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Research Article

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Stability and Shelf-Life of Plasma Bubbling Treated Cow Milk

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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 a new technology for the preservation and decontamination of food products. The present study was conducted for the scrutinization of microbial and physicochemical characteristics of plasma bubbled raw cow milk and an extensive comparison was observed for boiled raw cow milk, commercially (pasteurised and UHT) milk. Further, storage study (shelf-life) was done for the plasma bubbled raw cow milk and compared with raw cow milk sample (control). The bubbling of plasma was generated at a voltage of 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 with a volume of 100 mL of the sample operated at room temperature. A declined in microbial cell was observed for coliform and yeast at 200V, 10L/h, 15 min time interval. The pH value of 15 min plasma bubble treated sample was increased significantly to 6.85. While, a slight decrease in value was noticed in total soluble solids (TSS) and titratable acid (TA) after exposure to plasma bubbling. Further, a nondetrimental effect was observed for the nutrient content of plasma bubbling of milk. The result indicates that plasma bubbling at generated at 200V, 10L/h,100mL,15 min treatment enhances the milk quality. However, plasma bubbling: based on indirect dielectric barrier discharge (DBD) may use as a successful decontamination technology without affecting the physicochemical properties which could have a future perspective on industrial food applications.

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

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1. INTRODUCTION

Milk is a physiological liquid combination of highly biological active compounds with protein and fat[1]. All over the world cow milk is predominant consumed in different forms and also highly perishable with rich in biological nutritive value[2-3]. Simultaneously, the hazardous effect of milk due to pathogen contamination is also well studied. Generally, the microbes observed in dairy are coliform, Escherichia coli, Staphylococcus aureus, yeast and mould[4]. Previous studies have analysed that the diseases caused by the of milk contamination include bovine mastitis[5], diarrhoea[6], milk-borne diseases caused in the dairy industry[7] due to the lack of proper 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[7]. 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[8]. Also, thermal processes involve many changes in the milk composition such as flavour and colloidal properties[9-10-11]. 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 [12-13]. The cold plasma efficiency has been well documented in the sanitation of food[14]. The important characteristics for the deactivation of microorganisms are the major source of free radicals, ions, electrons generated during treatment of non-thermal plasma. Generally, the radicals like reactive oxygen species (ROS) can directly deactivate microorganisms by gaseous phase[15]. Further, the potentiality of non-thermal plasma is executed to food like milk for the deactivation of bacteria, E. coli[16-17]. Recently, a study on milk sterilization was observed by dielectric barrier discharge (DBD) but the change in physicochemical properties was found[18]. Further, on low pressure plasma was detected for milk decontamination where a reduced value in nutrient content such as fat was observed[19]. In contradictory to the finding, a preliminary investigation of based on plasma bubbling of milk was experimented where very negligible changes was noticed for physicochemical property on milk[20]. These findings confirmed that the non-thermal plasma set up playing a major role for the quality of the product.Generally, non-thermal plasma or cold plasma is generated by both direct as well as indirect methods. In direct cold plasma application: the generation of a large variety of reactive species, with a short life span (~milliseconds) reactive species, surface plasma reactions (etching and deposition) were observed[21]. In indirect (remote plasmas), contains longer-living reactive species such as nitric oxide or ozone contact the food, while the generation of plasma was done in a separate chamber[22]. In indirect cold plasma, the quantum of heat transmission of heat to the sample is reduced. So far, an achievement for direct cold plasma has well experimented in the sector of food science while the setup for indirect cold plasma is demanding[23]. Again, consumers have looked forward for dairy products which are nutritious, minimally processed, safe, healthy and economical longer shelf life[24]. Therefore, the focus of the study aimed towards on a novel plasma bubbling set-up based on indirect DBD method and was established to observe the bubbling of plasma effect on the decontamination of microbial cell as well as the physicochemical properties during extension of shelf-life. Further, a comparative study was observed on different decontamination method such as boiled and commercialized (UHT, pasteurized) milk with fresh raw cow milk (control) and plasma bubbling of milk for quality evaluation.

2. MATERIALS AND METHODS

2.1 Plasma Bubbling set up

A non-thermal plasma system named as plasma bubbling based on indirect DBD set up was developed[25]. Briefly, indirect 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 generator of plasma 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: such as voltage, air flow rate and time interval.

2.2. Analysis of milk sample

In this study, fresh raw cow milk, from the local market was analysed: followed by plasma bubbling treatment at voltage 200V, air flow rate 10 L/h, the sample volume 100mL, with treatment time intervals of 5, 10 and 15 min. An extensive comparison of plasma bubbled milk was made with the fresh raw cow milk (control sample), boiled fresh cow milk at 100°C for 10 minutes[26], commercialized (UHT and Pasteurized) milk sample.

2.3 Microbial analysis

The bubbled plasma milk, control, boiled, commercialised (UHT and pasteurized) milk was tested for coliform and yeast counts by violet red bile agar[27] and chloramphenicol yeast glucose agar[28]. Incubated by incubator (CI-10 plus LED Version BOD Incubator, REMI, Madurai, India) at 37°C for coliform. While for yeast the temperature set up was 25°C[29-30]. The calculation was represented as log CFU/mL[30.]

2.4 pH analysis

After giving plasma bubbling treatment to the milk sample, the pH was measured and comparison was made with the boiled, commercialized (UHT and pasteurised) along with the control milk sample using pH meter (Laqua, Horiba Scientific, Singapore) and operated at ambient temperature[31] to observe the changes occurred on the hydrogen concentration for the milk sample.

