Bacterial inactivation by using non thermal argon plasma jet and its application study for non thermal raw milk processing

Tota Pirdo Kasih1, Dave Mangindaan1, Afifah Septia Ningrum2, C Sebastian3, D Widyaningrum3

1 Professional Engineer Program Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480.
2 Industrial Engineering Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480.
3 Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta, Indonesia 11480.

Corresponding author: tkasih@binus.edu

Abstract. The present paper deals with the research about bacterial decontamination by using non thermal argon plasma jet and exploring the argon plasma jet application study for non thermal raw milk processing. Escherichia coli and Staphylococcus aureus bacteria were chosen in this research to be treated by argon plasma jet system, due to those types of bacteria are usually contained in raw milk. The bacteria that have been cultured in agar media was irradiated by argon plasma jet at various plasma treatment time to understand the effect of the jet on the bacteria growth. It was found that the growth of bacteria was inhibited proportionally with the plasma treatment time in their appropriate agar media. This was clarified by the increasing of inhibition zone diameter of bacteria’s growth in petri dish. From this result, attempting the application of the developed plasma system for raw milk processing by adopting the system to generate plasma under liquid milk. Argon plasma discharge inside the milk solution can be generated by applying 1.5 kVp-p electrical AC power and the operation temperature could be constantly kept under 32°C even after 15 min underwater plasma treatment to confirm non thermal processing. Investigating the effect of plasma treatment time on non thermal raw milk processing has been done, however all the results show the achievement still less comparable with the conventional raw milk processing, thermal pasteurisation.

Keywords: Argon plasma jet, bacterial decontamination, non thermal processing, raw milk

1. Introduction

Fresh cow's milk is a natural food ingredient which is like nutrition containing complete nutrients such as protein (3.5%), fat (3.9%), lactose (4.9%), minerals, and vitamins (0, 7%). The nature of these nutrients is easily digested and absorbed completely[1]. During the storage of fresh cow's milk, there is a decrease in protein content which can be influenced by several factors such as the growth of microorganisms in milk. Contamination of microorganisms contained in fresh cow's milk can be caused when in the udder, also when the milk is taken from the nipple. The cow hole for taking milk at the end of the teat is not closed and is usually wet. The next contamination can be affected by cow dung and body and less clean tools and
the environment (floor, water and air)[2]. Although consuming milk has good benefits for the body, the dangers of drinking milk that is not properly processed can cause the most dangerous infectious diseases caused by contaminated milk, including campylobacteriosis, salmonellosis, yersiniosis and tuberculosis. More than 90% of all cases of milk-related disease are reported from bacterial microorganisms contaminated in milk. Therefore, it is necessary to have a standard procedure to process raw milk properly, deactivate this pathogen before milk is consumed, either by pasteurization or ultrahightemperature (UHT)[3].

In general, processing raw milk is processed by pasteurization. Pasteurization is a common technique for removing microorganism populations in raw milk [4]. According to the International Dairy Federation, pasteurization of milk is carried out by using a heating process below the boiling point of milk with pasteurization at a temperature of 63°C for 30 minutes and a temperature of 72°C for 15 seconds to extend the shelf life of milk. But according to observations pasteurization heat treatment can change the chemical composition of milk protein, lipids and carbohydrates[5]. Then the further processing of raw milk to eradicate the microorganisms contained in raw milk is sterilization. Sterilization in milk is a process of killing all microorganisms by heating for the purpose of freeing milk from all destructive microorganisms. This sterilization process is carried out by heating the milk in an airtight and high-pressure container with a temperature of 105°C to 121°C, for about 15 minutes. The pressure drop in the process aims to increase the temperature in the airtight container so that the microorganisms present in milk can be removed due to this high temperature increase [6]. All the milk processing processes that have been mentioned above are generally known to be able to effectively eliminate the microorganism population in raw milk, but because it is carried out in thermal conditions there will also be a decrease in the nutritional content of the milk. Therefore, many researchers are currently carrying out various research methods to develop alternative technologies for processing non-thermal raw milk, including using non-thermal plasma technology.

