ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.1.L283-288

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Food Science and Technology

# Impact Of Plasma Bubbling On Cow Milk: Microbial Reduction And Improvement Of Milk Quality

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**Abstract:** Raw milk has high nutritive value and is used as an important dietary supplement for humans. But, microbial contamination in milk has been a problem for era, while existing thermal technology often deteriorates the quality of milk and its valuable ingredients. Again, consumer demand for least processing technology without degradation of the product quality forced the scientist to develop novel methods relevant to the application of non-thermal technology which does not create any detrimental effect to the composition of milk are now being under scrutiny. One of the novel methods is plasma bubbling technique and is not yet examined for liquid foods like milk. In the present study, the plasma bubbling system was established for the decontamination of raw cow milk. The plasma bubbling was generated at voltage of 160V, for 5, 10 and 15 minutes (min) and was evaluated for microbial reduction at an air flow rate of 5 and 10 Litre/hour (L/h). It accounted for a maximum 1.33 log reduction for coliforms at 160V, flow rate of air was 10 L/h, for a 100mL of milk sample with 15 min exposure time, while for yeast the log reduction was 1.40. The plasma bubbled milk was analysed for its quality evaluation such as pH, acidity, colour and lactose content of milk. The value of pH was found to be 6.77 at 160V, 10L/h, 15 min and 100mL of sample volume while the control value of pH was 6.60. The findings from this study revealed that the atmospheric plasma bubbling system could be used for the pasteurisation of raw cow milk by reducing the microbial load without compromising milk quality. This work on novel atmospheric plasma bubbling is an initiative for the pasteurization of raw cow milk, which could have a potential impact on the food industry in future.

**Key Words:** Plasma bubbling, Safety, Quality, Milk

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**Received On** 22 December, 2021
**Revised On** 25 January, 2022
**Accepted On** 27 January, 2022
**Published On** 31 January, 2022

**Funding** This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

**Citation** Samarpita Dashab and R. Jaganmohana , Impact Of Plasma Bubbling On Cow Milk: Microbial Reduction And Improvement Of Milk Quality.(2022).Int. J. Life Sci. Pharma Res.12(1), L283-288 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.1.L283-288

## 1. INTRODUCTION

Milk is a very good source of carbohydrates, fatty acids, high-quality protein content and various micronutrients, such as vitamins and minerals[1]. It was reported that by 2025, global per capita dairy consumption will increase to 12.5%[2]. Besides, dairy products are considered as a high value of nutrition along with health-promoting foods for the prevention of several diseases such as sarcopenia, metabolic syndrome, osteoporosis, cardiovascular disease, cognitive decline and digestive disorders[3]. But before consumption, the milk should be subjected to thermal processing which improves microbial safety but they extensively damage the nutritional and physicochemical properties[4-6]. However, recent interest in the consumption of raw milk has led to the scientist for consideration of alternative technologies that will not compromise the quality and safety of milk i.e., nonthermal technique, which can meet microbial food safety as well as enhance the physical, nutritional, sensory characteristics of the products and helps to preserve the unstable bioactive compounds[7,8]. The generation of non-thermal (cold plasma) plasma occurs at the non-equilibrium condition when the temperature of the electron is higher than the heavy particles[9]. Plasma contains a mixture of particles, molecules, ions and reactive oxygen species which acts simultaneously for the decontamination of the target[10]. This characteristic of plasma has led researchers to evaluate different cold plasma generation devices which can be adjusted accordingly to maintain a suitable condition for different applications. Cold plasma application in the food sector includes sterilization, decontamination, pesticide reduction, property modification and germination[11]. Cold plasma has shown decontaminating properties for various materials such as poultry, milk, water, living cells, meat, fresh fruit and vegetables, because of its capacity to kill microorganisms, including bacteria, yeasts, fungi and algae. It has become a well-known technology in the field of the food industry[12-17]. The key characteristics for the decontamination of microorganisms are the UV light, source of free radicals, ions, electrons generated during cold plasma treatment. Radicals like reactive oxygen species (ROS) can directly decontaminate microbes by gaseous phase[18]. However, in the case of liquid foods, the effect of plasma is complex because of its free-flowing nature[19]. Several studies have been conducted in recent years on liquid food for the decontamination purpose as well as for the quality evolution. Thus, the demand is increasing for establishing a novel approach for the pasteurization of liquid foods. The various non-thermal approaches were conducted for milk quality evolution such as ultraviolet light[20], ultrasound[21], cold plasma[22] and pulsed electric field[23] which have been studied for the replacement of the thermal pasteurization. Previously, several studies have focused on the decontamination of milk by using cold plasma technology[9,17-24]. Despite many studies conducted on decontaminating the capacity of cold plasma, there is limited scrutiny for the effect of the fourth state of matter (cold plasma) on the food products. The aim of the study is the use of cold atmospheric plasma gas, which has been bubbled through raw cow milk at different input flow rate of air and time with a constant voltage and to investigate the effect of input parameters on microbial and physicochemical properties of raw cow milk such as pH, titratable acidity, total soluble solids, colour.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Setup

