STABILITY AND SHELF-LIFE OF PLASMA BUBBLING TREATED COW MILK

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Running Head: Plasma Bubbling for cow milk pasteurisation

ABSTRACT

The demand of consumers for naturality of food with minimal processing was forced to the scientists for the discovery of non-thermal plasma which is now an emerging technology for the preservation and decontamination of highly perishable food such as milk. In this study, the microbial and physicochemical characteristics of plasma bubbling of raw cow milk were analysed and a comparison of boiled raw cow milk, commercially available pasteurised and

UHT milk was observed. Again, shelf-life study was experimented for the plasma bubbling of milk with respect to raw cow milk sample (control). The plasma bubbling was generated at a voltage (200V), the flow rate of air 10 litres/hour (L/h) and applied to fresh cow milk for 5, 10, and 15 minutes (min) of time interval with 100 mL of the sample volume at room temperature. A significant reduction was observed in coliform and yeast at 200V, 10L/h, 15 min time interval of treatment. The pH of the milk was increased significantly to 6.85 after exposure to plasma bubbling. Whereas, a decreasing value was noticed in colour, total soluble solids (TSS) and titratable acid (TA) with respect to time given by the plasma bubbling. The result of the study indicates that plasma bubbling at (200V, 10L/h,100mL,15 min) treatment improves the quality of milk. This study shows that indirect dielectric barrier discharge (DBD) (plasma bubbling) may offer an effective microbial reduction in barley without affecting the quality attributes. The plasma bubbling processing is an initiative for cow raw milk pasteurisation, which could have a future perspective on industrial food applications.

Keywords: Plasma bubbling, Cow milk, Quality, Food processing, Shelf life

1. INTRODUCTION

Milk is a physiological liquid combination of highly biological active compounds with protein and fat (Ebringer, Ferenčík and Krajčovič, 2008). Cow milk is predominant consumed all over the world in different forms and also highly perishable with rich in biological nutritive value (Rowlands, 1952; Council and others, 1988). Simultaneously, the hazardous effect of milk due to pathogen contamination is also well studied. Generally, the microbes present in milk are coliform, Escherichia coli, Staphylococcus aureus, yeast and mould (Ghodeker, Dudani and Ranganathan, 1974). Previous studies have analysed that the diseases caused by the

contamination of milk include bovine mastitis (Camacho *et al.*, 2005)[5], diarrhoea (Lambertini *et al.*, 2015), milk-borne diseases found in the dairy industry (Dhanashekar, Akkinepalli and Nellutla, 2012) due to the improper handling of dairy products. According to a previous report, per year in the USA and India, the rate of infection is 48 million due to microbes that are present in food (Dhanashekar, Akkinepalli and Nellutla, 2012). Generally, preservation of milk evolved from chilling, boiling, pasteurization, ultra-heat treatment (UHT) process. It has been proven by studies that the pasteurization of milk can degrade the nutritional quality of milk (Macdonald *et al.*, 2011). Also, thermal processes involve many changes in the milk composition such as flavour, microbial load, and its colloidal properties (Ajmal *et al.*, 2018)(Deeth and Datta, 2011)(Nair, Dalgleish and Corredig, 2013). Whereas, past researchers introduced a novel and effective non-thermal technology to food science. One of the non-thermal technologies is cold plasma, used for sterilization purposes: owing to its major impact on microbes (Ikawa, Kitano and Hamaguchi, 2010)(Kim *et al.*, 2013). The efficiency of cold plasma has been well documented in the sanitation of food (Basaran, Basaran-Akgul and Oksuz, 2008). The key characteristics for the deactivation 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 (Yu *et al.*, 2006). The effectiveness of non-thermal plasma is applied to liquid food like milk for the inactivation of bacteria as well as for E. coli (Kim *et al.*, 2011)(Gurol *et al.*, 2012). Recently, another study on milk sterilization was observed by dielectric barrier discharge (DBD) but the change in physicochemical properties was found (Wu *et al.*, 2021). Further, a preliminary investigation of plasma bubbling of milk was experimented where very negligible changes was observed for physicochemical of milk (Samarpita dash and R. Jaganmohan, 2022).

