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Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma

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ABSTRACT

This study evaluated the microbial and physicochemical characteristics of milk that was treated with encapsulated dielectric barrier discharge (DBD) plasma. Encapsulated DBD plasma was generated in a plastic container (250 W, 15 kHz, ambient air) and DBD plasma treatment was applied to milk samples for periods of 5 and 10 min. The total aerobic bacterial count in the untreated control sample was 0.98 log CFU/mL. Following plasma treatment, no viable cells were detected in the milk samples. When milk samples were inoculated with *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium, plasma treatment for 10 min resulted in a reduction in bacterial counts by approximately 2.40 log CFU/mL. The pH of the sample milk was found to decrease after the 10-min plasma treatment. Hunter color L^* and b^* values of milk increased, and the a^* value decreased as a result of the plasma treatment. The production of 2-thiobarbituric acid reactive substances increased slightly, but not significantly, following plasma treatment. The results of this study indicate that encapsulated DBD plasma treatment for less than 10 min improved the microbial quality of milk with slight changes in physicochemical quality of milk.

1. Introduction

Recently, atmospheric pressure plasma (APP) has been investigated as a non-thermal, bacterial-inactivation technique in food processing (Kim, Yong, Park, Choe, & Jo, 2013). An electrical discharge, such as plasma, can be an efficient source of electrons, ions, heat, UV light, an electric field, and/or free radicals, depending on the discharge conditions. Such attributes of plasmas are known to be capable of inactivating bacteria or other microorganisms; therefore, plasmas have been used widely for sterilization (Ikawa, Kitano, & Hamaguchi, 2010).

Numerous studies of plasma-based sterilization techniques have focused on the inactivation of microorganisms that are immobilized on the surface of solid foods, to which plasmas are directly applied. Under such conditions, microorganisms are often destroyed physically by impinging energetic ions and/or electrons; alternatively, microbial DNA is severely damaged by UV light from plasmas (Laroussi & Leipold, 2004). Reactive oxygen species (ROS) and other free radicals in the gas phase have also been reported to damage the microorganisms directly (Yu et al., 2006). For example, Song et al. (2009) reported that, on sliced cheese and ham that had been inoculated with *Listeria monocytogenes*, large-area APP effectively reduced or eliminated the bacteria. Kim et al. (2011) and Ragni et al. (2010) found that changes in microbial quality in bacon and eggshells occurred in response to treatment with APP. Moreover, a study performed by Kim et al. (2013) demonstrated that dielectric barrier discharge (DBD) plasma has potential for use in sanitizing pork loins through the inactivation of foodborne pathogens; however, the effect was limited to few category of food products.

There are many processed foods where it is important to perform sterilization under liquid conditions. When bacteria are inoculated in to a liquid food, ions and electrons cannot directly interact with the bacteria; this is because they are strongly absorbed by the liquid at the gas–liquid interface (Ikawa et al., 2010). This has been demonstrated previously, both with direct plasma

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treatment, where plasma is generated directly in, or in close contact with, liquids, and by indirect plasma treatment, where the plasma is generated in close vicinity to the liquid but without direct contact to the liquid's surface (Oehmigen et al., 2011). However, very few studies have investigated the possibility of using plasma to enhance the quality and safety of liquid foods (Gurol, Ekinci, Aslan, & Korachi, 2012; Ikawa et al., 2010; Surowsky, Fröhling, Gottschalk, Schlüter, & Knorr, 2014).

Raw or partially pasteurized milk is highly nutritious; it contains lipids, proteins (casein, whey), carbohydrates (lactose), amino acids, vitamins, and minerals (calcium) that are essential for the nutritional requirements of young mammals (Claeys et al., 2013; Hill, Smythe, Lindsay, & Shepherd, 2012). However, because of its nutritional properties, milk is also a good growth medium for a variety of spoilage and, potentially pathogenic, microorganisms including *Escherichia coli* O157:H7, *L. monocytogenes*, and *Pseudomonas* spp. (Sharma, Bremer, Oey, & Everett, 2014).