Cold plasma bubbling system was developed indigenously and was used with flow rates of air at 5 and 10 litres per hour (L/h) while using atmospheric air as feed gas[25]. Plasma was generated at voltage 160V and was bubbled through 50, 100 and 150 mL of raw cow milk for 5, 10 and 15 min. The overall experimental set up was depicted in (Fig 1). Previously observed parameters for cold plasma were established with slight modification[25].

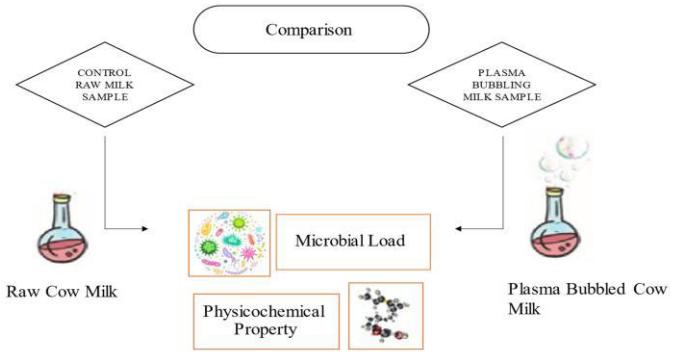


Fig 1. Schematic diagram of the overall experimental design

### 2.2 *Microbial Analysis*

In the present study, microbes analysed for milk samples were coliform, yeast[26]and was observed by using the spread plate method in triplicates[27]. The control and treated cow raw milk samples were tested for coliform using violet red bile agar[28]. For yeast, chloramphenicol yeast glucose agar was used[29]. The experimental analysis was performed in triplicate and the result was represented as log CFU/mL[26].

### 2.3 *pH analysis*

The pH of the raw cow milk and plasma bubbled cow milk sample was analysed by using the pH meter (Laqua PH1100, Horiba Scientific, Singapore) at ambient temperature[30].

### 2.4 Colour measurement

Plasma bubbled cow milk and fresh raw cow milk was poured into a cuvette cell (64 mm) and the colour of the cow milk was evaluated by colourimeter (Colour Flex EZ System, 45/0 LAV); the colour values L* (lightness), a*(redness) and b*(yellowness) were determined[31].

### 2.5 Titratable acidity

The titratable acidity was analysed by using 5 drops of 1% w/v phenolphthalein indicator and were added to the conical flask containing 20 mL of cow milk. The mixture was titrated using 0.1 N NaOH and the % of titratable acidity was calculated[30].

### 2.6 Total soluble solids

The TSS content was measured at room temperature by a digital refractometer (Erma, Japan, 0-80° Brix)[32].

## 3. STATISTICAL ANALYSIS

SPSS version 22 statistical software (SPSS, Inc., United States) was used to analyse the results. The replication of all the experimental analyses was three times. The statistical analysis was done by using a one-way analysis of variance (ANOVA). The significant differences among the mean values were determined by performing Duncan's multiple comparison tests at a confidence level of $p < 0.05$.

## 4. RESULTS AND DISCUSSION

### 4.1 Microbial Analysis

In this study, it was observed that the microbial reduction is based upon the processed parameters such as the input time, volume and flow rate of air. A log reduction of 1.33 was observed at (160 V), with a flow rate of air (10 L/h), 100 mL of the volume of cow milk and 15 min treated time in the plasma bubbling system. While for yeast 1.4 log reduction was observed for 160V, 10L/h, 100mL and 15 min treated parameters. This could be due to the generation of hydroxyl radicals HO˙ during cold plasma, which damages the cell wall[33]. Non-thermal plasma produces gas such as ozone, nitric oxide, oxygen, hydroxyl radicals and could able to directly interact with the bacterial membrane[14]. Another study revealed that ROS and RNS plays a vital role in microbial cell death by breaking the double bond of the lipid bilayer present on the microbial cells, thus strong oxidative stress occurred which damages the transportation of macromolecules to the cell, therefore pathogen inactivation was observed[34]. Again, it was observed that after exposure to cold plasma, different types of stress could be responsible for the death of yeast cell[35]. A comparison of the microbial load was depicted (Fig 2).