2.5 Titratable Acidity

Titratable acidity was performed[32]. Briefly, sample volume (20mL) was taken and 1% w/v phenolphthalein indicator (5 drops) were mixed to the sample. This mixture was followed by titration using 0.1N NaOH and represented in % of lactic acid[33].

2.6 Total soluble solids

The presence of quantity of dissolved solids in milk was calculated by TSS. The content of TSS was noticed at ambient temperature i.e., room temperature (30±5°C) by digital refractometer (Erma, Japan, 0-80^{O} Brix). Distilled water was

used for the calibration of refractometer before measurement and represented as °Brix[34].

2.7 Nutrient Content

The protein content is determined using protein nitrogen content of milk Kjeldahl method[35]. Fat content of milk was analysed using[36].

2.8 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 used with a rotation speed of 200rpm. The measurements for treated and control milk samples were detected at 25±0.1°C[37].

2.9 Shelf-Life Study

The storage study was observed for the plasma bubbled sample and a comparison was made in accordance to the control sample. All the samples were stored in the refrigerator at 4°C with glass jars and the milk samples were withdrawn once a week[38-39] to the observe the important parameters i.e., analysis of microbial count, pH, TA, viscosity and TSS at the interval of 0, 7, 14, 21, 28 and 35th days.

3. STATISTICAL ANALYSIS

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

4. RESULT AND DISCUSSION

4.1 Microbial Analysis

The result of viable cell count of coliform and yeast was monitored in the treated, control and boiled milk sample which was compared with the commercialised (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 statistically reduction ($P<0.05$) i.e., no microbial cells were observed for M3 treated sample; the control sample value was 6.38±0.002 and 4.42±0.002. These findings observed that the cold plasma system (plasma bubbling) established in this study can reduce of microbes in milk satisfactorily. A significant microbial cell decontamination was obtained for coliform and yeast after plasma bubbling. While a successful decontamination was observed for sample M3. This could be explained due to the creation of reactive species during generation of plasma: thus, showing a better efficacy on microbial cells. Previous studies reported that the air flow rate and time in plasma bubbling plays a vital role for the generation of hydroxyl radical ($^{\bullet}OH$)[26], which might an important factor for death of microbial cell[40]. Again, the commercialized UHT and pasteurized milk decontaminates from all microorganisms, but already it has been reported that the physical properties were affected during the shelf-life study of UTH and pasteurized milk[9-8].The time interval of 15min was found as the final treatment time because of its satisfactory deactivation of microbial load. Specifically, in milk products: result of non-thermal/ cold plasma application on microbes depends on various factors such as target species of microorganism, time interval, input power, gas and food composition[41]. The decontamination of microbes by using cold plasma was not fully observed since the nature of plasma is highly complex[42]. Basically, the acceptable range of coliform in milk is 0-1000/ mL at 24h with 37°C[43] which was owned by the bubbled plasma treated sample M3. Previous report put forth that the yeast cells are found in both raw and pasteurised milk[44-45-46-47] but at a low population mostly 10^3 cells /mL[48], while the study observed at M3 treated sample yeast cells were not detected which indicates a good finding.

4.2 pH

The control value of pH was 6.66 ± 0.015 (Fig 3). While a statistically significant ($P<0.05$) increase was found in pH after exposure to plasma bubbling. The pH value 6.85±0.01 in M3 which is showing a gradual increase with an increase in time interval. This could be explained due to the increase in hydroxyl '$^{\bullet}OH$ radicals' generation during cold plasma generation[49]. A contradictory result was observed in cold plasma applied milk sample[19]. For boiled samples: result showed decrease value in hydrogen concentration as it may be explained that changes observed in the gelation behaviour during heating of milk[50].

4.3 Titratable acidity

In the post plasma bubbled milk sample with the increase in time interval, a significant decreasing value of lactic acid (%) was observed at M3 treated sample i.e., 0.121±0.002 (Fig 3), whereas, the control sample's TA value was 0.144±0.002. This could be explained because of the increase of $OH^{\bullet}$ radical formation during cold plasma treatment which was accountable for the decomposition of an additional water molecule into the sample[51]. While, a contradictory to this study observed an increase in TA value in tiger nut milk after exposure to cold plasma[32]. The boiled milk showed significant ($P<0.05$) increase in TA value i.e., 0.15±0.002. The gradual increase in TA value of boiled milk sample could be owned due to the high heat treatment which was responsible for the lactose degradation[52-53].

4.4 Total soluble solids

The value of TSS was decreased after a long time of exposure to plasma bubbling. The value of °Brix in 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 for the ozonolysis, happening throughout plasma exposure which made cleavage of the glycosidic bond and helps to de-polymerisation of macromolecule[54-55]. A comparative description was mentioned for the physicochemical property of milk (Fig 3). This contrast, previous report revealed that no significant change was observed in nut milk of tiger after exposure to cold plasma[32]. The TSS value for boiled sample is non significantly decrease ($P <0.05$) 10.1±0.001. The slight decrease in TSS could be explained due to evaporation of water from milk during boiling.