Pasteurization of milk is the most effective technique in use today that destroys pathogenic bacteria and inactivates some enzymes, extending the shelf life of the product (Silva et al., 2010). However, the high temperatures used to inactivate pathogenic bacteria, as in ultra-high temperature (UHT) pasteurization, can have an effect on many quality factors of the milk, such as flavor and nutritional content are affect (Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009). To overcome these disadvantages, non-thermal technologies including chemical treatment, high hydrostatic pressure applications, and pulsed electric field processing have been developed. However, in comparison with heat-based treatments, these technologies also have some drawbacks, including the high cost of application, the requirement of specialized equipment, the generation of undesirable residues, extended processing times, and lower efficiencies (Shamsi, Versteeg, Sherkat, Wan, & Wan, 2008; Yang et al., 2012).

Therefore, the objective of this study was to evaluate the microbial safety and possible physicochemical quality changes of commercial milk following treatment with atmospheric pressure encapsulated DBD plasma.

2. Materials and methods

2.1. Sample preparation and plasma treatment

Whole milk was purchased from a local market in Seoul (Maeil Co., Pyeongtaek, Korea), a day prior to the experiment and stored in a refrigerator at 4 °C.

An encapsulated DBD plasma source was fabricated using a rectangular, parallelepiped plastic container (137 × 104 × 53 mm) (Fig. 1). The actuator was made of copper electrodes and a polytetrafluoroethylene sheet was attached to the inner walls of the container. A bipolar square-waveform voltage at 15 kHz was applied to one electrode while the other electrode was grounded. The plasma was generated inside the container with an input power of 250 W. The levels of ozone produced during DBD plasma generation were measured using a UV ozone photometer (UV–H; Aeroqual Co., Auckland, New Zealand) at an absorbance of 254 nm. Milk (10 mL) was placed in a glass dish at the bottom of the container; it was treated with the DBD plasma source for 5 and 10 min.

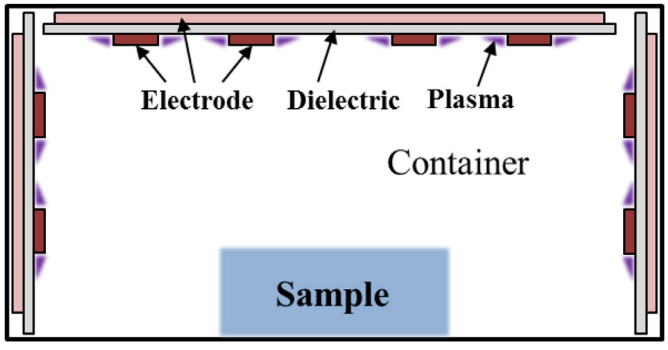

Fig. 1. Schematic diagram of the experimental system for the generation of encapsulated dielectric barrier discharge plasmas.

2.2. Microbial analysis

The prepared sample (5 mL) was mixed for 2 min in a sterile stomacher bag containing 45 mL of sterile saline solution (0.85%) using a stomacher BagMixer® 400 (Interscience Co., Saint Nom, France). Total plate count agar was prepared for counting the total number of aerobic microbes (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37 °C for 48 h, and the colony forming units (CFU) per mL were counted at a dilution of 30–300 CFU per plate.

2.3. Inoculation test

2.3.1. Sterilization, test pathogens, and inoculation

Prior to inoculation, the sample was sterilized using electron-beam irradiation (35 kGy at 2.5 MeV) with a linear electron beam RF accelerator (EB-Tech, Daejeon, Korea). Three pathogens, *E. coli* (KCTC 1682), *L. monocytogenes* (KCTC 3569), and *Salmonella* Typhimurium (KCTC 1925), were used in this study; they were obtained from the Korean Collection for Type Cultures (KCTC, Daejeon, Korea). *E. coli*, *L. monocytogenes*, and *S.* Typhimurium were cultivated to mid log phase in tryptic soy broth (Difco Laboratories), tryptic soy both containing 0.6% yeast extract (Difco Laboratories), and nutrient broth (Difco Laboratories) at 37 °C for 48 h, respectively. The cultures were then centrifuged (3000 rpm for 10 min at 4 °C) using a refrigerated centrifuge (model VS-5500; Vision Scientific Co., Seoul, Korea). The resulting pellet was washed twice with sterile saline solution (0.85%) and was resuspended in the same saline solution. The viable cell density was measured and was approximately 10^8 CFU/mL. The samples (5 mL each) were inoculated with 100 μL of this solution.

