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Extension of Raw Cow Milk Shelf Life by Microplasma Discharge

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Abstract: Cow's milk, the universal nutrient, is being stored and supplied, which seeks proper preservation. The prevalent milk preservation procedure of refrigeration, is effective only for two days, and after which, it starts to contaminate due to the growth of various milk-laden bacteria. This bacterial overload has to be inactivated properly to increase its shelf life, and is been achieved effectively using microplasma, a single-step, cost-effective and chemical-free process. Raw milk was treated for 5, 10, and 13 seconds in microplasma discharge. After 13 seconds of microplasma treatment, E. Coli, Pseudomonas, and S. Aureus bacteria got reduced at a respective rate of 89.93, 84.55, and 94.19% for in raw milk. The reactive species formed during microplasma discharge disrupts the structural integrity of bacterial cells and inactivates it, thereby enhancing the milk shelf life. Treated samples remained in good condition for 8 days. Thus, microplasma discharge increases the shelf life of milk by quickly inactivating the bacterial load.

Keywords: Microplasma, Cow milk, Shelf life, Reactive species

1. Introduction

Milk is an essential and healthy food for humans. It is a nutrient-rich natural food obtained from the mammary glands of mature mammals. It contains proteins, carbohydrates, fat, vitamins, and minerals. Milk of cow, sheep, goat, buffalo, camel, and donkey are used by humans to meet the daily nutrient intake. Apart from the milk, lots of by-products such as gee, cheese, yoghurt, curd, butter, buttermilk, cream, skimmed milk, condensed milk, etc., are also prevalently used for the dietary purpose. 100 ml of whole milk contains 68 calories, whereas semi-skimmed and skimmed milk contain 47 and 35 calories, respectively [1-2].

Raw milk can be stored fresh at room temperature for up to two hours. As raw milk is an excellent nutrient medium, the indigenous and exogenous microorganisms easily multiply in it leading to contamination, souring and spoilage. Some of these microorganisms are pathogens and cause infections in humans. The pathogenic bacteria present in raw milk are *E. Coli, Staphylococcus, Pseudomonas, Salmonella, Bacillus cereus, Mycobacterium tuberculosis*, etc

[3]. These bacteria are majorly responsible for milk spoilage, and consuming the same causes various infections such as food poisoning, pneumonia, bone joint pain, soft tissue infections, inflammatory conditions, respiratory problems, and urinary tract infections. The severity of infections depends on the immunity of affected individual. Thence, pathogens in raw milk has to be removed to extend its shelf life and ensure safe consumption. The methods used for the reduction of these pathogens are pasteurization, sterilization, and non-thermal processes [4-5].

In pasteurization, the pathogens are killed, which also breaks down the vitamins, but in sterilization, there is a total destruction of microorganisms. The loss of nutrient value is higher in sterilization compared to pasteurization. In the thermal process, there is a decrease in nutrition level, whereas non-thermal processes like Bactofugation, Microfiltration and Ultrasound sterilization are costly and the shelf life is shorter compared to thermal process. It is a challenge to extend the shelf life of raw milk without altering and affecting its nutritional composition [6, 7].

Plasma is an emerging technology that is used in the synthesis of nanomaterials, surface modification, waste management, welding, spraying, etc [8]. Non-thermal plasma is a type of plasma that needs low power, process time, and cost-effective. It is widely used in dye degradation, nanoparticle synthesis, sterilization of food items, waste water treatment, plasma activated water, seed germination, and wound healing treatments [9-18].

Microplasma is a non-thermal plasma method where the plasma is confined to millimeter scale in at least one dimension. It is a miniature version of plasma. Since, it is at micro level, the surface-to-volume ratio is increased compared to other plasma. It is easy to handle, and is stable at low and normal temperatures [19]. It contains reactive species that bombard the cells of pathogens. Since it is a low-temperature and chemical-free method, the nutrient content of raw milk would remain unaltered [20].

In this study, the growth of harmful bacteria was inhibited by treating raw cow milk using microplasma discharge method. The treated and untreated milk samples were then subjected to pH test, storage test, and microbiological test.

2. Experimental procedure

The experimental setup (Figure 1.) consists of two stainless steel electrodes, and sample in quartz chamber was tightly closed by rubber cork [10-12]. The AC power supply is applied between the grounded electrode, and to the capillary electrode. The grounded electrode was immersed in the milk sample, whereas the capillary electrode was kept 3mm above the surface of milk sample to produce microplasma discharge. Air was used as plasma-producing gas, and a mass flow controller, controlled its flow rate [19].

