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Full Research Paper

Numerical calculation of the lethality of becteria in bottled milk under cold plasma treatment
Running title: Effect of cold plasma on milk bacteria

A. Ranjbar Nedamani[1]*

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Abstract

In recent years, cold plasma is one of the expected alternatives for post-harvest treatments and post-harvest management of products. A surface discharge plasma system was used for investigating the destruction time of *Bacillus cereus*, *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Clostridium botulinum* in bottled milk. The simulation was performed by COMSOL a3.5 software for a two-dimensional geometry. The collected experimental data were simulated in COMSOL software. The *k* factor of microorganism deactivation data was used to validate the simulated data. Results showed that the production of reactive oxygen species during plasma treatment increases with time and extends to the entire container. The concentration of reactive oxygen species (at the output of the plasma probe) at the beginning of the production was high, and at the end when they leave the free surface of the milk, the concentration decreased. Increasing the initial temperature of milk sample, from 50 to 80℃, can cause significant changes in the amount of ozone from 125 mol/m^3 to 266 mol/m^3, respectively (p <0.05). However, voltage changes in these two temperatures did not show a significant effect on ozone concentration. Also, immediately upon the initiation of plasma treatment, plasma destruction begins where the concentration of active species is higher. It is shown that among the four studied bacteria, *Bacillus stearothermophilus* has the highest resistance against cold plasma, and after that other bacteria have shown similar resistance. Finally, it can be concluded that the deep plasma treatment in bottle can make it possible to overcome the surface limitation of cold plasma treatment.

Keywords: Cold plasma; Milk; Sterilization; Pasteurization.

Introduction

All necessary nutritional compounds and energy for human can be found in milk (Balthazar et al., 2017). On the other hand, Milk is high in water and this makes it suseptible to bacterial deterioration and some bacteria such as *Escherichia coli*, Salmonella, *Listeria monocytogenes*, can easily grown in milk. This leads to decrease in nutrional and quality characteristics of milk (Dash & Jaganmohan, 2022). One of the ways to reduce these unacceptable changes, is using thermal processes such as pasteurization and sterilization. Thermal processing can inactivate the microorganisms but also can exert negative effect on milk. For example, some interactions, changes in protein structure, browning

۱. Assistant Professor, Department of Biosystem Engineering, Sari Agricultural Sciences & Natural Resources University. Sari, POB:578, I.R.IRAN.

(Corresponding Author Email: a.ranjbar@sanru.ac.ir)
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reactions, nutritional loss which ultimately reduces the qaulity of processed milk (Eazhumalai et al., 2021; Sharma & Singh, 2022; Wu et al., 2021).

In some cases, the interaction between serum proteins and proteins of fat glubule membrane was reported due to thermal processing (Kim & Jiménez-Flores, 1995). Also, the increasing of heating temperature can staibize proteins with long chains. It is reported that thermal processing of milk can change the second structure of proteins (Farrell Jr et al., 2001).

In recent years, cold plasma is considered one of the the expected alternatives for post-harvest treatments and post-harvest management of products (Misnal et al., 2022). Cold plasma technology (CP) is a non-thermal physical process that has a high potential for application in the food industry (Jiang et al., 2016). Because this technology can easily be used on a large scale and does not leave any dangerous chemical residues, while it destroys or inactivates pathogens without thermal damage to food (Misnal et al., 2022).

Cold plasma showed a high effieciency of inactivation of microorganisms (Niveditha et al., 2021; Soni et al., 2021).

Table 1- Physical characteristics of milk and ozone

Property	The amount of characteristics	Unit	Refrence
	Milk		
Density	((0.3*T[1/degC])+(0.03*T^2[1/degC^2])+(0.7*4.1)+(0.01*4.1^2)+1034.5)[kg/m^3]	kg/m³	(Bakshi & Smith, 1984)
Viscosity	(2721.5/T[1/degC])+()^2.8 (0.1*4.1)-8.9	Pa·s	(Bakshi & Smith, 1984)
Relative penetration	60		(Ghanem, 2010)
	Ozone		
Ozone density	2.14	kg/m³	(Wang et al., 2020)
The speed of ozone gas monement in fluid	0.003	m/s	(Wang et al., 2020).
Effective diffusion coeficient of ozone gas in fluid	1.74×10^{-9}	m^2/s	(Wang et al., 2020).
Ozone gas movement vector	8.33×10^{-6}	-	(Wang et al., 2020).
Diameter of ozone bubbles	3.21	mm	(Wang et al., 2020).