2.3.2. Microbial analysis

Inoculated samples were plasma-treated and then mixed in a sterile stomacher bag as described above. Serial dilutions were prepared with the sterile saline solution. The media used for the recording the growth of *E. coli*, *L. monocytogenes*, and *S.* Typhimurium were typtic soy agar (Difco Laboratories), tryptic soy agar containing 0.6% yeast extract (Difco Laboratories), and nutrient agar (Difco Laboratories), respectively. Incubation of plates and colony counting were done as explained above.

2.4. pH

After treatment with encapsulated DBD plasma, the pH was measured using a pH meter (Model 750, iSTEC, Seoul, Korea). Calibration was performed using standard buffers provided by the manufacturer at pH 4, 7, and 10 at room temperature.

2.5. Color

Plasma-treated milk was poured into a cuvette cell (45 × 17 mm) and the color of the milk was evaluated using a Color Difference Meter system (Spectrophotometer CM-3500d, Konica

Minolta Sensing, Inc., Osaka, Japan); next, the Hunter color values, L^* (lightness), a^* (redness), and b^* (yellowness), were determined. The instrument was calibrated with a standard black and white plate before analysis. The Hunter values were monitored by a computerized system using SpectraMagic software (Konica Minolta Sensing, Inc.) and the measurements were performed in triplicate.

2.6. Fatty acid composition

The milk samples (10 mL) were mixed with 1 N KOH in ethanol (50 mL) before being heated in a water bath (60 °C) for 1 h. After cooling, 10 mL of each sample was transferred to a 50 mL tube, vortexed with distilled water and hexane (10 mL, each), and centrifuged at 3100 rpm for 10 min (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). The supernatant was removed and the remaining mixture was mixed again with 6 mL of HCl–distilled water solution (1:1, v/v). Following this, each mixture was added to hexane (5 mL), vortexed, and centrifuged at 3100 rpm for 10 min. The supernatant was then collected into a 15-mL tube. This same procedure was also followed after adding 5 mL of hexane to the original mixture. The resulting supernatant was again transferred to the same 15-mL tube; this was then evaporated using N_2 gas (99.99%). Subsequently, to each tube 1 N H_2SO_4 in methanol (5 mL) was and the tubes were heated in a water bath (50 °C) for 1 h. After cooling, 2 mL of distilled water and 3 mL of hexane were added to the same tubes, which were then centrifuged at 3100 rpm for 10 min. The top hexane layer containing fatty acid methyl esters (FAME) was transferred to another 15 mL tube, concentrated to a volume of 1.5 mL using N_2 gas (99.999%), and dehydrated through the addition of anhydrous Na_2SO_4 before being stored in a vial. The fatty acid composition of the samples was then analyzed using gas chromatography (HP 7890, Agilent Technologies, Santa Clara, CA, USA). A split inlet (split ratio 100:1) was used to inject the samples into a capillary column (30 m × 0.32 mm × 0.25 μm; Omegawax 320, Supelco, Bellefonte, PA, USA) and oven temperature was performed (130 °C for 5 min, increased to 170 °C at 2.5 °C/min, and maintained for 8 min, then increased to 190 °C at 5 °C/min, and maintained for 15 min, before finally being increased to 220 °C at 5 °C/min, and maintained for 30 min). The inlet temperature was 210 °C. N_2 gas was used as the carrier gas at a constant flow rate of 0.7 mL/min.

2.7. Lipid oxidation

Lipid oxidation was determined by calculating the levels of 2-thiobarbituric acid reactive substances (TBARS) values in the samples. Milk samples (3 mL) and 9 mL of distilled water were vortexed with 50 μL of butylated hydroxytoluene (7.2%). The sample (1 mL) was then transferred to a test tube and thiobarbituric acid (TBA)/trichloroacetic acid (TCA) solution (20 mM TBA in 15% TCA, 2 mL) was added. The tubes were then heated in a water bath at 90 °C for 30 min; after they had cooled, the tubes were centrifuged at 3000 rpm for 10 min. Using a spectrophotometer, the absorbance of the supernatant was measured at 532 nm and lipid oxidation was reported as mg of malondialdehyde per kg of sample.

2.8. Statistical analyses

The data were analyzed using SAS software (Release 8.01, SAS Institute, Inc., Cary, NC, USA). The statistical analysis was performed using a one-way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were determined by performing the Duncan's multiple comparison test at a confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported.