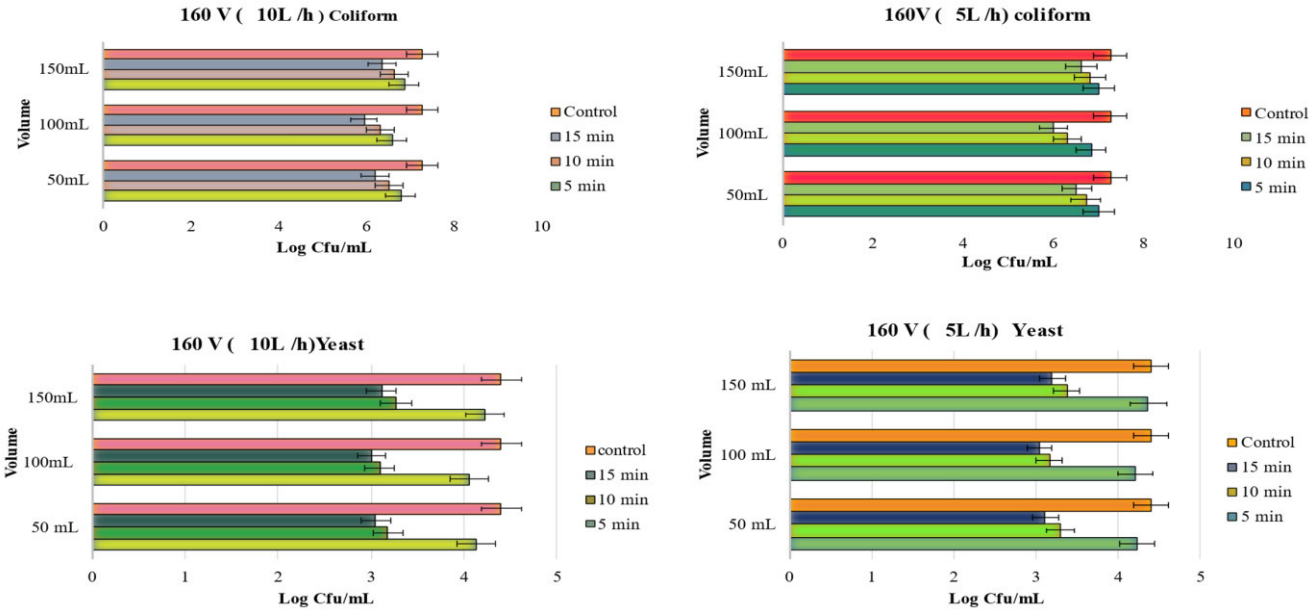


Fig 2. Reduction of microbial cell viability (Log Cfu /mL) control and treated sample.

### *4.2 pH*

The concentration of hydrogen is crucial in cow milk. In this study, the value of pH is slightly increasing in accordance with particular volume, high flow rate of air and time interval (Table 3). In the control sample, the pH value was 6.60±0.011 while in 160V, 10L/h, 100mL, and 15 min treatment time the pH value was 6.77±0.018. The volume, flow rate and time play an important role in the increase of pH value. Again, previously it was reported that HO˙ radicals are produced during non-thermal treatment which could be responsible for the pH[36].