By cold plasma technology, it is possible to processing foods under low temperature which is a high advantages of cold plasma treatment in food technologies. Also, this technology seems to be environmentaly fraindely. But it has some limitations such as the deactivation of microorganisms with CP, is a surface treatment and is not suitable to process the entire volume of material. Some studies numerically investigated the effect of cold plasma treatment in model systems (Ranjbar Nedamani, 2022; Ranjbar Nedamani & Hashemi, 2022). Numerical calculation can reduce the number of experiments and can make it possible to investigate a wide range of factors or conditions regardless the costs of experimental activities. Tabibian (2019) nnumericaly studied the CFD (computational fluid dynamics) modeling of fludiezed bed reactors combined with cold plasma jet for treatment of particles (Tabibian, 2019). Also Wang et al. investigated the deactivation of yeast in a model media by

numerical calculations (Wang et al., 2020). The aim of this work is to investigate the effect of cold plasma technology on bacterial deactivation in milk through a needle plasma in bottled milk.

Materials and method

Problem definition

The physical model of the plasma system

A surface discharge plasma system was used for this purpose. The reactor of this system was a quartz cylinder with a diameter of 1 cm and a height of 25 cm. A steel cover with a thickness of 1 mm and a height of 25 cm was used on the inner surface of the reactor and as a high-voltage discharge electrode. The liquid inside the bottle (milk) was also considered the neutral electrode (Wang et al., 2020). Electric discharge was performed to the electrode with the studied frequency and voltage. Plasma produces active species such as hydrogen peroxide, ozone, hydroxyl radicals, and oxygen radicals. Since ozone monitoring during operations is easy and a suitable indicator to check plasma conditions, ozone concentration was used as a simulation index in this study.

Definition of variables

Physical characteristics of milk

To perform the simulation, some physical characteristics of milk and variables such as ozone movement speed in the fluid, ozone effective diffusion coefficient, ozone gas density, ozone gas bubble diameter, and ozone movement vector in the fluid were used according to Table 1.

Simulation and Governing equations

The simulation was performed by Comsol a3.5 software for a two-dimensional geometry as shown in Fig. 1. Four modules of laminar bubble flow, dilut species transport (for air injected between electrodes in the valve), dilute species transport (to remove bacteria or test compound in the valve), and electric field (plasma generator) were solved.

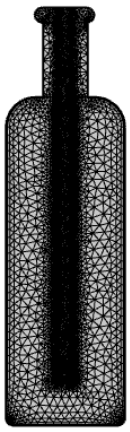


Fig. 1. The two-dimensional geometry of the milk bottle inside which the plasma generator system is placed.

Calculation of the the process destroying factor of K for pathogen deactivation in milk

During thermal processes, milk acts as a complex system of different compounds. A large amount of chemical, physical, and biochemical reactions occur in it. Some of these changes are of great importance because they can change the characteristics of milk. Others may change the nutritional value of milk and even increase its biological safety (De Jong, 2008).

Since in plasma treatment the non-thermal condition was used to destroy the microorganisms, it is believed that the incidence of temperature-affected reactions in milk will decreased. The reactions which may occure in milk during processing, can be divided into five categories: (1) destruction of microorganisms, (2) inactivation of enzymes,

(3) denaturation of proteins, (4) loss of nutritional compounds, and (5) formation of new compounds. Most of these reactions can be represented by a simple one-step irreversible reaction as A→B. by a standard reaction rate equation in the form of equation (1):

$$r_A = -kC_A^n, \quad r_B = -r_A \qquad (1)$$

Where r is the reaction rate (mol/m^3.s), k is reaction rate constant (m^3/mol.s), and n is reaction degree. The way in which the reaction rate constant is affected by temperature, is important in determining the extent of final transformations caused by heat treatment. For this reason, equations 2 and 5 were used.

$$k_{Bacillus\ Stearothermophilus} = 101.15 \exp\left(\frac{-345.4}{8314T}\right) \qquad (2)$$

$$k_{Clostridium\ Botulinum} = 107.5 \exp\left(\frac{-351}{8314T}\right) \qquad (3)$$

$$k_{Bacillus\ Coagulans} = 151.29 \exp\left(\frac{-509}{8314T}\right) \qquad (4)$$

$$k_{Bacillus\ Cereus} = 91.92 \exp\left(\frac{-294.5}{8314T}\right) \qquad (5)$$

Laminar bubble flow

This module was used to simulate the movement of ozone bubbles from the plasma reactor inside the valve and equations 6 to 9 were solved by the software.