Table 1
Total aerobic bacterial counts of milk following treatment with encapsulated dielectric barrier discharge plasma.

Treatment time (min)	log CFU/mL
0	0.94a
5	NDb[a]
10	NDb
SEM[b]	0.010

a,b Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Viable with no growth at a detection limit < 10^1 CFU/g.
[b] Standard errors of the mean ($n = 9$).

3. Results and discussion

3.1. Microbial analyses

The total aerobic bacteria count of the milk used in the study was measured at 0.98 log CFU/mL. No viable cells were detected in the samples that received treatment with plasma for both 5 and 10 min (Table 1). *E. coli*, *L. monocytogenes* and *S.* Typhimurium were initially loaded at levels of 6.28, 6.43, and 6.21 log CFU/mL, respectively, when inoculated. The application of encapsulated DBD plasma reduced the number of pathogens to 2.43, 2.40, and 2.46 log CFU/mL for *E. coli*, *L. monocytogenes*, and *S.* Typhimurium, respectively (Table 2).

DBD plasma can create specific types of ROS, such as oxygen atoms, ozone, metastable oxygen molecules, peroxide, superoxide, and hydroxyl radicals; all of these are bactericidal. These ROS have strong oxidizability and are known to oxidize bacteria cells (Birmingham, 2004; Kim et al., 2013). Ma, Zhang, Shi, Xu, and Yang (2008) reported that ROS play a significant role in the inactivation of microorganisms that have been treated with DBD plasma.

In particular, hydroxyl and oxygen radicals can cause damage to the cell wall. OH radicals can oxidize unsaturated fatty acids in the lipid layer of cell walls, cleave peptide bonds, and oxidize amino acid side chains (Surowsky et al., 2014). Oxygen radicals are involved in the oxidation of proteins and in plasma etching processes. Ultraviolet–visible spectroscopy has been used to prove the existence of both species, but only atomic oxygen exhibited increasing quantum yields as a result of an increase in oxygen concentration (Surowsky et al., 2014). A strong correlation with the ability of the species to inactivate bacteria can be seen here, since the level of inactivation also improved due to an increase in the atomic oxygen concentration. Besides the direct action of these radicals, they can also further form antimicrobial compounds, such as hydrogen peroxide, hydroperoxide radicals, and ozone (Ikawa et al., 2010; Liu et al., 2010). The ozone concentration of encapsulated DBD plasma used in this study was found to be in excess of 200 ppm (data not shown). It has previously been demonstrated

Table 2
Inactivation of foodborne pathogens inoculated into milk following exposure of the milk to atmospheric pressure encapsulated dielectric barrier discharge plasma.

Treatment time (min)	Pathogens (log CFU/mL)		
	Escherichia coli	*Listeria monocytogenes*	*Salmonella* Typhimurium
0	6.28a	6.43a	6.21a
5	4.76b	5.17b	4.74b
10	3.85c	4.03c	3.75c
SEM[a]	0.011	0.010	0.012

a–c Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean ($n = 9$).

Table 3
The pH changes in milk following treatment with encapsulated dielectric barrier discharge plasma.

Treatment time (min)	pH
0	6.90a
5	6.83a
10	6.60b
SEM[a]	0.035

a,b Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean ($n = 9$).

that, when DBD plasma was applied to sealed polyethylene bags containing 30% oxygen and 70% argon, the ozone levels inside the packages increased rapidly within a few seconds and this was linked with the inactivation of *Listeria innocua* (Rød, Hansen, Leipold, & Knøchel, 2012). Recently, Yong et al. (2014) suggested that a post-treatment storage duration following sealed DBD plasma can greatly enhance the pathogen inactivation efficiency of agar and cheese slices, which can be applied for the present system.

Surowsky et al. (2014) suggested that the inactivation of *Citrobacter freundii* by plasma is a result of the action of plasma-generated reactive compounds in apple juice, which partially penetrate the cytoplasmic membrane of the cells and/or permeabilizes the cells. Permeabilization might result from the degradation of specific proteins and lipids, which are known to be susceptible to oxidation, whereas reactive compounds that penetrate the cells damage RNA (and possibly DNA).