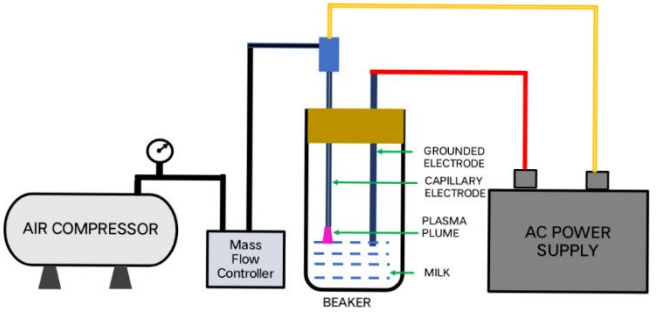

Figure 1. The schematic of microplasma discharge setup

10 ml raw cow milk was taken in the quartz chamber. The flow rate of air was adjusted to 0.35 standard litres per minute (SLPM) in the mass flow controller. The experiment was conducted at three different treatment times of 5, 10, and 13 seconds, whereas the flow rate remained unchanged. The microplasma discharge was exposed directly at the surface of the raw milk. After treatment, the milk samples were poured into 10 ml vials and then stored in refrigerator for storage test. The pH of milk was checked before and after the microplasma treatment using pH meter.

The *E. Coli, Pseudomonas,* and *S. aureus* present in milk before and after microplasma treatment was counted using plate count method in their respective differential culture media [15]. M. Lauryl sulphate agar (MLSA), Pseudomonas agar base (PAB), and Mannitol salt agar (MSA) were prepared for differential culture of *E. Coli, Pseudomonas, and S. Aureus*, respectively. 1 ml of treated (5, 10, and 13 seconds) and untreated (raw milk) samples were diluted separately in 50 ml of cooled sterile water, and 0.5 ml of this solution was pipetted and released to petri dish containing culture medium. For *E. Coli* and *Pseudomonas*, culture the petri dish was kept in oven for 24 hours at 33°C, and for *S. aureus*, the culture time was 48 hours at 33°C. fter the culture, the samples were subjected to microbiological test and counting as colony forming units (CFU).

3. Result and Discussion

After microplasma discharge treatment, the milk's colour remained unchanged, and the smell remained as fresh as it had been before the treatment. The pH of milk that had been exposed to microplasma found to have only slight changes. This signifies the milk's buffering nature [3].

The microbiological results shows that a huge reduction in bacterial growth at 13 seconds, compared to other treatment times. After 24 hours of incubation in oven, yellow

circlular colonies (Fig. 2) of *E. Coli* were observed in MLSA. The average count of *E. Coli* in the untreated raw milk was found to be 83,400 CFU/ml. After 5, 10 and 13 seconds of treatment 38.70, 75.42, and 89.93 percent of *E. Coli* bacteria were reduced, respectively.

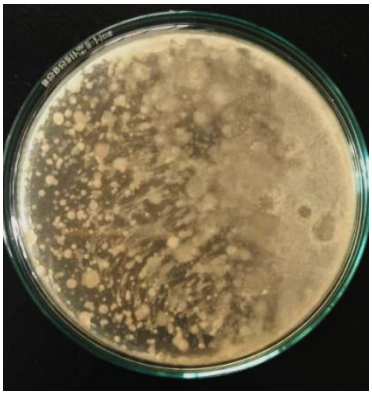

Figure 2. Image of *E. Coli* in MLSA

Figure 3. Image of *Pseudomonas* in PAB

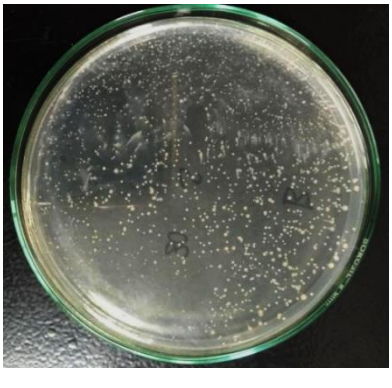

Figure 4. Image of S. aureus in MSA

Light yellow circle colonies of *Pseudomonas* (Fig. 3.) were observed in PSB after 24 hours of incubation in oven. The average count of *Pseudomonas* in untreated raw milk was 1, 15,200 CFU/ml. After 5, 10 and 13 seconds of treatment respectively 16.32, 48.96, and 84.55 percent of *Pseudomonas* bacteria was got reduced.

After 48 hours, the MSA produced yellow-circle colonies (Fig. 4.) of S. Aureus. The average count of S. Aureus in untreated raw milk was 2, 98,000 CFU/ml. After 5, 10 and 13 seconds of treatment 44.7, 78.46, and 94.19 percent of S. aureus bacteria were reduced, respectively.