$$\emptyset_l \rho_l \frac{\partial u_l}{\partial t} + \emptyset_l \rho_l (u_l.\nabla) u_l = \nabla.[-pI + \emptyset_l(\mu_l + \mu_T)(\nabla u_l + (\nabla u_l)^T)] + \emptyset_l \rho_l g + F \qquad (6)$$

$$\rho_1 \nabla.(u_l) = 0, \qquad u_l = u \qquad (7)$$

$$\frac{\partial \emptyset_g \rho_g}{\partial t} + \nabla. N_{\rho_g \emptyset_g} = -m_{gl}, \qquad \emptyset_g \rho_g = rhogeff \qquad (8)$$

$$N_{\rho_g \emptyset_g} = \emptyset_g \rho_g u_g, \qquad u_g = u_l + u_{slip} - \mu_T \frac{\nabla \emptyset_g}{\rho_l \emptyset_g} \qquad (9)$$

where l and g are related to liquid (milk) and gas (ozone), respectively.

The density of gas is negligible compared to the density of milk. Laminar flow equations were used to solve the rising of ozone bubbles inside the bottle. The density, diameter, and diffusion coefficient of ozone bubbles were considered according to Table 1.

Transport of diluted species

The deactivation of microorganisms depends on the amount of ozone and ions - formed by the plasma. The rate of this reaction can be calculated according to Fick's law in the form of equations 10 and 3-25:

$$\frac{\partial c_i}{\partial t} + \nabla.(-D_i \nabla c_i) + u.\nabla c_i = R_i \qquad (10)$$

$$N_i = -D_i \nabla c_i + u c_i \qquad (11)$$

During the simulation, the value of R_i (reaction rate) was defined according to equation 12:

$$R_i = -k_{reac} c_{o_3} \qquad (12)$$

Initial and boundary conditions

In solving this problem, the outer boundary at the top of the plasma-generating reactor was considered a free surface. For the simplicity of the calculations, the surface motions of the fluid were neglected. The border of entering ozone into the valve was considered at the end of the

reactor. The ozone flow rate was calculated according to equation 13:

$$n.N_1 = n.\left(uc_{0.j}\right) \quad (13)$$

Solving the problem

The COMSOL a3.5 software was used to solve four modules based on laminar flow. A system of Intel® Core TM i5-4300U, 2.50 GHz, RAM 4 GB, and Windows 10 64-bit were used for this purpose. The relative tolerance of solving the problem was 0.01 and data recording was done for ten minutes at one minute intervals. The normal mesh was used for geometry and the fine mesh was used for the reactor in 2D space as shown in Fig. 1.

Verification of the simulated model

The collected experimental data were simulated in Comsol software. The *k* factor of microorganism deactivation data were used to validate the simulated data. After investigating the best fitted simulated data through comparing the R^2, the accuracy of simulation was also validated.

Statistical analysis

To check the validity of the model and to detect regression coefficients and statistical significance, an analysis of variance was performed by ANOVA in Design Expert. The equation of the line, regression coefficients, and lack of fit were analyzed by statistical parameters R^2, *p-value* (at the level of 0.05), and *Adj-*R^2 .

Results and discussion

Verification of the simulated model

In microbial inactivation, if a semi-logarithmic plot of microbial population is plotted, a linear plot with slope *k* is obtained (Ibarz & Barbosa-Cánovas, 2002). Process temperature can change the slope of this graph. But in this study, the effect of the number of ions formed during plasma treatment and especially the amount of ozone concentration should be considered as an indicator to check the amount of air ionization. In particular, temperature changes can affect the amount of ozone gas produced by plasma treatment (Ranjbar Nedamani & Hashemi, 2022). Figure 2 shows the pre-treatment results for fitting the experimental and simulated data. The value of R^2= 0.9802 indicates the proper fit of these data with each other. In this way, the simulation conditions are well adapted to the real conditions and it will be possible to change the parameters in the simulation with the least error of the output data.