4.5 Nutrient content

(UHT and pasteurized) sample is given in the (Fig 4). The value of nutrient content in milk after plasma bubbling treatment for all the treatment time i.e., (M1, M2 and M3) were observed to be significantly not different (P>0.05) from the control sample. For protein and fat content, the value of control sample was 3.41±0.001% and 3.52±0.001% respectively. While, for M3 the value was 3.39±0.002 and 3.49±0.001% respectively. A similar finding was described in content of protein after cold plasma of milk[56]. Further, in a recent study, a nondetrimental effect was observed on cold plasma of milk[19]. In the present study, fat content of milk was slightly decreased could be due to the difference in colour value on milk after exposure to cold plasma for the generation of reactive species which have high oxidation ability[57]. A similar finding on the composition of milk fatty acid was observed[58]. A non-significant (P<0.05) increase value was noticed for boiled milk sample for fat content i.e., 3.53±0.003. The negligible increase in fat content of boiled sample could be due to waste of evaporated water during heat treatment[59]. While a decrease value in content of protein was observed after boiling of milk could be due to heat which was responsible for the whey protein denaturation: particularly beta lactoglobulins[60].

4.6 Storage study of milk

In the present study, a comparison of storage study was done for plasma bubbled milk i.e., 200V,10 L/h, 100mL at time interval of 5, 10 and 15 min represented as M1, M2 and M3 which compared with the control sample (Table 1). The shelf-life of the plasma bubbled samples were established by the initial microbial load for the control sample. During storage, there were a gradual increase of coliform and yeast in the control sample was observed 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[61] which is stable and storable at a low temperature ranging from (-60-0°C)[62]. Further, hydrogen peroxide is examined as an antimicrobial compound[12-63]. A similar finding of sudden decrease in microbial load was noticed on cold plasma of milk[16]. The spoilage calculation was done on the basis of 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 The protein and fat content of control, plasma bubbling treated sample, boiled sample along with commercialized 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[64-63].

4.7 Physiochemical properties of stored milk

During the period of storage study, control sample: the pH value decreased to 4.2±0.013 on 3rd day of storage; whereas a gradual increase in pH value was detected for the treated sample on the 7th day of storage for M2 and M3 plasma bubbling treated sample. The sudden rise in pH could be due to the OH$^{\bullet}$ radical production in cold plasma technology[49]: the closed glass vessel could maintain the OH$^{\bullet}$ radical formed on the non-thermal/cold plasma generation 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[65] (Table 2). The other parameters were also observed throughout the storage period in which % lactic acid increased with reference to the interval of time. 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. However, the increase in % lactic acid could be explained because of the high bacterial activity during storage[65]. Again, TSS (°Brix) increased significantly with progress in storage days, as it may be owing to the hydrolysis of components[66]. Further, the viscosity was noticed for M1, M2 and M3 with reference to control sample. In the initial day (0th day) the viscosity of the plasma bubbled 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 negligible decrease in viscosity value could be postulated due to high voltage during cold plasma generation which is accountable for the oxidation of lipid and protein[67]. A contradictory finding was noticed on cold plasma of milk[68]. Again, during storage days the value of viscosity remain constant which might explain 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 noticed during spoilage of milk could be explain due to denaturation of casein[37]. However, 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 respective

TABLE 1. Microbial Analysis during storage

Treatment	Stage of sampling	Storage days	Coliform (Log cfu/mL)	Yeast (Log cfu/mL)
	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}

200v *100mL*10lph		14	UD[a]	UD[a]
		21	UD[a]	UD[a]
		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[d]	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*]
200 V, 100 mL, 10 L/h	**M1**	0	6.83±0.012[e]	8.5±0.002[d]	0.130±0.002[e]	1.66±0.001[a]
		7	6.81±0.011[e]	8.7±0.011[f]	0.135±0.012[f]	1.66±0.002[a]
		14	4.1±0.015[a*]	11.6±0.001[j*]	0.144±0.010[f*]	7.31±0.002[e*]
	M2	0	6.87±0.001[f]	8.3±0.002[b]	0.126±0.002[c]	1.65±0.002[a]
		7	6.89±0.015[f]	8.4±0.001[c]	0.124±0.002[c]	1.65±0.003[a]
		14	6.74±0.011[d]	8.6±0.012[e]	0.130±0.013[d]	1.68±0.002[a]
		21	6.65±0.010[c]	9.1±0.014[g]	0.135±0.012[e]	2.38±0.002[b]
		28	4.0±0.012[a*]	11.2±0.001[j*]	0.144±0.011[f*]	7.30±0.001[e]
	M3	0	6.89±0.015[f]	8.2±0.001[a]	0.117±0.002[a]	1.64±0.001[a]
		7	6.9±0.012[g]	8.2±0.015[a]	0.116±0.012[a]	1.64±0.002[a]
		14	6.79±0.011[d]	8.4±0.012[c]	0.126±0.002[c]	1.66±0.002[a]
		21	6.68±0.015[c]	8.6±0.010[e]	0.126±0.001[c]	1.68±0.003[a]
		28	6.60±0.012[b]	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*]	7.27±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- and 15-min treatment respectively