### *4.3 Colour*

A difference in colour value was observed after being exposed to plasma bubbling treatment with a low volume of sample, high flow rate and higher treatment time. A significant decrease was observed for L*, a* and b* value at 160V, 10L/h, 50mL, 15min processed parameters with respect to control colour value of milk (Table 2). A decrease in colour difference could be explained due to the partial degradation of pigment after exposure to cold plasma treatment[37-38]. Previously, it was observed that a slight colour

change has occurred in milk after exposure to cold plasma[39]. In the present study, it was observed that the volume of the sample plays a major role in the colour difference in 50mL of volume; the colour value decreases more than that of 100mL and 150mL

. ***Table 2***. *Colour value of cow milk before and after plasma*

| Time (Minutes) | Voltage(V) | Flow Rate of air (L/h) | Volume (mL) | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|---|---|
| **Control** | | | | | | |
| **5** | 160 | 5 | 50 | $88.76^{e}$±0.013 | $-0.91^{s}$±0.012 | $14.23^{f}$±0.013 |
| | | | 100 | $88.72^{d}$ ± 0.005 | $-1.02^{p}$± 0.012 | $14.21^{f}$ ± 0.005 |
| | | | 150 | $88.74^{e}$± 0.005 | $-0.97^{q}$ ± 0.00 | $14.22^{f}$± 0.006 |
| | | 10 | 50 | $88.76^{e}$± 0.012 | $-0.95^{r}$ ± 0.01 | $14.23^{f}$ ± 0.006 |
| | | | 100 | $88.70^{d}$± 0.005 | $-1.15^{m}$ ± 0.011 | $14.18^{e}$± 0.012 |
| | | | 150 | $88.71^{d}$± 0.006 | $-1.06^{n}$ ± 0.005 | $14.20^{e}$ ± 0.005 |
| **10** | 160 | 5 | 50 | $88.74^{e}$ ± 0.006 | $-1.00^{o}$ ± 0.005 | $14.21^{f}$± 0.005 |
| | | | 100 | $88.66^{c}$± 0.00 | $-1.28^{j}$± 0.006 | $14.15^{d}$ ± 0.00 |
| | | | 150 | $88.69^{d}$ ± 0.006 | $-1.12^{k}$± 0.017 | $14.17^{e}$ ± 0.005 |
| | | 10 | 50 | $88.70^{d}$± 0.00 | $-1.08^{l}$± 0.00 | $14.19^{e}$± 0.11 |
| | | | 100 | $88.64^{c}$± 0.017 | $-1.33^{g}$± 0.023 | $14.12^{d}$± 0.006 |
| | | | 150 | $88.67^{c}$ ± 0.00 | $-1.21^{h}$ ± 0.011 | $14.13^{d}$ ± 0.005 |
| **15** | 160 | 5 | 50 | $88.68^{c}$± 0.006 | $-1.14^{i}$± 0.00 | $14.16^{e}$± 0.12 |
| | | | 100 | $88.57^{a}$± 0.006 | $-1.59^{d}$ ± 0.005 | $14.07^{c}$ ± 0.011 |
| | | | 150 | $88.60^{b}$± 0.023 | $-1.48^{e}$± 0.00 | $14.10^{d}$ ± 0.028 |
| | | 10 | 50 | $88.61^{b}$± 0.023 | $-1.30^{f}$ ± 0.011 | $14.11^{d}$± 0.012 |
| | | | 100 | $88.53^{a}$± 0.017 | $-1.76^{a}$± 0.005 | $14.02^{a}$ ± 0.028 |
| | | | 150 | $88.56^{a}$ ± 0.01 | $-1.64^{b}$ ± 0.028 | $14.05^{ab}$ ± 0.011 |
| | | | | $88.59^{b}$± 0.011 | $-1.53^{c}$ ± 0.006 | $14.09^{b}$ ± 0.012 |

*The results were expressed as mean ±S.D. Mean values followed by different letters in the same row indicate significant differences ($p < 0.05$).*

### *4.4 Titratable acidity*

After exposure to plasma bubbling with a particular volume, high flow rate along with increasing in time interval, a gradual decrease in titratable acidity (TA) value of the milk was observed. The lowest value of titratable acidity was observed at 160V,10 L/h, 100mL, 15 min i.e., 0.127±0.015% w/v lactic acid whereas the control sample value of lactic acid was 0.144±0.012% w/v (Table 3). This could be explained due to an increase of hydroxyl $HO^{\bullet}$ radicals formed by the decomposition of an additional water molecule during cold plasma[40].

### 4.5 Total soluble solids

In this study it was observed that with increasing flow rate and time the value of TSS was decreasing. A prominent decrease value of TSS was observed at 160V, 10 L/h, 100mL, 15 min i.e., 8.9° Brix; while the value for control was 10° Brix (Table 4). The decrease in TSS could be explained due to the formation of ozone during cold plasma generation which is responsible for de-polymerization of the macromolecule and thus responsible for the decrease in TSS value[33-41].