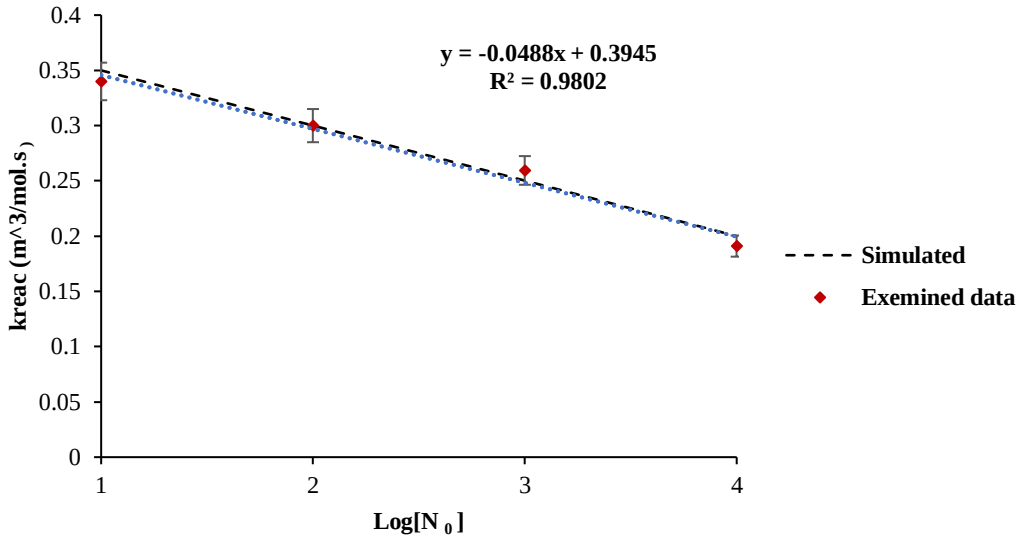
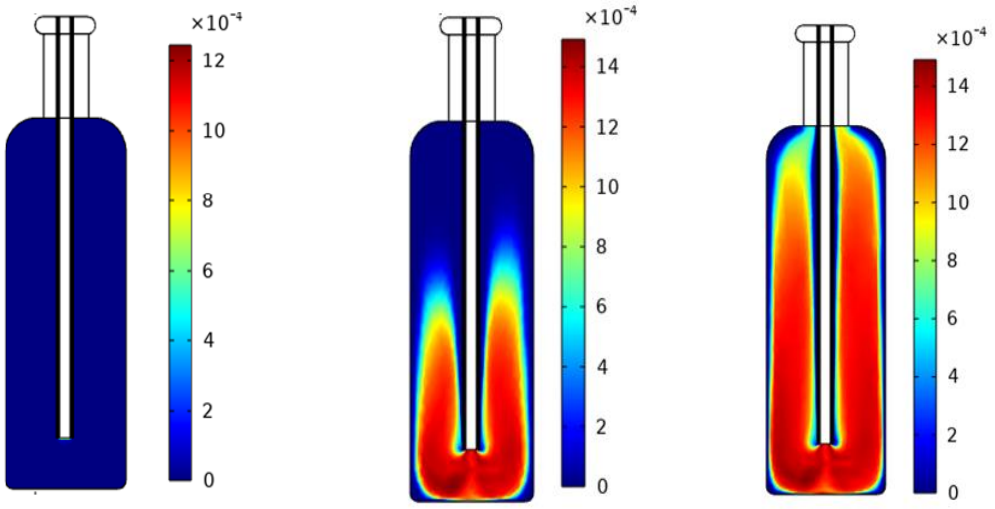


Fig. 2. Fitting the simulated and experimental data to verify the simulation.

Reactive species concentration

Figures 3 and 4 show the changes in ozone concentration as an indicator of reactive species production during plasma treatment. According to Fig. 3, the production of reactive oxygen species during plasma treatment increases with time and extends to the entire container. These active species come from the surface of the milk in the bottle to the outside of the bottle in the form of bubbles, and after production, they quickly leave the radical state to react on microorganisms or possibly other consumption compounds due to their short life span. Thus, the concentration at the beginning of the production of reactive oxygen species (at the output of the plasma probe) was high, and at the end when they leave the free surface of the milk, the concentration decreased. This bubbly movement towards the top of the active species causes the flow and smooth corrosion of the milk inside the bottle, but the speed of displacement is small due to the low speed of air injection into the probe, and it will not cause turbulence flow and had no affect on the fat cells of the milk.



(a **(b)** **(c)**

Fig. 3. The gas component (ozone) inside the milk bottle at time zero (a), 5 minutes (b), and 10 minutes (c).