Plasma is simply defined as an ionized gas and is known as the fourth phase of matter after solid, liquid and gas phases[7]. Gas becomes plasma when the addition of heat or energy causes a large number of atoms to release some or all of the electrons. Plasma technology is starting to be applied in the industrial, medical, biomedical and agricultural sectors[8]. Plasma can kill vegetative cells and bacterial endospores. Non-thermal plasma has been used mostly for water and wastewater treatment, surface sterilization and environmental control. Increased non-thermal use of Plasma is being developed to inactivate vegetative foodborne pathogens on different surfaces. Several studies began using nonthermal plasma for pasteurization of liquid foods such as juice or milk in the early 2000s [9]. In this study, we will develop the use of non-thermal plasma for pasteurization of milk using underwater plasma treatment. Developing a method of releasing plasma gas bubbles in the milk liquid that allows efficient and selective production of reactive species is required. Using this methodical approach, the high selective production of hydrogen peroxide (H₂O₂) and nitrate (NO₃) is obtained from argon and air gas, respectively. Hydrogen peroxide is well known for having a bactericidal and preservative effect in milk. This method involves the discharge of argon gas bubbles in the milk resulting in a hydrogen peroxide concentration lower than 0.05% at ambient temperature to suit the processing of heat sensitive raw milk. The use of non-thermal plasma for pasteurization of milk with the aim of killing microbes to extend the shelf life of milk without reducing the nutrients contained in fresh milk.

2. Experimental

2.1. Argon plasma jet for bacterial decontamination

Agar media containing Escherichia coli and Staphylococcus aureus as a model of bacteria present in raw cow’s milk was treated by the argon plasma jet, powered by a homemade high voltage AC power supply that has been developed before [10] was carried out at atmospheric pressure. The picture of the argon plasma jet can during bacterial decontamination test can be looked out at figure1. This experiment was carried out to know the effect of the non-thermal argon plasma jet on the growth process of bacteria on agar media in a petri dish. From the argon plasma jet treatment at variations in plasma treatment time, we want to see the effect on bacterial growth measured by approximating the diameter of inhibition zone on the agar after incubation for 24 hours at 37°C. The plasma jet was operated by flowing 2 L/min gas argon and applied voltage 6.7 KVp-p and the distance between the edge of silicon cover to the surface of the agar media was 8 mm. It was found that the operating temperature of the argon plasma jet was at ambient temperature or around 28-30°C, as measured by thermal imaging camera FLIR-TG165. This confirms
that the generated argon jet plasma is the typical nonthermal, as expected for the non thermal pasteurization process, with the target operating temperature below 40°C.

2.2. Argon plasma jet for non thermal process of raw milk

Based on the argon plasma jet system above, we adopt the similar system for the generation of argon plasma jet with underwater mode (the plasma was developed underwater) with the basic consideration that even generated underwater, the expected operating temperature of the plasma should be under 40°C, so that the pasteurization process can occur in non-thermal conditions. The schematic diagram of the non-thermal underwater plasma system developed in this experiment can be seen at figure 2, which adopted from the development of the argon jet plasma system at atmospheric pressure. 60 ml raw milk was poured in a glass container, then a plasma system was inserted into the container with the settings like the Figure 2. 1 L/min Argon gas was flowed into the milk through the SUS cylinder that has 3 mm inner diameter, before the AC power supply electrified the plasma generation under the liquid milk.

3. Result and Discussion

3.1. Bacterial inactivation

Figure 3 shows the test results of the effect of argon plasma jet treatment on the bacterial growth at various treatment time to the E. Coli as well as to S. aureus as the bacterial model, clarified by the measurement of diameter of inhibition zone in the petri dish. Figure 4 shows the associated photograph of the test of plasma treatment for both bacteria in petri dish. From the figure, it can be seen that the diameter of inhibition zone of growth of E. coli and S. aureus bacteria has increased with the prolong of treatment time of argon plasma jet. At the same condition of plasma treatment, argon plasma jet gave a little bit higher effectiveness to inhibit the growth of E. coli than S. aureus in the agar media. Similar result has been reported previously by the other research group when using DBD plasma jet with the plasma treatment time ranging from 3 to 15 min [11]. This result confirms that the argon plasma treatment could provide a non thermal handling to inhibit bacterial growth population in their appropriate living media, such as agar, fruit juice and milk. By using this consideration, we tried to eliminate or inhibit the growth of bacteria in raw cow’s milk, in which E. coli and S. aureus bacterias and other microorganisms are commonly contained in it.
3.2. Study of non thermal milk processing