***Table 3.*** *Physicochemical Property of control and plasma bubbled milk sample.*

| Time (Min) | Voltage (V) | Flow rate of air (L/h) | Volume (mL) | pH | TA | TSS |
|---|---|---|---|---|---|---|
| Control | | | | $6.60^{a}$±0.011 | $0.144^{d}$±0.012 | $10.2^{d}$±0.002 |
| 5 | 160 | 5 | 50 | $6.61^{a}$±0.011 | $0.139^{cd}$±0.012 | $10.0^{c}$±0.00 |
| | | | 100 | $6.62^{a}$±0.015 | $0.138^{c}$±0.014 | $9.9^{c}$±0.003 |
| | | | 150 | $6.61^{a}$±0.02 | $0.139^{cd}$±0.021 | $10.1^{bc}$±0.013 |
| | | 10 | 50 | $6.62^{a}$±0.021 | $0.137^{c}$±0.022 | $9.9^{c}$±0.014 |
| | | | 100 | $6.63^{a}$±0.012 | $0.136^{c}$±0.010 | $9.8^{c}$±0.011 |
| | | | 150 | $6.62^{a}$±0.010 | $0.137^{c}$±0.011 | $9.9^{c}$±0.006 |
| 10 | 160 | 5 | 50 | $6.64^{ab}$±0.010 | $0.134^{b}$±0.011 | $9.8^{c}$±0.013 |
| | | | 100 | $6.65^{b}$±0.011 | $0.130^{ab}$±0.009 | $9.5^{b}$±0.005 |
| | | | 150 | $6.63^{a}$±0.011 | $0.135^{bc}$±0.009 | $9.8^{c}$±0.007 |
| | | 10 | 50 | $6.66^{bc}$±0.017 | $0.132^{b}$±0.018 | $9.6^{b}$±0.015 |
| | | | 100 | $6.70^{c}$±0.015 | $0.129^{a}$±0.012 | $9.4^{b}$±0.011 |
| | | | 150 | $6.65^{b}$±0.011 | $0.133^{b}$±0.010 | $9.7^{b}$±0.021 |
| 15 | 160 | 5 | 50 | $6.71^{c}$±0.003 | $0.130^{ab}$±0.005 | $9.2^{a}$±0.005 |
| | | | 100 | $6.74^{cd}$±0.010 | $0.128^{a}$±0.011 | $9.1^{a}$±0.008 |
| | | | 150 | $6.70^{c}$±0.014 | $0.131^{b}$±0.013 | $9.3^{a}$±0.006 |
| | | 10 | 50 | $6.72^{c}$±0.016 | $0.129^{a}$±0.015 | $9.0^{a}$±0.002 |
| | | | 100 | $6.77^{d}$±0.018 | $0.127^{a}$±0.015 | $8.9^{a}$±0.005 |
| | | | 150 | $6.71^{c}$±0.001 | $0.129^{a}$±0.002 | $9.1^{a}$±0.011 |

*The results were expressed as mean ±S.D. Mean values followed by different letters in the same row indicate significant differences ($p < 0.05$).*

## 5. CONCLUSION

In the present study, plasma bubbling treatment of raw cow milk was done at a constant voltage (160V) and 1.33, 1.40 log reduction for coliforms and yeast was achieved at 160V, 100mL, 10L/h and 15min treatment respectively. While, atmospheric plasma bubbling technology maintain the physicochemical quality of milk with a non-detrimental effect. The plasma bubbling parameter at a voltage 160V, 10L/h flow rate of air, sample volume of 100mL and 15 min of time interval considered as the good parameter set up among the other set up parameters. However, the current study is a preliminary experiment for the plasma bubbling treatment for cow milk. Further optimization of process parameters along with milk quality and safety need to be examined. Again, the seasonal atmospheric gas needs to determine for better understanding the feed gas for cold plasma and its reaction with respect to the seasons.

## 6. ACKNOWLEDGEMENTS

The author acknowledges IIFPT, for the technical support and proving infrastructure to carry out the research work. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

## 7. AUTHORS CONTRIBUTION STATEMENTS

Mrs Samarpita Dash conceived the practical work and wrote the manuscript. Dr R. Jaganmohan conceived the manuscript idea. All the authors discussed the methodology and results.

## 8. DECLARATION OF COMPETING INTEREST

The author declares there is no conflict of interest.

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