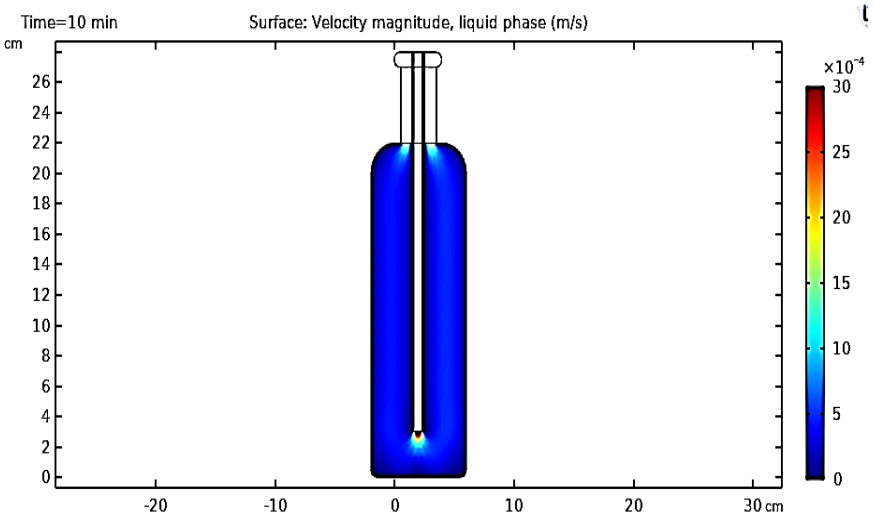


Fig. 4. The speed of milk movement during plasma treatment (m/s), Y and X axis are height and width of geometry, respectively.

Fig. 5. shows the changes in ozone concentration during plasma treatment. As displayed in this figure, it is necessary to achieve complete lethality of the microorganism, it is better to carry out plasma treatment continuously; because the effect of plasma treatment will be practically lost when the active species of oxygen is used up or expires.

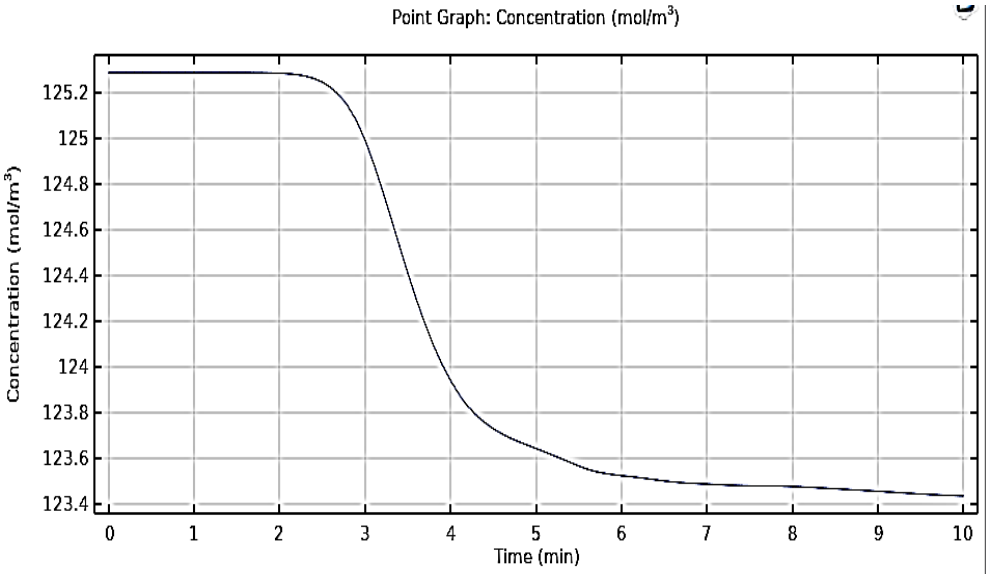


Fig. 5. Changes in ozone concentration inside the milk bottle during plasma treatment

Fig. 6. shows the relationship between ozone concentration and changes in temperature and voltage studied in this study. According to the figure, changes in initial temperature had a greater effect on the amount of active species production than changes in voltage. Increasing the initial temperature of the milk sample, from 50 to 80℃, can cause significant changes (p<0.05) in the amount of ozone from 125 mol/m^3 to 266 mol/m^3, respectively. However, voltage changes in these two temperatures did not show a significant effect on ozone concentration.