Cold plasma is generated by both direct and indirect methods. So far, an achievement for direct cold plasma has well experimented in the field of food science while the setup for indirect cold plasma is demanding (Misra *et al.*, 2011). Until now, there has not been any study conducted on indirect DBD with prolonged shelf-life. Further, consumers have looked forward for dairy products which are nutritious, minimally processed, safe, healthy, and economical along with a long shelf life (Mir, Shah and Mir, 2016). In spite of many studies on cold plasma of milk was conducted there is limited study on cold plasma on milk shelf-life extension. Therefore, this study focused on a novel plasma bubbling set-up based on indirect DBD method and was established to observe the effect of bubbling of plasma on the microbial decontamination as well as the physicochemical properties of raw cow milk during extension of shelf-life and a comparative study on different decontamination method used for milk pasteurization along with raw cow milk and plasma bubbling of milk was conducted for milk quality evaluation.

2. MATERIALS AND METHODS

2.1 Experimental set up

A cold plasma system named as plasma bubbling was developed by (Aparajhitha and Mahendran, 2019). Briefly, an encapsulated DBD plasma source was created using a cylindrical aluminium container named as plasma generator with two-port: one inlet and another outlet port. The inlet port was connected to an air pump through a pipe. The air pump was supplied with atmospheric gas used as feed gas and the plasma generator converted the atmospheric gas (feed gas) to free radicals. The outlet port was connected from the plasma generator to the sample through a pipe and was immersed to the sample (Fig 1). However, some modification of parameters on plasma bubbling system for generation of plasma was done in this study such as voltage and flow rate.

2.2. Analysis of milk sample

In the present study, fresh raw cow milk collected from the local market of Thanjavur was analysed: followed by plasma bubbling treatment at voltage 200V, flow rate of air 10 L/h, the volume of 100mL of milk sample with treatment time intervals of 5, 10 and 15 min. The samples were treated with plasma bubbling system with some modification on the plasma parameters (Aparajhitha and Mahendran, 2019)[22]. An overall schematic diagram of the study was represented in (Fig1). An extensive comparison of plasma bubbled milk was done with the fresh raw cow milk (control sample), boiled fresh cow milk at 100°C for 10 minutes (Metwally *et al.*, 2011)[23], commercialized (UHT and Pasteurized) milk sample.

2.3 Microbial analysis

The plasma bubbling of milk, control, boiled, commercialised (UHT and pasteurized) milk was tested for coliform and yeast counts by violet red bile agar (Ray and Speck, 1978) and chloramphenicol yeast glucose agar (Torkar and Teger, 2006). Incubation was done by incubator (CI-10 plus LED Version BOD Incubator, REMI, Madurai, India) at 37ºC for coliform while for yeast the temperature set up was 25°C(Wells *et al.*, 1991)(Marshall, Poulson-Cook and Moldenhauer, 1998). The calculation was represented as log CFU/mL (Marshall, Poulson-Cook and Moldenhauer, 1998).

2.4 pH analysis

After giving plasma bubbling treatment to the milk sample, the pH was measured and compared with the control, boiled, commercialized (UHT and pasteurised) milk using the pH meter (Laqua, Horiba Scientific, Singapore) at ambient temperature (Alves *et al.*, 2018) for the detection of changes in the concentration of hydrogen in the sample.

2.5 Titratable Acidity

Titratable acidity was performed (Muhammad *et al.*, 2019). Briefly, 20mL of the sample was taken and 5 drops of 1% w/v phenolphthalein indicator were added to the conical flask. This mixture was subjected to titration by using 0.1N NaOH and represented in % of lactic acid (binti Zakaria *et al.*, 2020).

2.6 Total soluble solids

The amount of dissolved solids present in milk was measured by total soluble solids (TSS). The TSS content was measured at room temperature (30±5ºC) by a digital refractometer (Erma, Japan, 0-80°Brix). The refractometer was calibrated with distilled water before measurement and represented as °Brix (Cavalcanti *et al.*, 2008).

2.8 Nutrient Content

The protein content in milk is determined using protein nitrogen content of milk Kjeldahl method (Barbano, Lynch and Fleming, 1991). Fat content of milk was analysed using (Kleyn *et al.*, 2001).

2.9 Viscosity

The viscosity of milk was measured using Viscometer (DV-I PRIME, AMETEK, Brookfield, USA) equipped with a thermostatically controlled water. The rotor SC4-2 was used with a rotation speed of 200rpm. The measurements for control and treated samples were observed at 25±0.1°C (Ting *et al.*, 2016).