Gurol et al. (2012) found that their corona discharge system, equipped with a 9 kV AC power supply and two tungsten electrodes, significantly reduced the number of *E. coli* cells in different types of milk (whole, semi-skimmed, and skimmed) by approximately 4 log cycles after a 20-min treatment. Montenegro, Ruan, Ma, and Chen (2002) were able to inactivate greater than 5 log units of *E. coli* O157:H7 at a frequency of less than 100 Hz by using 4000 pulses of 9000 V peak voltage without any obvious temperature increase occurring. Several researchers have reported that physical parameters (input power, type of discharge, etc.), microbiological parameters (Gram-negative or -positive, bacteria or fungi, cell concentration of bacteria, etc.), and sample parameters (type of sample, humidity, etc.) might influence the inactivation potential of plasma (Deng et al., 2007; Kim et al., 2013).

3.2. pH

Typically, the pH of a solution is important in determining a system's decontamination capabilities (Gurol et al., 2012). Following treatment with encapsulated DBD plasma, the pH decreased slightly (Table 3). It was observed an increase in the acidity of the liquid due to the action of the plasma (Bruggeman et al., 2008). This can be attributed to the multistep reactions of the plasma-generated reactive species, including NO_x, O, and O_3, with water at the gas–water interface (the quasi-steady gas cavity surface, as well as on the surfaces of micro droplets of liquid inside the gas cavity) (Liu et al., 2010).

Liu et al. (2010) demonstrated that the pH value of sterile water decreased to approximately 3.2 following treatment with an atmospheric-pressure air plasma microjet for approximately 6 min; additionally, they noted that it took longer for the pH value to stabilize at 4.5 and 4.2 in sterile water with LB culture and in sterile water with a bacterial suspension, respectively. Using a low-frequency DBD plasma in air, Tang, Lu, Larousii, and Dobbs (2008) observed a similar immediate and pronounced pH decrease in deionized water, and a slightly delayed and less pronounced pH decrease in an Alga-Gro medium. The authors of this study attributed the differences to the buffering capacity of the liquids.

Table 4
Hunter color values of milk treated with encapsulated dielectric barrier discharge plasma.

Treatment time (min)	Hunter color value		
	L^*	a^*	b^*
0	89.52b	−2.39a	5.72c
5	89.49b	−2.43b	5.99b
10	89.70a	−2.47c	6.19a
SEM[a]	0.011	0.003	0.005

a–c Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean ($n = 9$).

Table 5
Fatty acid composition of milk following treatment with encapsulated dielectric barrier discharge plasma.

Fatty acid	Treatment time (min)			SEM[a]
	0	5	10	
Butyric acid ($C_{4:0}$)	0.07a	0.07ab	0.06b	0.003
Caproic acid ($C_{6:0}$)	0.14	0.14	0.15	0.009
Caprylic acid ($C_{8:0}$)	0.33ab	0.31b	0.33a	0.005
Capric acid ($C_{10:0}$)	1.55	1.59	1.60	0.018
Lauric acid ($C_{12:0}$)	3.77	3.84	3.84	0.065
Myristic acid ($C_{14:0}$)	12.22	12.25	12.29	0.039
Myristoleic acid ($C_{14:1}$)	0.85	0.88	0.87	0.018
Palmitic acid ($C_{16:0}$)	35.72	37.00	35.64	0.734
Palmitoleic acid ($C_{16:1}$)	1.42	1.47	1.41	0.303
Stearic acid ($C_{18:0}$)	16.47	17.09	16.44	0.349
Oleic acid ($C_{18:1}$)	24.33	24.34	24.28	0.030
Linoleic acid ($C_{18:2}$)	2.83	2.90	2.76	0.067
Linolenic acid ($C_{18:3}$)	0.27	0.29	0.29	0.012

a,b Different online letters within the same column indicate significant differences ($p < 0.05$).
[a] Standard errors of the mean ($n = 9$).

3.3. Color

Changes in the Hunter color values of milk treated with encapsulated DBD plasma are shown in Table 4. The L^* and b^* values were increased, whereas the a^* values were decreased following plasma treatment. Gurol et al. (2012) reported that a longer exposure to plasma (20 min) resulted in slightly higher color differences, with a ΔE value of 0.52; this suggests that the application of plasma resulted in a slightly noticeable color difference in milk. According to Walstra, Geurts, and Wouters (2006), a high pressure-induced increase in the L^* value is due to an increase in the number of fat globules, which can diffract light more effectively. However, we were unable to locate any references discussing the mechanisms of changes in the color of milk following treatment with plasma.