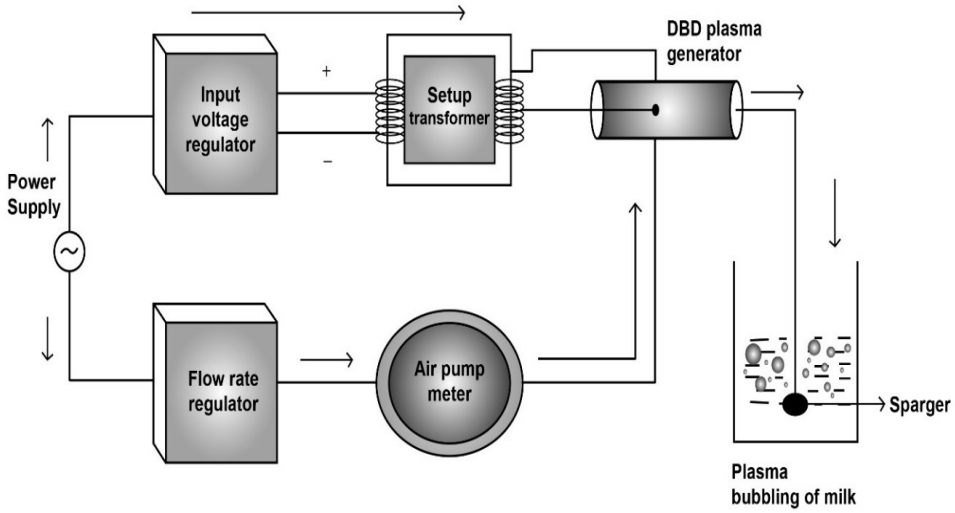

Fig 1. Schematic representation of plasma bubbling system.

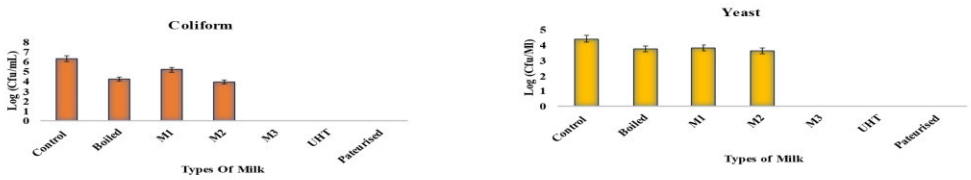

Fig 2. A comparative study on microbial cell viability of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).

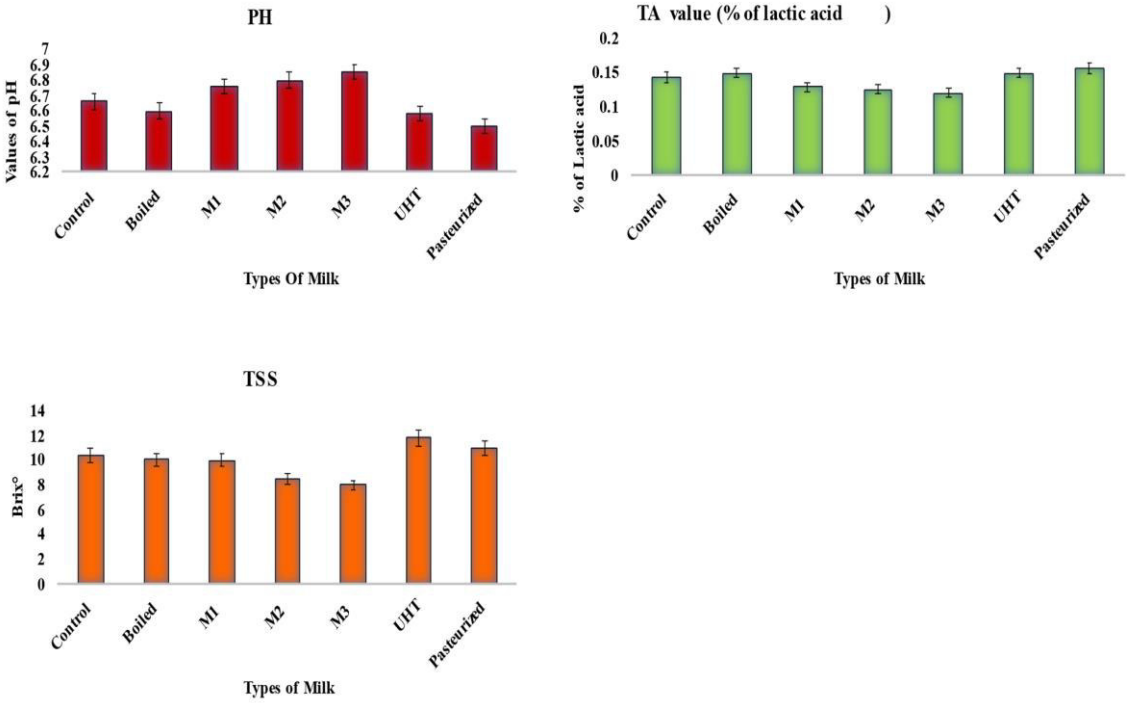

Fig 3. A comparative study on physicochemical property of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).

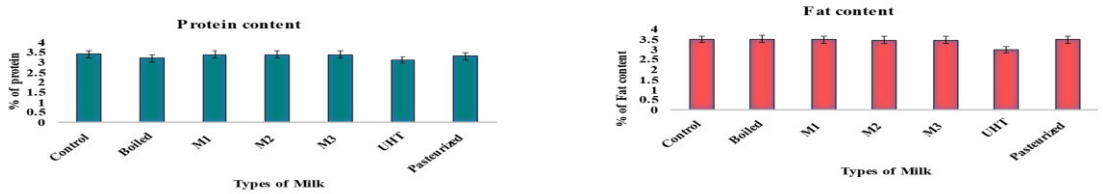

Fig 4. A comparative study on nutrient content of plasma bubbling treated milk with control, boiled, Commercialized (UHT and pasteurised milk).

