- [14] Syamsir E. 2002. Studying the stability of the antimicrobial activity Atung seed extract (*Parinarium glaberrimum* Hassk) with technical ethyl acetate solvent during storage against *S. aureus* [thesis]. Bogor: Graduate Program, Bogor Agricultural University.
- [15] Jay JM. 1996. *Modern Food Microbiology*. Ed 4th. Florence: Chapman & Hall. Int; Thomson Publ.
- [16] Lynn MJ, Bohach GA. 2001. *Staphylococcus aureus*. Inside Doyle MP, Beachat LR, Montville TJ, editor. *Food Microbiology: Fundamentals and Frontiers*. Washington DC: ASM Pr.
- [17] Gemmel GC, Lorian V. 1996. Effect of low concentration of antibiotics on bacterial ultrastructure, virulence and susceptibility to immunodefence: clinical significance. Inside: V Lorian. *Antibiotics in Laboratory Medecine*. Ed 4th. London: Williams & Wilkins.
- [18] Naufalin R. 2005. Study of antimicrobial properties kecombrang flower extract (*Nicolaia*

- speciosa Horan) against a variety of microbial pathogens and food destroyer [dissertation]. Bogor: Graduate Program, Bogor Agricultural University.
- [19] Franklin T, Snow GA. 2005. Biosynthesis Inhibitors of Nucleic Acids. Inside Franklin TJ, Snow GA. Biochemistry and Molecular Biology of Antimicrobial Drug Action. Ed 6th. London: Springer.