3.4. Fatty acid composition

The fatty acid composition of the milk, calculated based on the peak areas is shown in Table 5. The major fatty acids identified in the sample were myristic acid, palmitic acid, stearic acid, and oleic acid. Palmitic acid was found to be the most prevalent fatty acid in all the samples. The concentrations of the various fatty acids in the milk did not change following treatment with plasma with the exceptions of butyric acid and caprylic acid. It has been reported that free radicals or ROS, including ozone, react with unsaturated fatty acids and break down the double bonds, resulting in cellular injury (Goldstein, Lodi, Collinson, & Balchum, 1969). However, these results suggest that encapsulated DBD plasma causes

Table 6
Levels of 2-thiobarbituric acid reactive substances in milk following treatment with encapsulated dielectric barrier discharge plasma.

Treatment time (min)	TBARS value (mg malondialdehyde/kg)
0	0.027
5	0.028
10	0.029
SEM[a]	0.001

[a] Standard errors of the mean ($n = 9$).

negligible fatty acid composition changes in milk. Similarly, several studies have demonstrated that there were no significant changes in the fatty acid composition of beef jerky and goat milk after being treated with plasma and UV irradiation, respectively (Kim, Lee, Choi, & Kim, 2014; Matak et al., 2007).

3.5. Lipid oxidation

The level of TBARS was used to determine the inhibition of lipid oxidation in the non-thermally treated samples. The TBARS level showed no difference following encapsulated DBD plasma treatment (Table 6). Kim et al. (2013) have previously reported that the level of TBARS in He + O_2-DBD plasma-treated pork loin were greater than those in non-treated pork loin. Joshi et al. (2011) suggested that ROS were produced by plasma-induced oxidative stress; their study demonstrated that intact *E. coli* cells and isolated membrane-rich fractions undergo lipid peroxidation in a manner that is proportional to the amount of plasma energy. Irradiation, another emerging non-thermal technology, results in the acceleration of lipid oxidation (Kim et al., 2010). This is an indication that the irradiation process results in the production of hydroxyl radicals, formed mainly by water radiolysis, and that this causes the oxidation of lipids (Lee et al., 2005). Radicals generated by plasmas are known to accelerate the production of peroxides, which are formed as intermediate products of lipid oxidation; this may be the cause of the increase in TBARS in the encapsulated DBD plasma-treated samples. The generation of ozone, which occurred as a result of treatment with encapsulated DBD plasma, may also affect lipid oxidation in milk (Goldstein et al., 1969). However, the present encapsulated DBD plasma system for 10 min did not cause the increase of TBARS value under these possibilities.

A power supplier is an essential component to operate plasma system. In commercial level, its price would be comparable to ordinary sterilization devices. The food container designed in this study consists of a dielectric layer and the custom-made metallic patterned electrode. Cost of this container can be estimated as similar to that of commercial polypropylene container when it is produced in large quantities or in large volume at commercial plant due to the low cost of the additional dielectric and metallic layers. In addition, average power consumption of the DBD plasma operation is approximately 5 W; energy efficiency is high. This will reduce the cost of whole operation.

In consumer's point of view, pasteurization of milk may result in a positive or negative effect on the sensory attributes of end product (Cruz et al., 2012). In preliminary sensory test, the slight changes in flavor and taste of encapsulated DBD plasma-treated milk were observed when compared with non-treated milk (data not shown). However, the first objective of this study was to establish the sterilization conditions of APP device for milk. An optimization of the processing method to minimize the changes in sensorial qualities of plasma-treated milk must be conducted to meet the consumer's satisfaction before industrial application using proven methodology (Cruz et al., 2012).

4. Conclusion

Our findings suggest that the developed encapsulated DBD plasma can be used to improve the microbial safety of milk. DBD plasma treatment for less than 10 min might be the optimum treatment as most of the quality attributes were not changed under this treatment conditions compared to control. However, this plasma device must be developed further to minimize the observed slight changes in quality attributes of milk. In addition, more detailed studies are recommended to assess the quality such as sensory evaluation of milk after plasma treatment and its suitability for industrial application.

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