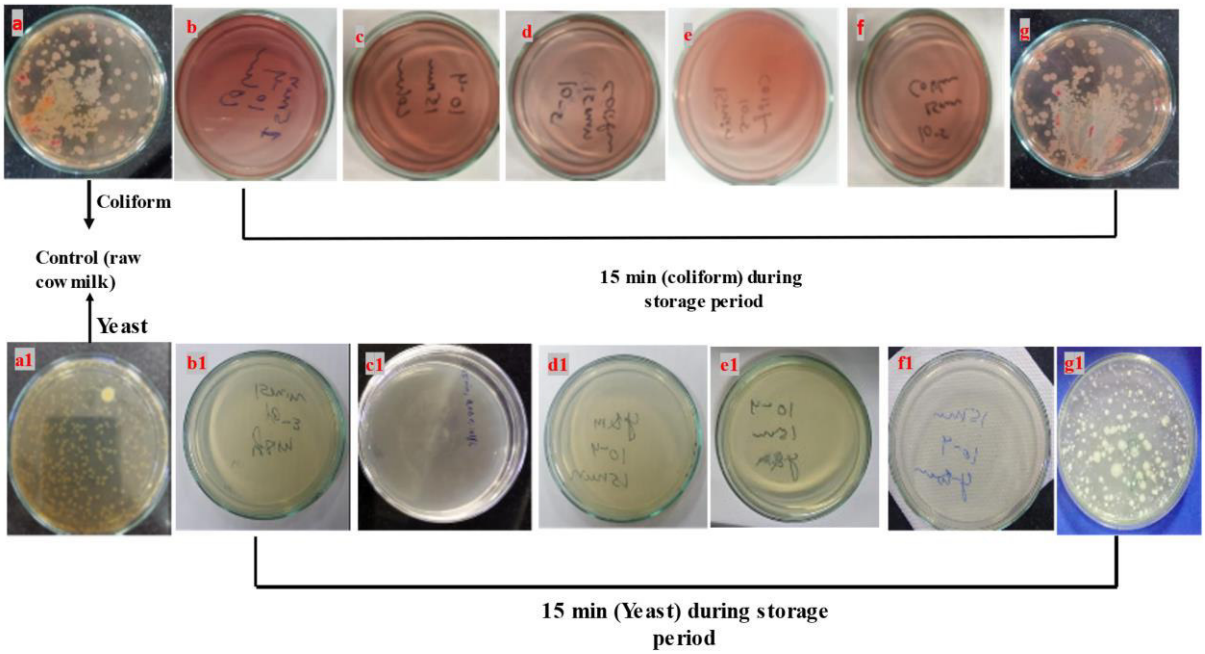

Fig 5. A comparative study on coliform viable cell count in raw milk and M3 (15 min, 10L/h, 200V) treated sample during storage period a) control (untreated), b) M3 on 0th day, c) M3 on 7th day, d) M3 on 14th day, e) M3 on 21st day, f) M3 on 28th day and g) M3 on 35th day (spoiled). For yeast viable cell count in raw milk and M3 treated sample during storage study a1) control (untreated), b1) M3 on 0th day, c1) M3 on 7th day, d1) M3 on 14th day, e1) M3 on 21st day, f1) M3 on 28th day and g1) M3 on 35th day (spoiled).

5. CONCLUSION

Next to natural raw cow milk the attribute of milk is maintained by non-thermal 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 bubbled system was able to decontaminate microbes like coliform, yeast satisfactorily. The treatment time was played a crucial role in the decontamination of microbial load from the milk sample. This technology efficiently reduced the number of colonies in milk with one month shelf life on 200volt, air flow rate of 10 L/ h, 100mL sample volume and time interval of 15 min, without any negative effect. A non-detrimental effect was observed for physicochemical and nutrient content of plasma bubbled milk. To better understand the quality of plasma bubbling on milk, further sensory analysis and protein structural analysis should be done.

6. ACKNOWLEDGEMENTS

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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 result.

8. CONFLICT OF INTEREST

The author declares there is no conflict of interest

9. REFERENCE

1. Ebringer L, Ferenčík M, Krajčovič J. Beneficial health effects of milk and fermented dairy products--review. Folia microbiol. 2008;53(5):378-94. doi: 10.1007/s12223-008-0059-1, PMID 19085072.
2. Rowlands A. Bacteriological Standards for Perishable Foods: (c) Rowlands A. Bacteriological standards for perishable foods. Milk and dairy products. J R Sanit Inst. 1952;72(4):404-10. doi: 10.1177/146642405207200422, PMID 12981677.
3. National Research Council. Designing foods: animal product options in the marketplace. Natl. Acad. Sci (Wash DC). 1988.
4. Ghodeker DR, Dudani AT, Ranganathan B. Microbiological quality of Indian milk products. J Milk Food Technol. 1974;37(3):119-22. doi: 10.4315/0022-2747-37.3.119.
5. Camacho AT, Guitian FJ, Pallas E, Gestal JJ, Olmeda S, Goethert H, Telford S, Spielman A. Serum protein response and renal failure in canine Babesia annae infection. Vet Res. 2005;36(5-6):713-22. doi: 10.1051/vetres:2005026. PMID 16120247.
6. Lambertini E, Karns JS, Van Kessel JAS, Cao H, Schukken YH, Wolfgang DR, Smith JM, Pradhan AK. Dynamics of Escherichia coli virulence factors in dairy herds and farm environments in a longitudinal study in the United States. Appl Environ Microbiol. 2015;81(13):4477-88. doi: 10.1128/AEM.00465-15, PMID 25911478.
7. Dhanashekar R, Akkinepalli S, Nellutla A. Milk-borne infections. An analysis of their potential effect on the