2.9.1 Shelf-Life Study

The storage study of the plasma bubbled sample was compared with the control sample. All the milk samples were stored in the refrigerator at 4°C with glass jars and the samples were withdrawn once a week (Girling, 2003)(Sepulveda *et al.*, 2009) for the evaluation of important parameters of the milk i.e., analysis of microbial count, pH, TA, viscosity and TSS at the interval of 0, 7, 14, 21, 28 and 35th days.

2 statistical Analysis

The result was analysed by using SPSS version 22 statistical software (SPSS, Inc., United States). All the experimental analyses were replicated three times. The statistical analysis was carried out by using a one-way analysis of variance (ANOVA). When significant deviations were detected, the differences among the mean values were determined by executing Duncan's multiple tests of comparison at a confidence level of $p < 0.05$.

4. RESULT AND DISCUSSION

4.1 Microbial Study

The result of viable cell count of coliform and yeast was monitored in the plasma bubbling, control and boiled milk sample which was compared with commercially available UHT and pasteurized milk samples (Fig 2). Plasma bubbling treated milk sample for 5, 10 and 15 min at (200V, 10L/h,100mL) was mentioned as M1, M2 and M3 respectively. A declined in microbial cell viability was observed for M3 treated sample while the control value was 6.38±0.002. These results indicate that the plasma bubbling system used in this study can reduce the number of microbes in milk with an increase of interval of time. Again, the commercialized UHT milk decontaminates from all microorganisms, but already it has been studied that the physical properties were affected during the shelf-life study of UTH milk (Deeth and Datta, 2011).

A significant reduction was observed in coliform, yeast after plasma bubbling. While the treated sample M3 was able to decontaminate microbes from cow raw milk samples successfully. This could be explained due to the development of reactive species during plasma generation: thus, showing a better efficacy on microbial cells. Previous studies reported that with an increase in time and flow rate in plasma bubbling, the hydroxyl radical ($^{\bullet}OH$) generation was increased (Metwally *et al.*, 2011), which could be responsible for the destruction of microbial cell (Dobrynin *et al.*, 2009).

The time interval of 15min was taken as the final treatment time because of its satisfactory deactivation of microbial load. Specifically, in dairy products, the result of cold plasma treatment on microbes depends on various factors such as target species of microorganism,

time interval, input power, gas and food composition (Coutinho *et al.*, 2018). The inactivation of microbes by using cold plasma was not fully observed since the nature of plasma is highly complex (Moreau, Orange and Feuilloley, 2008).

Basically, the acceptable range of coliform in milk is 0-1000/ mL at 24h with 37°C ('FSHN 312 : Applied Microbiology', 2018) which was owned by the plasma bubbling treated sample M3. Previously it has been observed that the yeast cells are found in both raw and pasteurised milk (Foster *et al.*, 1957)(Randolph *et al.*, 1973)(JONES and LANGLOIS, 1977)(Fleet and Mian, 1987) but at a low population mostly 10^3 cells /mL (Fröhlich-Wyder, 2003), while in this study at M3 treated sample yeast cells were not observed which indicates a good finding.

4.2 pH

The hydrogen concentration is the most important factor in milk. The control value of pH was 6.66 ± 0.015 (Fig 3). While a significant increase was observed in pH after the application of plasma bubbling. The pH value 6.85±0.01 in M3 which is showing a gradual increase with respect to the time. This could be due to the increase in hydroxyl ˙OH radicals' generation during cold plasma generation (Canal, Tampieri and Ginebra, 2021). The result obtained for boiled and commercialised (pasteurised and UHT) milk samples showed a small change in hydrogen concentration that could be explained by the changes in gelation behaviour during heating of milk (Vasbinder and De Kruif, 2003).

4.3 Titratable acidity

In the post plasma bubbled milk sample with respect to the time interval, a decreasing value of % of lactic acid was observed at M3 treated sample i.e., 0.121±0.002 (Fig 3), whereas the control sample TA value was 0.144±0.002. This could be explained due to the increase of OH radical formation during cold plasma treatment which is responsible for the decomposition of an additional water molecule into the sample(Guo, Huang and Wang, 2015).

4.4 Total soluble solids

The % of TSS was decreased after a long time of exposure to plasma bubbling. The valu of °Brix of control sample was 10.4±0.003 while at M3 treated sample the value decreased to 8±0.001. This decrease in °Brix value could be explained due to the ozonolysis, happening during plasma exposure which made cleavage of the glycosidic bond and helps to de-polymerisation of macromolecule (Ben'Ko, Manisova and Lunin, 2013)(Almeida *et al.*, 2017). A comparative description of the physicochemical property of milk (Fig 3).