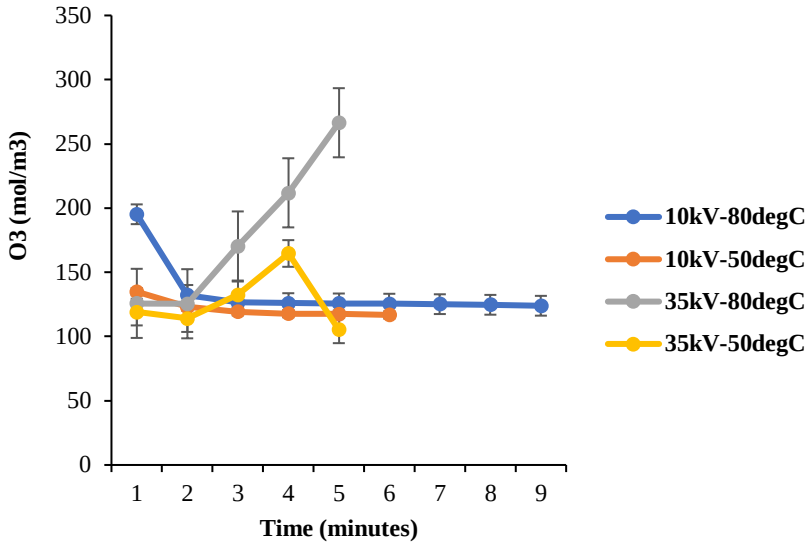
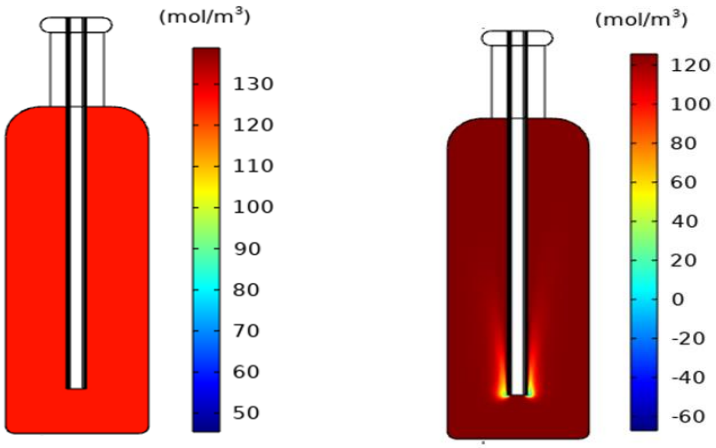


Fig. 6. The amount of ozone concentration produced at different voltages and temperature.

Temperatures of cold plasma treatment

The study of Bahreini et al. (2021) on the inactivation of microorganisms in milk also did not show a relationship between voltage and destruction rate (Bahreini et al., 2021). In a previous study on the inactivation of yeast, it was also found that ozone concentration has a direct relationship with temperature (Ranjbar Nedamani, 2022). The reason for this was perhaps due to the selection of high voltages for operation. Study by Aslan (2016) showed that the effect of voltage on sterilization can be investigated by changing the applied voltages to 1.5 kV, 3 kV, and 5 kV with a fixed frequency of 500 Hz for 3 minutes (Aslan, 2016). He reported that under the same application conditions, the level of bacterial growth obtained at voltages of 3 kV was significantly (p<0.05) lower than that at 1.5 kV and 5 kV. Therefore, it can be concluded that applying a low voltage to the plasma to inactivate all bacteria in the milk, had low effect. Hence, the plasma microbial inactivation efficiency improved and increased with applying higher voltage. In this regard, Wu et al. (2021) reported that a voltage of more than 70 V with 120 seconds of treatment was similar to the ultra-high temperature (UHT) sterilization process and better than pasteurization (Wu et al., 2021). They also reported that all bacteria in the plasma treated group showed varying degrees of destroying and even broken mycelium morphology. Since bacteria had a broken morphology, the bacterial cell can leak out and this will lead to the destruction of the microorganism. These changes in the microorganism concentration inside the milk bottle during plasma treatment are shown in Fig. 7.



(a **(b)**

Fig. 7. Microorganism concentration inside the milk bottle at zero time (a) and immediately after the start of plasma treatment (b).

As shown in the Fig. 7, immediately upon the initiation of plasma treatment, plasma destruction begins where the concentration of active species is higher. With the development of active species, the destruction of microorganisms develops throughout the bottle. The feature of using a long cylindrical probe that is placed in the center of the bottle is that in this way, the speed of distribution of plasma species from depth to the surface will be wide. While other plasma treatment methods, because plasma is considered a surface disinfection method, have limitations and are unable to treat samples with a depth greater than a certain limit, especially if the sample is liquid and with a large volume. In addition, Fig. 8. shows the inactivation time of *Bacillus cereus*, *Bacillus coagulans*, *Bacillus stearothermophilus*, and *Clostridium botulinum* during treatment of bottled milk with cold plasma.