milk industry. GERMS. 2012;2(3):101-9. doi: 10.11599/germs.2012.1020, PMID 24432270.
8. Macdonald LE, Brett J, Kelton D, Majowicz SE, Snedeker K, Sargeant JM. A systematic review and meta-analysis of the effects of pasteurization on milk vitamins, and evidence for raw milk consumption and other health-related outcomes. J Food Prot. 2011;74(11):1814-32. doi: 10.4315/0362-028X.JFP-10-269.
9. Datta N, Deeth HC. Diagnosing the cause of proteolysis in UHT milk. LWT Food Sci Technol. 2003;36(2):173-82. doi: 10.1016/S0023-6438(02)00214-1.
10. Nair PK, Dalgleish DG, Corredig M. Colloidal properties of concentrated heated milk. Soft Matter. 2013;9(14):3815-24. doi: 10.1039/C2SM27540F.
11. Ajmal M, Nadeem M, Imran M, Abid M, Batool M, Khan IT, Gulzar N, Tayyab M. Impact of immediate and delayed chilling of raw milk on chemical changes in lipid fraction of pasteurized milk. Lipids Health Dis. 2018;17(1):190. doi: 10.1186/s12944-018-0843-0, PMID 30115071.
12. Ikawa S, Kitano K, Hamaguchi S. Effects of pH on bacterial inactivation in aqueous solutions due to low-temperature atmospheric pressure plasma application. Plasma Processes Polym. 2010;7(1):33-42. doi: 10.1002/ppap.200900090.
13. Kim HJ, Yong HI, Park S, Choe W, Jo C. Effects of dielectric barrier discharge plasma on pathogen inactivation and the physicochemical and sensory characteristics of pork loin. Curr Appl Phys. 2013;13(7):1420-5. doi: 10.1016/j.cap.2013.04.021.
14. Basaran P, Basaran-Akgul N, Oksuz L. Elimination of Aspergillus parasiticus from nut surface with low pressure cold plasma (LPCP) treatment. Food Microbiol. 2008;25(4):626-32. doi: 10.1016/j.fm.2007.12.005, PMID 18456118.
15. Yu H, Perni S, Shi JJ, Wang DZ, Kong MG, Shama G. Effects of cell surface loading and phase of growth in cold atmospheric gas plasma inactivation of Escherichia coli K12. J Appl Microbiol. 2006;101(6):1323-30. doi: 10.1111/j.1365-2672.2006.03033.x, PMID 17105563.
16. Kim B, Yun H, Jung S, Jung Y, Jung H, Choe W, Jo C. Effect of atmospheric pressure plasma on inactivation of pathogens inoculated onto bacon using two different gas compositions. Food Microbiol. 2011;28(1):9-13. doi: 10.1016/j.fm.2010.07.022, PMID 21056769.
17. Gurol C, Ekinci FY, Aslan N, Korachi M. Low temperature plasma for decontamination of E. coli in milk. Int J Food Microbiol. 2012;157(1):1-5. doi: 10.1016/j.ijfoodmicro.2012.02.016, PMID 22622128.
18. Wu X, Luo Y, Zhao F, M SM, Mu G. Influence of dielectric barrier discharge cold plasma on physicochemical property of milk for sterilization. Plasma Process Polym. 2021;18(1). doi: 10.1002/ppap.201900219.
19. Manoharan D, Stephen J, Radhakrishnan M. Study on low-pressure plasma system for continuous decontamination of milk and its quality evaluation. J Food Process Preserv. 2021;45(2):0-1. doi: 10.1111/jfpp.15138.
20. Samarpita dash and R. Jaganmohan. Impact of plasma Bubbling On Cow Milk: microbial Reduction and quality Improvement, Life J Sci. Int. Pharm Res. 2022;12, 283–288. doi: 10.22376/ijpbs/lpr.2022.12.1.
21. Misra NN, Jo C. Applications of cold plasma technology for microbiological safety in meat industry. Trends Food Sci Technol. 2017; 64:74-86. doi: 10.1016/j.tifs.2017.04.005.
22. Surowsky B, Schlüter O, Knorr D. Interactions of non-thermal atmospheric pressure plasma with solid and liquid food systems: a review. Food Eng Rev. 2015;7(2):82-108. doi: 10.1007/s12393-014-9088-5.
23. Misra NN, Tiwari BK, Raghavarao KSMS, Cullen PJ. Nonthermal plasma inactivation of food-borne pathogens. Food Eng Rev. 2011;3(3-4):159-70. doi: 10.1007/s12393-011-9041-9.
24. Mir SA, Shah MA, Mir MM. Understanding the role of plasma technology in food industry. Food Bioprocess Technol. 2016;9(5):734-50. doi: 10.1007/s11947-016-1699-9.
25. Aparajhitha S, Mahendran R. Effect of plasma bubbling on free radical production and its subsequent effect on the microbial and physicochemical properties of Coconut Neera. Innov Food Sci Emerg Technol. 2019;58. doi: 10.1016/j.ifset.2019.102230, PMID 102230.
26. Metwally AM, Dabiza NM, El-Kholy WI, Sadek ZI. The effect of boiling on milk microbial contents and quality. J Am Sci. 2011;7:110-4.
27. Ray B, Speck ML. Plating procedure for the enumeration of coliforms from dairy products. Appl Environ Microbiol. 1978;35(4):820-2. doi: 10.1128/aem.35.4.820-822.1978, PMID 348110.
28. Godič Torkar K, Golc-Teger S. The presence of some pathogen microorganisms, yeasts and moulds in cheese samples produced at small dairy-processing plants. Acta Agric Slov. 2006;1:37-51.
29. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, Downes FP, Martin ML, Griffin PM, Ostroff SM. Isolation of Escherichia coli serotype O157: H7 and other Shiga-like-toxin-producing E. coli from dairy cattle. J Clin Microbiol. 1991;29(5):985-9. doi: 10.1128/jcm.29.5.985-989.1991, PMID 2056066.
30. Marshall V, Poulson-Cook S, Moldenhauer J. Comparative mold and yeast recovery analysis (the effect of differing incubation temperature ranges and growth media). PDA J Pharm Sci Technol. 1998;52(4):165-9. PMID 9752711.
31. Alves MN, Nesterenko PN, Paull B, Haddad PR, Macka M. Separation of superparamagnetic magnetite nanoparticles by capillary zone electrophoresis using non-complexing and complexing electrolyte anions and tetramethylammonium as dispersing additive. Electrophoresis. 2018;39(12):1429-36. doi: 10.1002/elps.201800095, PMID 29579343.
32. Muhammad AI, Li Y, Liao X, Liu D, Ye X, Chen S, Hu Y, Wang J, Ding T. Effect of dielectric barrier discharge plasma on background microflora and physicochemical properties of tiger nut milk. Food Control. 2019;96:119-27. doi: 10.1016/j.foodcont.2018.09.010.
33. Binti Zakaria Z, Yun WS, Alias N, Noor SNM, Mustapha Z, Hussin N, Yusoff NAM. Physicochemical composition, microbiological quality and consumers'