4.5 Nutrient content

The protein and fat content were and the raw milk (control) is given in the (Fig 4). Protein and fat content in milk after plasma bubbling treatment for all the treatment time were found to be significantly not different (P>0.05) from the control. A similar findings of protein content after cold plasma was observed on milk (Mcauley *et al.*, 2016). Further, the fat content of milk was slightly decrease which could be due to the colour difference on milk after exposure to cold plasma due to the reactive species which have high oxidation ability (Gavahian *et al.*, 2018).

4.6 Storage study of milk

In the present study, a comparison of storage study was done for bubbled milk i.e., 200V,10 L/h, 100mL at time interval of 5, 10 and 15 min which compared with the control sample (Table 1). Plasma bubbling is a non-thermal technology based on indirect DBD, where the treatment used to carry out at room temperature due to this reason control sample was compared with the plasma bubbled milk sample. The shelf life of the plasma bubbled milk samples was established by taking into account the initial microbial populations of the control sample. During storage, there were an increase of coliform and yeast counts in the control sample and spoilage was declared on the 3rd day of storage. The coliform and yeast count of the control sample were 6.38 and 4.42 respectively, for the 0th day. During storage period in the plasma bubbling treated sample: a drastically reduction was observed on microbial load for M1, M2 and M3 (Fig 5). The sudden reduction of microbial cell could be described by the hydrogen peroxide produced by cold plasma (Yan *et al.*, 2017) which is stable and storable at a low temperature ranging from (-60-0ºC)(Cass, Paris and Stock, 1966) Further, hydrogen peroxide is considered an antimicrobial compound (Ikawa, Kitano and Hamaguchi, 2010)(Liu *et al.*, 2010). A similar finding of sudden decrease in microbial load was observed on cold plasma milk (Kim *et al.*, 2011).

The spoilage was calculated based on the activation of microorganisms. The milk sample treated for M1 was spoiled on 14 days of storage while for M2 and M3 the spoiled was declared on the 28th day 35th day of the exposure respectively, the spoilage of milk could be described by activation of coliform which helps in the fermentation of lactose by producing of gas and acid (Kornacki, 2001) (Liu *et al.*, 2010).

4.7 Physiochemical properties of stored milk

During storage, for the control milk: the pH decreased to 4.2±0.013 on 3rd day of storage; whereas a gradual increase in pH was observed for the treated sample on the 7th day of storage for M1, M2 and M3 plasma bubbling treated sample. The sudden increase in pH could be due to the $OH^{\bullet}$ radical production in cold plasma technology (Canal, Tampieri and Ginebra, 2021): the closed glass vessel could maintain the $OH^{\bullet}$ radical formed during cold plasma during storage at refrigerator condition. However, the M1 spoiled on the 14th day of storage, M2 on the 28th day and M3 on 35 days of storage with pH value 4.1±0.015, 4.0± 0.012, 4.1±0.015 respectively, the decrease could be explained due to the acidification of milk (Adrian, Rodica and Iuliana Cretescu, 2014)(Table 2). The spoilage of milk was calculated based on the activation of microorganisms and pH value. The other parameters of physicochemical properties were also observed during the storage period in which % lactic acid increased with respect to time of treatment. The value of % lactic acid for M3 was 0.144±0.002 on the 35th day of storage while control showed a value of 0.202±0.013 on the 3rd day of storage: the increase in % lactic acid could be explained due to the high bacterial activity during storage (Adrian, Rodica and Iuliana Cretescu, 2014). Again, TSS increased significantly with progress in storage days, which could be owing to the hydrolysis of components (Mittal and Bajwa, 2014). The control milk shelf life was of 3 days while for plasma bubbling treated milk the shelf life was 14, 28 and 35 for M1, M2 and M3 respectively.

The viscosity was observed for M1, M2 and M3 with respect to control sample. In the initial day (0th day) the viscosity of the plasma bubbling treated milk statistically showed a non-significant value i.e., negligible decrease. M1, M2 and M3 value was 1.66, 1.65 and 1.64cP respectively while the value of control was 1.67cP. The slight decrease in viscosity value could be postulated due to high voltage during cold plasma generation which is responsible for the oxidation of protein and lipid (Sarangapani *et al.*, 2017). Again, during storage days the value

of viscosity remain constant which could be due to lower temperature specifically at 2-5°C on refrigerator condition which was able to deferred the time for viscosity change and responsible to maintain water retention ability of macromolecular substances in milk. Eventually, an increased value was observed during spoilage of milk could be due to the denaturation of casein (Ting *et al.*, 2016).