It should be noted that compared to the use of two electrodes formation with 5-10 mm in between with a cone shapes and sharp angles at the ends of the two electrodes to generate underwater plasma, the non-thermal plasma system using gas flow (in this study argon gas) is intended to avoid poisoning milk due to electrodes wear problem during emitting the plasma inside the liquid milk. Moreover, the operation temperature of the plasma generated under solution by flowing the argon gas was found to be constant in ambient, in which the electrical energy delivered between the electrodes is more likely to be utilized for generation of more radical species during plasma interaction with the liquid instead of heating the solution itself, thus the plasma underwater with gas argon flowing was chosen in this research.

It is generally known that the easiness of the generation of plasma underwater is closely related to the amount of the minerals contained in the liquid itself. Underwater plasma is difficult to be generated when using pure water which has no mineral content. The compensation is that the applied voltage used is higher than the applied voltage utilized to generate plasma in liquids of mineral water that have a TDS around 200. On the contrary, it is very easy to generate plasma underwater in a liquid with has much content of minerals. The measure of the amount of minerals contained in a liquid is usually measured by the value of total dissolve solid (TDS) of the liquid. Liquids that have a large TDS value are usually followed by a large electroconductivity (EC) value. The same things applies on the contrary, a liquid with

Figure 3. Test results of the effect of argon plasma jet treatment at several time in the E. coli and S. aureus bacterial model on the diameter of inhibition zone of bacterial growth in the petri dish.

Figure 4. Photograph of the effect of argon plasma jet treatment at several time at (a) E. coli and (b) S. aureus bacteria in the petri dish.
a small TDS value then the EC value of the fluid is also small. The TDS value of the raw milk that measured was 1230 ppm, high enough to generate underwater plasma easily. For the purpose of raw milk processing with non thermal plasma, in this experiment, argon plasma jet was generated inside the milk when the utilized applied voltage was 1.5 kV p-p. The disadvantage of this system can be seen in figure 5 and figure 6 below, where the plasma parameter setting must be right to avoid the excess of applied voltage which potentially causes the burning of the silicon material in liquid milk.

Furthermore, using the schematic diagram as shown above and paying attention to the optimal applied voltage, the influence of non-thermal argon plasma jet treatment to 60 ml raw milk was investigated with a variation of treatment time (100, 300 and 600 seconds). The results of the non-thermal plasma treatment processes were then compared with raw cow's milk treated with the conventional process, namely thermal pasteurization. After being given the treatment, the cow's milk is stored in a bottle, then put in a refrigerator with a temperature of about 4°C, to see the comparison of the results with pasteurized thermal milk (figure 7).

From the non-thermal experiments of Argon jet plasma on raw milk, it was found that there wereagglomeration product that appeared to the surface of the milk after 2 days of storage in the refrigerator for all types of plasma treated time. On the other hand, raw milk which has undergone thermal pasteurization does not occur agglomeration. From the provisional results of this experiment, it is still necessary to set the right plasma parameters so that the results obtained are at least similar to the processes in thermal pasteurization, however operated at non thermal temperatures or below 30°C. By
using this non-thermal processes as an effort to keep nutrient content that usually degraded when using thermal processes. Further research is certainly still needed to ensure that this non-thermal plasma system is feasible to use for the pasteurization process with a larger volume as expected.

4. Conclusion

Through this paper we have shown that argon plasma jet treatment that has operating temperature lower than 30°C has a significant on the decontamination of E. coli and S. aureus bacteria. The bacterial inactivation efficiency of the argon plasma jet measured by the diameter of inhibition zone of the bacteria growth in petri dish shows that the diameter of inhibition zone was increased by the elongate of plasma treatment time. Adopting the system in generating plasma underwater for raw milk processing also shown the similar operating temperature around 30°C with the purpose to decontaminate the microorganism contained in liquid milk. This initial experiments reveals that plasma treated milk for 100, 300 and 600 seconds still not achieved similar characteristics compared to the pasteurised one. The formation of the agglomeration product that appeared at the surface of the milk after 2 days storage at refrigerator exhibits the quality of plasma treated raw milk still need improvements.

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