acceptability of raw and pasteurized locally produced goat milk. Mal. J Fundam Appl Sci. 2020;16:475-82.
34. Cavalcanti AL, Fernandes LV, Barbosa AS, Vieira FF. pH, titratable acidity and total soluble solid content of pediatric antitussive medicines. Acta Stomatol Croat. 2008;42.
35. Barbano DM, Lynch JM, Fleming JR. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: collaborative study. Journal of AOAC INTERNATIONAL. 1991;74(2):281-8. doi: 10.1093/jaoac/74.2.281.
36. Kleyn DH, Lynch JM, Barbano DM, Bloom MJ, Mitchell MW DH Mercer F Monahan D Peat B Petit M. Determination of fat in raw and processed milks by the Gerber method: collaborative study. J AOAC Int. 2001;84(5):1499-508. doi: 10.1093/jaoac/84.5.1499, PMID 11601470.
37. Ting K, Liu YF, Tian-Li G, Lu-Hua Z. Relationships between viscosity and the contents of macromolecular substances from milk with different storage styles. fst. 2016;4(4):49-56. doi: 10.13189/fst.2016.040401.
38. Girling PJ. Packaging of food in glass containers. Food packaging technology. In: Coles R, editor, Dereck Mc Do-well, Mark J Kirwan editors. Food packaging Technology. Oxford: Blackwell Publishing, CRC Press; 2003. p. 152-73.
39. Sepulveda DR, Góngora-Nieto MM, Guerrero JA, Barbosa-Cánovas GV. Shelf life of whole milk processed by pulsed electric fields in combination with PEF-generated heat. LWT Food Sci Technol. 2009;42(3):735-9. doi: 10.1016/j.lwt.2008.10.005.
40. Dobrynin D, Fridman G, Friedman G, Fridman A. Physical and biological mechanisms of direct plasma interaction with living tissue. New J Phys. 2009;11(11). doi: 10.1088/1367-2630/11/11/115020, PMID 115020.
41. Coutinho NM, Silveira MR, Rocha RS, Moraes J, Ferreira MVS, Pimentel TC, Freitas MQ, Silva MC, Raices RSL, Ranadheera CS, Borges FO, Mathias SP, Fernandes FAN, Rodrigues S, Cruz AG. Cold plasma processing of milk and dairy products. Trends Food Sci Technol. 2018;74:56-68. doi: 10.1016/j.tifs.2018.02.008.
42. Moreau M, Orange N, Feuilloley MGJ. Non-thermal plasma technologies: new tools for bio-decontamination. Biotechnol Adv. 2008;26(6):610-7. doi: 10.1016/j.biotechadv.2008.08.001, PMID 18775485.
43. EAS. (East African standards) raw cow milk specifications. EAS 67:2006. ICS 67.100 HS04014.20.00 (Eas. Vol. 67; 2000).
44. FOSTER EM, NELSON FE, SPECK ML, DOETSCH RN, OLSON JC. Dairy microbiology. Prentice-hall, Inc., Dairy Sci. Vol. 29. New York; 1957. PMID 439452.
45. Randolph HE, Chakraborty BK, Hampton O, Bogart DL. Microbial counts of individual producer and commingled grade A raw milk. J Milk Food Technol. 1973;36(3):146-51. doi: 10.4315/0022-2747-36.3.146.
46. Jones FT, Langlois BE. Microflora of retail fluid milk products 1. J Food Prot. 1977;40(10):693-7. doi: 10.4315/0362-028X-40.10.693, PMID 30736235.
47. Fleet GH, Mian MA. The occurrence and growth of yeasts in dairy products. Int J Food Microbiol. 1987;4(2):145-55. doi: 10.1016/0168-1605(87)90021-3.
48. Fröhlich-Wyder MT. Yeasts in dairy products. Yeasts Food. 1970 (2003):209-37. doi: 10.1016/B978-1-85573-706-8.50013-7.
49. Tampieri F, Ginebra MP, Canal C. Quantification of plasma-produced hydroxyl radicals in solution and their dependence on the Ph. Anal Chem. 2021;93(8):3666-70. doi: 10.1021/acs.analchem.0c04906, PMID 33596048.
50. Vasbinder AJ, De Kruif CG. Casein–whey protein interactions in heated milk: the influence of pH. Int Dairy J. 2003;13(8):669-77. doi: 10.1016/S0958-6946(03)00120-1.
51. Guo J, Huang K, Wang J. Bactericidal effect of various non-thermal plasma agents and the influence of experimental conditions in microbial inactivation: a review. Food Control. 2015;50:482-90. doi: 10.1016/j.foodcont.2014.09.037.
52. Raynal-Ljutovac K, Park YW, Gaucheron F, Bouhallab S. Heat stability and enzymatic modifications of goat and sheep milk. Small Rumin Res. 2007;68(1-2):207-20. doi: 10.1016/j.smallrumres.2006.09.006.
53. Prasantha BDR, Wimalasiri KMS. Effect of HTST thermal treatments on end-use quality characteristics of goat milk. Int J Food Sci. 2019;2019:1801724. doi: 10.1155/2019/1801724, PMID 31915676.
54. Ben'Ko EM, Manisova OR, Lunin VV. Effect of ozonation on the reactivity of lignocellulose substrates in enzymatic hydrolyses to sugars. Russ J Phys Chem. 2013;87(7):1108-13. doi: 10.1134/S0036024413070091.
55. Almeida FDL, Gomes WF, Cavalcante RS, Tiwari BK, Cullen PJ, Frias JM, Bourke P, Fernandes FAN, Rodrigues S. Fructooligosaccharides integrity after atmospheric cold plasma and high-pressure processing of a functional orange juice. Food Res Int. 2017;102:282-90. doi: 10.1016/j.foodres.2017.09.072, PMID 29195950.
56. McAuley CM, Singh TK, Haro-Maza JF, Williams R, Buckow R. Microbiological and physicochemical stability of raw, pasteurised or pulsed electric field-treated milk. Innov Food Sci Emerg Technol. 2016;38:365-73. doi: 10.1016/j.ifset.2016.09.030.
57. Gavahian M, Chu YH, Mousavi Khaneghah AM, Barba FJ, Misra NN. A critical analysis of the cold plasma induced lipid oxidation in foods. Trends Food Sci Technol. 2018;77:32-41. doi: 10.1016/j.tifs.2018.04.009.
58. Kim HJ, Yong HI, Park S, Kim K, Choe W, Jo C. Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. Food Control. 2015;47:451-6. doi: 10.1016/j.foodcont.2014.07.053.
59. Elhasan SM, Bushara AM, Abdelhakam KE, Elfaki HA, Eibaid AI, Farahat FH, Sukrab AM. Effect of heat treatments on physico-chemical properties of milk samples. J Acad Ind Res, (JAIR). 2017;6:40-6.
60. Rolls BA, Porter JWG. Some effects of processing and storage on the nutritive value of milk and milk products. Proc Nutr Soc. 1973;32(1):9-15. doi: 10.1079/PNS19730003, PMID 4594175.