5.**Conclusion**

Next to natural raw cow milk the quality of the milk is maintained by cold plasma technology introduced to food industry. The freshness, safety and natural characteristic are observed in plasma bubbling. So far, no other technology has been produced favourable property along with microbial safety concerns simultaneously. The plasma bubbling system was able to decontaminate microbes like coliform, yeast satisfactorily. The treatment time was played a major role in the decontamination of microbial load from the milk sample. The plasma bubbling generated system efficiently reduced the number of colonies in milk with one month shelf life on 200volt, 10 L/ h, 15 min, 100mL without any negative effect. To better understand the quality of plasma bubbling on milk, further sensory analysis should be done.

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7. AUTHORS CONTRIBUTION

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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TABLE 1. Microbial Analysis during storage

Treatment	Stage of sampling	Storage days	Coliform	Yeast
	Control	0	6.38±0.002[d]	4.42 ±0.012[d]
		3	SP*	SP*
	M1	0	5.23 ±0.012[c]	3.86 ±0.002[c]
		7	UD[a]	UD[a]
		14	7.45 ±0.02[e]*	6.51 ±0.03[e]*
	M2	0	4.3 ±0.002[b]	3±0.001[b]
		7	UD[a]	UD[a]
		14	UD[a]	UD[a]
		21	UD[a]	UD[a]

200v *100mL*10lph		28	7.49 ±0.04^{f}*	6.53 ±0.03^{g}*
	M3	0	UD^{a}	UD^{a}
		7	UD^{a}	UD^{a}
		14	UD^{a}	UD^{a}
		21	UD^{a}	UD^{a}
		28	UD^{a}	UD^{a}
		35	7.45 ±0.02^{e}*	6.51 ±0.02^{f}*

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

UD: Represents undetectable cell count

SP and * values: Represent spoiled sample

M1, M2 and M3: represents 5, 10 and15 min treatment respectively.

TABLE 2. Physiochemical property of storage study

Treatment	Stage of sampling	Storage days	pH	TSS (°Brix)	TA (% of lactic acid)	Viscosity (cP)
	Control	0	6.76±0.015^{h}	10.4±0.003^{i}	0.144±0.002^{f}	1.67±0.002^{a}
		3	4.2±0.013^{a*}	11.9±0.015^{j*}	0.202±0.013^{h*}	7.22±0.002^{f*}
	M1	0	6.83±0.012^{j}	8.5±0.002^{d}	0.135±0.002^{e}	1.66±0.001^{a}
		7	6.76±0.011^{h}	8.7±0.011^{f}	0.135±0.012^{f}	1.66±0.002^{a}
		14	4.1±0.015^{c}*	11.6±0.001^{j}*	0.144±0.010^{f}*	4.31±0.002^{e*}
	M2	0	6.87±0.001^{j}	8.3±0.002^{b}	0.126±0.002^{c}	1.65±0.002^{a}
		7	6.89±0.015^{k}	8.4±0.001^{c}	0.126±0.002^{c}	1.65±0.003^{a}
		14	6.74±0.011^{g}	8.6±0.012^{e}	0.13±0.013^{d}	1.68±0.002^{a}
		21	6.65±0.010^{e}	9.1±0.014^{g}	0.135±0.012^{e}	2.38±0.002^{b}