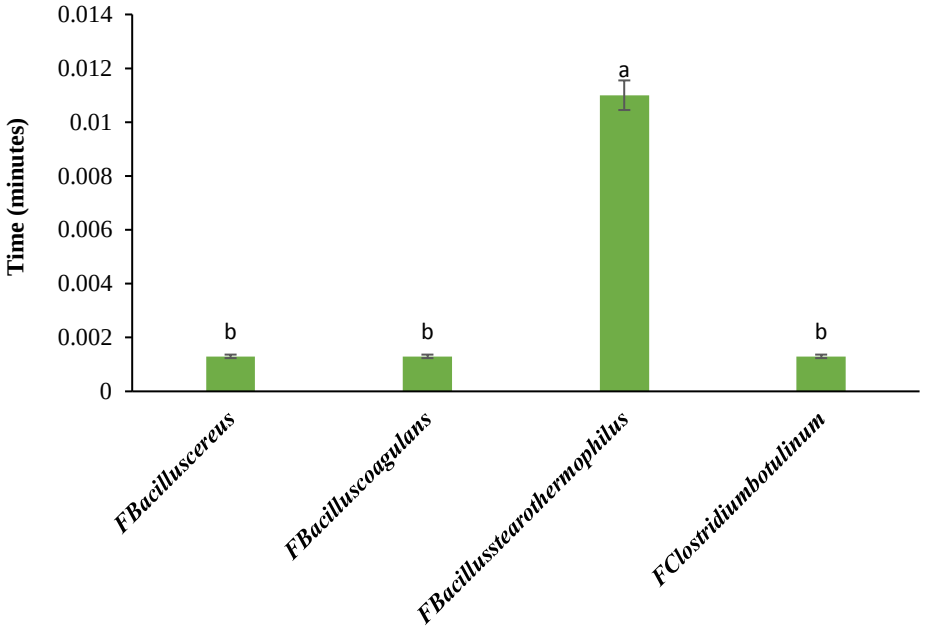


Fig. 8. Inactivation time of studied microorganisms in milk.

It is shown in the figure that among the four studied bacteria, *Bacillus stearothermophilus* has the highest resistance against cold plasma, and after that other bacteria have shown similar resistance. Although many studies have focused on the decontamination ability of cold plasma technolog, limited research has been reported on the effect of this technology on cow's milk. Wu et al. (2021) investigated the destruction of *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* with the help of DBD plasma (Wu et al., 2021). They reported that after 120 seconds of treatment at 80 V, the inactivation rate of all three bacterial species was 100%. Gurol et al. (2012) also reported that treatment with corona-type plasma at a voltage of 9 kV was able to destroy 54% of the microbial population of *Escherichia coli* within 3 minutes (Gurol et al., 2012). Kim et al. (2015) investigated the rate of destruction of *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella typhimurium* using encapsulated-DBD plasma during periods of 5 and 10 minutes. They reported that the treatment was able to reduce the microbial population by 20 log CFU/ml after 10 minutes (Kim et al., 2015). Bahreini et al. (2021) also reported that by using the plasma jet system in the inactivation of *Staphylococcus aureus* and *Escherichia coli* in milk, voltage changes during plasma treatment did not affect the microbial population of *Escherichia coli* (Bahreini et al., 2021). Cold plasma has different effect on different types of microorganisms. Since the microorganisms had determined and specific characteristics, the CP treatment showed different effects on microorganism even in the same strains. The stationary phase of microorganism growth or spores are more resistance to CP (Liao et al., 2017). Also, the gram-negative bacteria are more sensetive than gram-positive bacteria to CP treatment (Liao et al., 2017; Schlüter & Fröhling, 2014). Because in the outer part of gram-positive membrane, there is a thick peptidoglycon structure. On the other hand, in gram-negative bacteria, the elecrostatic force of CP overcomes the strenght of its membrane tensile and thus the cell wall of bacteria will destroyed (Nishime et al., 2017). The nature of material which used in CP treatment affect the process effeciency. The CP is a surface treatment in most solid food materials and the active produced species penetrate to solid food based on the water content, prosity and also physici-chemical properties (Surowsky et al., 2013). But in liquid foods, more volume should be treated with CP and if it is possible, the penetration depth of reactive species had low importance on process efficiency. Kim et al. (2015) reported that they found a strong relationship between the ability of these species to inactivate the microorganisms because with the increase in atomic oxygen concentration, the level of inactivation of microorganisms also increased. Lioa et al. (2018) have reported that in addition to the direct effect of these active species in the inactivation of microorganisms, the formation of antimicrobial compounds such as hydrogen peroxide, hydroperoxide radicals, and ozone also destroy microorganisms (Liao et al., 2018). What should be noted here is that the amount of milk fat has no effect on the inactivation of microorganisms in milk. There is limited studies regarding the effect of milk fat content on microbial deactivation of milk. Gurol et al. (2012) reported that fat content of milk, had no effect on the susceptibility of *Escherichia coli* by plasma treatment, thus revealing the potential advantage of this disinfection system for possible use in fat-content food materials. They suggested in pulsed electric field processig of milk, fat can make a protection against pulsed electric and thus can had a reverse effect on microorganisms. But in cold plasma treatment, there is no pulsed electric effect of field and only the gas species can attak the micribial cell and destroy it. Thus it can be concluded that the atmospheric conditions of CP operation had a great effect on the final efficiency of it (Surowsky et al., 2014).