61. Yan D, Cui H, Zhu W, Talbot A, Zhang LG, Sherman JH, Keidar M. The strong cell-based hydrogen peroxide generation triggered by cold atmospheric plasma [sci rep:10831]. Sci Rep. 2017;7(1):10831. doi: 10.1038/s41598-017-11480-x, PMID 28883477.
62. Cass OW, Paris JP, Stock AM. Research on the stability of high strength H2O2. Du Pont DE NEMOURS (El) AND CO. DE: WILMINGTON Publishing; 1966.
63. Liu F, Sun P, Bai N, Tian Y, Zhou H, Wei S, Zhou Y, Zhang J, Zhu W, Becker K, Fang J. Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. Plasma Processes Polym. 2010;7(3-4):231-6. doi: 10.1002/ppap.200900070.
64. Kornacki JL. Enterobacteriaceae, coliforms and Escherichia coli as quality and safety indicators. Compend Methods Microbilogical Exam Foods. 2001:69-82.
65. Căpriță A, Căpriță R, Crețescu I. The effects of storage conditions on some physicochemical properties of raw and pasteurized milk. J Agroaliment Processes Technol. 2014;20:198-202.
66. Mittal S, Bajwa U. Effect of heat treatment on the storage stability of low calorie milk drinks. J Food Sci Technol. 2014;51(9):1875-83. doi: 10.1007/s13197-012-0714-z, PMID 25190842.
67. Sarangapani C, Ryan Keogh DR, Dunne J, Bourke P, Cullen PJ. Characterisation of cold plasma treated beef and dairy lipids using spectroscopic and chromatographic methods. Food Chem. 2017;235:324-33. doi: 10.1016/j.foodchem.2017.05.016, PMID 28554643.
68. Wu X, Luo Y, Zhao F, M SM, Mu G. Influence of dielectric barrier discharge cold plasma on physicochemical property of milk for sterilization. Plasma Process Polym. 2021;18(1). doi: 10.1002/ppap.201900219