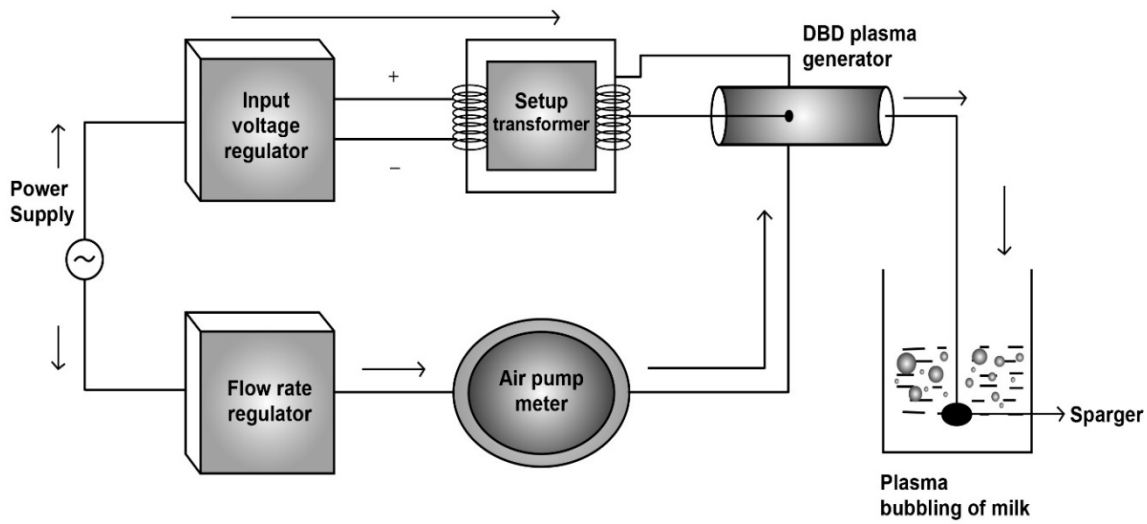
200 V, 100 mL, 10 L/h		28	4.0±0.012[a]*	11.2±0.001[j]*	0.144±0.011[f]*	4.30±0.001[e]
	M3	0	6.89±0.015[k]	8.2±0.001[a]	0.117±0.002[a]	1.64±0.001[a]
		7	6.9±0.012[k]	8.2±0.015[a]	0.117±0.012[a]	1.64±0.002[a]
		14	6.79±0.011[i]	8.4±0.012[c]	0.126±0.002[c]	1.66±0.002[a]
		21	6.68±0.015[f]	8.6±0.010[e]	0.126±0.001[c]	1.68±0.003[a]
		28	6.60±0.012[e]	9.2±0.011[h]	0.132±0.012[e]	2.20±0.001[b]
		35	4.1±0.013[a]*	11.1±0.013[j]*	0.144±0.002[f]*	4.27±0.001[d]*

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

SP and * values: represent the spoilage of the sample

M1, M2 and M3: represents 5, 10 and15 min treatment respectively.

Fig 1. Schematic representation of plasma bubbling system.



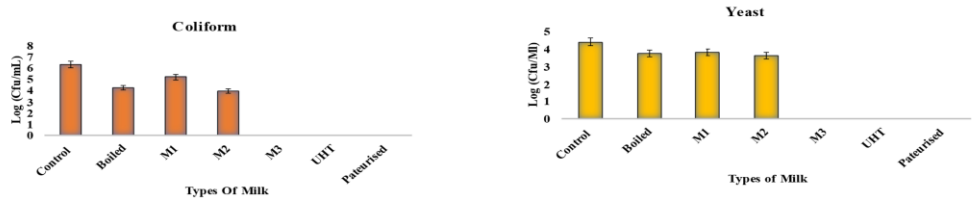


Fig 2. A comparative study on microbial cell viability of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).

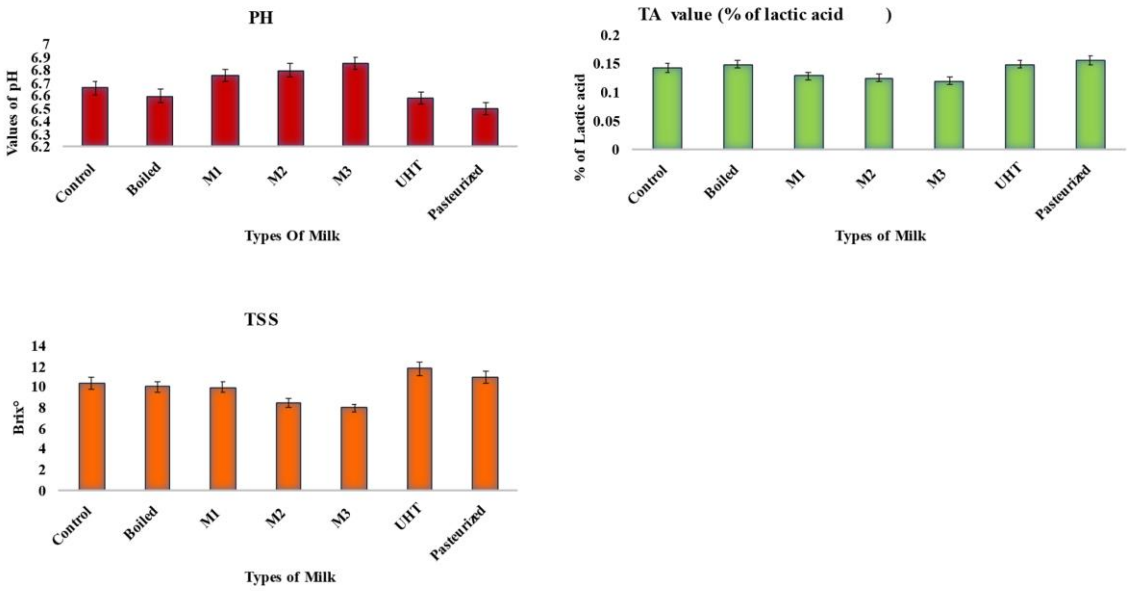
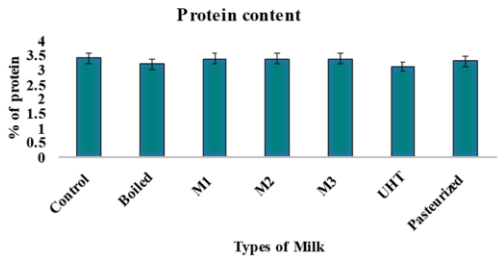