Conclusion

It is important to know that the CP is an surface treatment and before selecting the type

of CP apparatus, the type of food material should be considered. Also, the type of microorganism, the microbial population, and the growth phase of microorganism are effective on the final inactivation effeciency of CP. To stablish a widespread adoption of cold plasma in dairy industry, there is some limitations. We know that the thermal processes are the validated classic methods in food industry and all of the food machinary are designed based on thermal processes. Replacing the CP treatment with conditional thermal processes in food industry needs to overcome or determining the CP limitations. Different studies are trying to find the CP effect, its limitation, and its benefits in different food products or raw material. It can be concluded that this new technology can be applied to reduce the cost of processing and preserving the nutritional and physicochemical characteristics of food.

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Data Availability Statement

Data will be made available on request.

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محاسبه عددی کشندگی باکتری در شیر بطری شده تحت تیمار پلاسمای سرد

آزاده رنجبر ندامانی[1*]

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چکیده

در سال‌های اخیر، پلاسمای سرد یکی از جایگزین‌های مورد انتظار برای تیمارهای پس از برداشت محصولات هستند. در این مطالعه، یک سیستم تخلیه سطحی برای جستجوی زمان نابودی *باسیلوس سرئوس*، *باسیوس کوآگولانس*، *باسیلوس استئاروترموفیلوس* و *کلستریدیوم بوتولینیوم* در شیر بطری شده استفاده شد. شبیه سازی توسط نرم‌افزار COMSOL ورژن ۳/۵a برای یک هندسه دو بعدی اجرا شد. داده‌های آزمایشی جمع‌آوری شده در نرم‌افزار شبیه‌سازی شدند. فاکتور k حاصل از داده‌های غیرفعال‌سازی میکروارگانیسم برای تأیید داده‌های شبیه‌سازی استفاده شد. نتایج نشان دادند تولید گونه‌های فعال اکسیژن طی تیمار پلاسمای سرد، با افزایش زمان افزایش می‌یابد و در کل ظرف پخش می‌شود. غلظت این گونه‌ها در ابتدای تولید یعنی در لحظه خروج از پروب پلاسما، بالا بوده و در انتها که سطح آزاد شیر را ترک می‌کنند، کاهش می‌یابد. با افزایش دمای اولیه نمونه شیر از ۵۰ به ۸۰ درجه سانتی‌گراد، می‌توان تغییرات بارزی در مقدار ازون مشاهده کرد. اما تغییرات ولتاژ در این دو دما اثر بارزی بر غلظت ازون نداشت. همچنین بلافاصله با آغاز تیمار پلاسما، تولید پلاسما نیز آغاز شده و میزان غلظت گونه‌های فعال در آن لحظه بیشترین مقدار است. نشان داده شده است که در بین چهار باکتری مورد مطالعه، *باسیلوس استئاروترموفیلوس* بیشترین مقاومت را در برابر پلاسمای سرد داشته و باکتری‌های دیگر بعد از آن قرار می‌گیرند. در نهایت می‌توان نتیجه گرفت که تیمار پلاسما در عمق بطری، این امکان را ایجاد می‌کند که محدودیت کاربرد سطحی تیمار پلاسمای سرد رفع شود.

واژه‌های کلیدی: پلاسمای سرد، شیر، استریلیزاسیون، پاستوریزاسیون.

۱- استادیار، گروه مهندسی بیوسیستم، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری.
(*- نویسنده مسئول: Email: a.ranjbar@sanru.ac.ir)