In all the above three bacteria, maximum reduction rate was obtained at 13 seconds of plasma treatment (Figs. 5 & 6). Though E. Coli and Pseudomonas are gram-negative bacteria and S. aureus is a gram-positive bacterium, thus this method is effective in reducing both the type of bacteria present in. The Free radicals generated during microplasma discharge, plays a key role in diminishing the bacterial colonies [15]. Due to the potential difference between the capillary electrode and the sample's surface, air gets ionized to create the plasma. The Reactive oxygen and reactive nitrogen species are produced when the electrons interact primarily with oxygen and nitrogen present in air [9, 10, 19]. The microorganisms in the milk get collided with the reactive species and are adsorbed on their surface. These components create an opening on the membrane of cell, through which the inner fluids of microorganisms are released. Due to this pore formation, reactive species could directly damage the inner parts of microorganisms, such as DNA, proteins and others cell components [1, 20].Thus, the population of microorganisms was reduced by the microplasma discharge treatment [14,15].

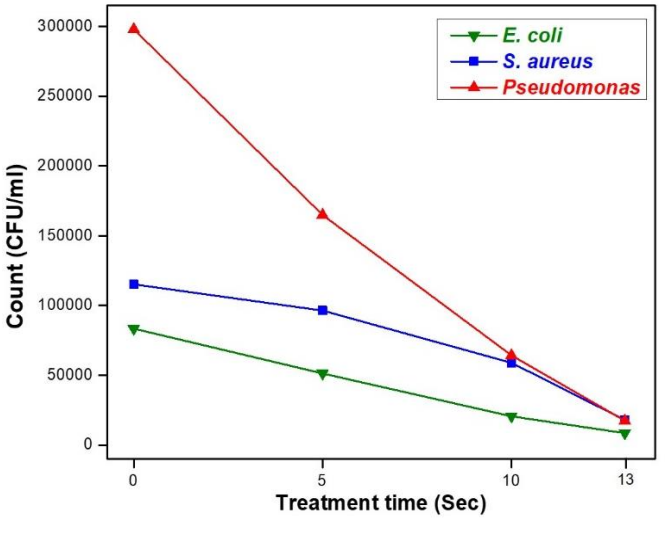

Figure 5. Bacterial count vs treatment time

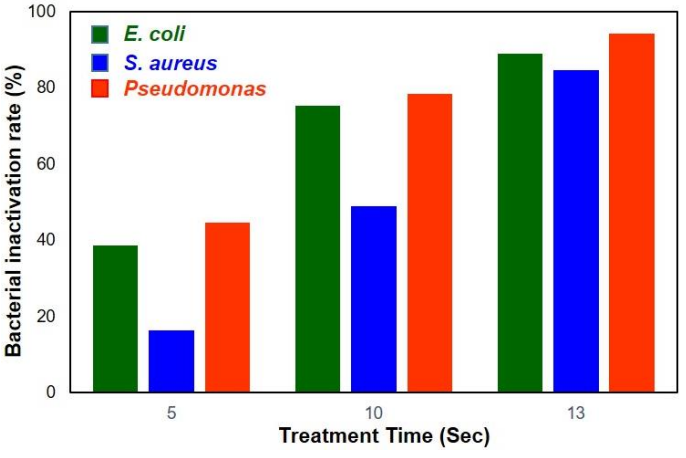

Figure 6. Percentage of bacterial inactivationvs treatment time

The microplasma discharge on milk shows very little change in pH value for all treatment times. The pH of untreated milk is 6.72, whereas the pH of treated milk is 6.71, 6.70, and 6.70 for treatment times of 5, 10, and 13 seconds, respectively. Since the change in pH value is very small (0.02), it is negligible.

The treated milk samples were stored in refrigerator. After 2 days, the untreated milk has a slight solid consistency, smelled like curd (an odour), and the pH was below 6.4. The treated milk was good for up to 8 days in the refrigerator, smelled good, and had a good liquid consistency. After 8 days, the consistency changed slightly, as did the smell. After 12 days, the treated milk smells like curd, the consistency is slightly solid, and the pH is just below 6.4.

4. Conclusion

The shelf life of raw cow milk was extended by reducing E. Coli, S. Aureus, and Pseudomonas present in the raw cow milk using Microplasma Discharge method. The reactive species in plasma break down the bacterial cells. From the results, with a treatment time of 13 seconds, there were an 88.93, 84.55, and 94.19 percent reduction in the microbial growth of *E. Coli, Pseudomonas, and S. aureus,* respectively. The pH fluctuates relatively little, and treated milk had a shelf life of up to 8 days. Therefore, this method can be a safe, chemical-free and cost-effective method suitable for the decontamination of liquid food items, especially milk.

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