Fig 3. A comparative study on physicochemical property of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).



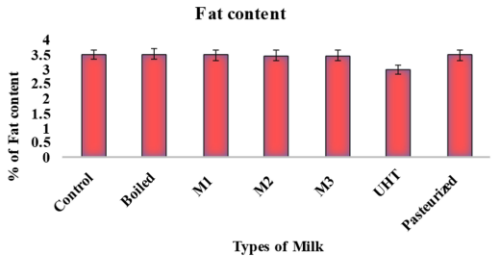


Fig 4. A comparative study on nutrient content of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).

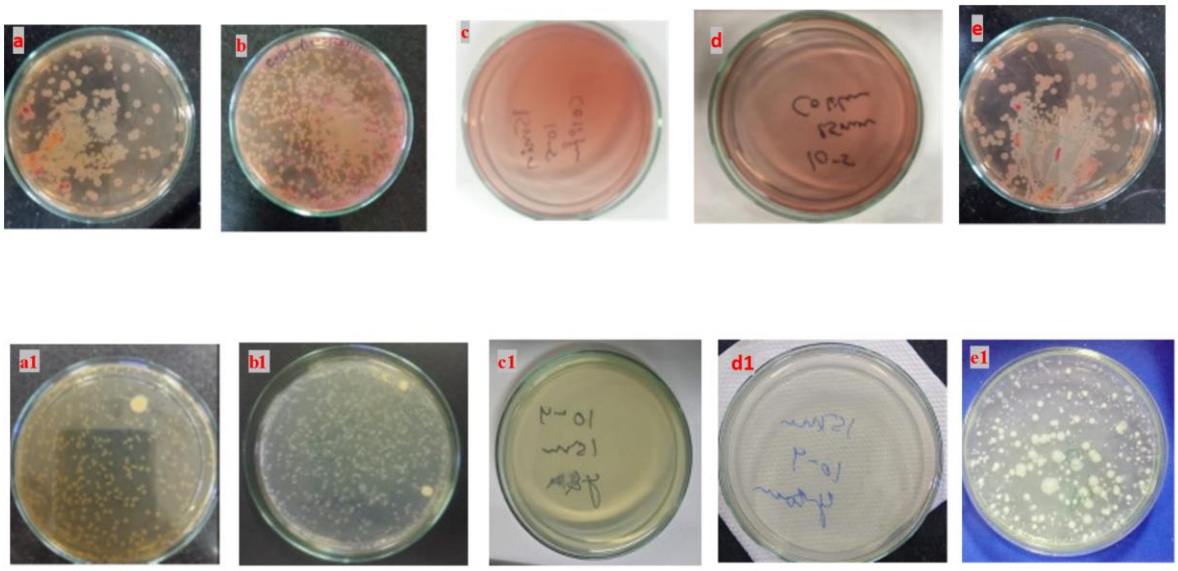


Fig 5. A comparative study on microbial colony reduction in raw milk and M3 treated sample during storage period a) control (untreated), b) spoiled of control milk on 3rd day c) M3 on 0th day, d) M3 on 28th day and e) M3 on 35th day.