Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Numerical Calculation of the Denaturation of Enzymes, Nutritional Proteins, and Occurrence of Browning Reaction in Bottled Milk under Cold Plasma Treatment

Azadeh Ranjbar Nedamani[1*]

Assistant Prof. of Biosystem Engineering Dept. in the Sari Agricultural Sciences & Natural Resources University.

ARTICLE INFO

Article History:

Received:2023/1/31
Accepted: 2024/1/3

Keywords:

Browning reactions,
Cold plasma,
Enzyme deactivation,
Milk,
Nutritional Proteins

DOI: 10.22034/FSCT.21.146.42

*Corresponding Author E-Mail:
a.ranjbar@sanru.ac.ir

ABSTRACT

The aim of this work, was studying the effect of cold plasma treatment on enzymes and nutritional proteins denaturation, and ocurrance of browning reactions of bottled raw milk. 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 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 as neutral electrode. The time of inactivation of catalase, alkaline phosphatase, lipase, peroxidase, and protease enzymes, bovine serum albumin, immunoglobulins, alpha lactalbumin, beta lactalbumin, lysine and thiamine were investigated. The simulation was performed by COMSOL a3.5 software for a two-dimensional geometry. The results showed the deactivation time of catalase, phosphatase, and lipase is highly low while the peroxidase and protease show the longest deactivation time. However the final deactivation time of all enzymes is highly low compared with thermal treatments. The peroxidase diactivated at 0.9 min and protease deactivated at 2 minutes after plasma treatment. The other enzyme deactivation time were 0.5 seconds. Also, the protein and amino acid denaturation time has a significant difference at $p< 0.05$. The inactivation time of lysine amino acid was shorter than other cases studies in this work, and beta-lactalbumin protein had the longest denaturation time. Also, the time of starting the browning reaction under plasma treatment was 3.4 minutes. It can be concluded that the studied cold plasma condition have no negative effect on proteins and color of milk.

[DOI: 10.22034/FSCT.21.146.42]

1-Introduction

In all different parts of the world, cow's milk is used directly or processed into other milk products. It consists of water, lactose, fat, protein, vitamins, and minerals [1]. Milk safety and increasing its shelf-life are often established by pasteurization or sterilization fermentation, and rendering.

Destruction of microorganisms in food is one of the biggest challenges of food processing industries. Because despite the introduction of new technologies, they often have to use heat to destroy them. In some cases, to destroy the target microorganism, the use of high temperatures such as sterilization causes the destruction of nutritional compounds on a large scale. Also, the traditional methods of pasteurization and sterilization significantly affect the quality of milk and lead to enzymatic browning, loss of vitamins and taste, and structural changes in proteins. In addition to that, the change in taste, smell, texture, color, and appearance is also one of the negative results of using heat to make food healthy [1 and 2]. On the other hand, today, consumers of food industry products are looking for products with minimum processing and fresher, and dairy products are not excluded from this point of view. Since dairy products have high nutritional composition. Thus, the use of new food preservation technologies that can deactivate harmful microorganisms and enzymes with minimal heating is receiving attention. Among these methods, high hydrostatic pressure, pulsed electric field, ultrasound, and cold atmospheric plasma can be mentioned [1, 2, and 3].

In recent years, cold plasma has been one of the expected alternatives for post-harvest treatments and post-harvest management [2]. Cold plasma technology (CP) or non-thermal plasma is a non-thermal physical process that has a high potential for use in the food industry [3]. 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 the food [2]. Milk contains all the necessary compounds to meet the nutritional and energy needs of the human body [4]. However, due to the high water activity, milk is an excellent environment for the growth of bacteria such as *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, and other bacteria that cause nutritional loss of milk and reduce its quality and safety [5].

In general, thermal processing of milk is a necessary technology to ensure the microbial safety of milk for human consumption. The microbial inactivation process can immediately destroy most pathogenic microorganisms in raw milk and increase its shelf life. However, it has always been determined that the effects of this heating in milk have been the occurrence of a series of interactions and changes in the composition and structure of milk, which ultimately affect the sensory characteristics, nutritional quality, and physicochemical properties. humidity, pH, color indicators) has changed it [6]. Kim and Jimenez-Floures (1995) reported a significant interaction between milk serum proteins (such as beta-lactoglobulin) and proteins of the milk fat globule membrane during the heating of milk protein [7]. By increasing the heating temperature, a stable change occurs in the long protein chains, and the secondary structure of the protein changes [8].

[DOI: 10.22034/FSCT.21.146.42]

In dairy products, the adequacy of plasma treatment depends on factors such as the type of microorganism, input power, treatment time, gas composition, and food composition [8]. Therefore, in this study, the optimal conditions for the destruction of the desired microorganisms, as well as the extent of the destruction of nutritional compounds such as nutritional proteins, lysine, and immunoglobulin, and the occurrence of browning reactions will be investigated. To reduce laboratory costs, with little experimental data, simulation operations are performed in COMSOL, and then the data are analyzed by Design Expert software for optimization. The results of this research will be useful for removing the initial treatments before experiments to conduct studies on other qualitative and sensory parameters of raw milk.

2-Materials and Methods

2.1. 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 as a high-voltage discharge electrode. The liquid inside the bottle (milk) was considered a neutral electrode [9]. Electric discharge was performed to the electrode with the studied frequency and voltage. Plasma produces active species such as hydrogen peroxide, ozone, hydroxyl radical and oxygen radical. Since ozone monitoring during operation is easy and a suitable indicator to check plasma conditions, ozone concentration was used as a simulation index in this study.

2.2. Factors determining

2.2.1. Milk physical properties

For COMSOL simulation, some physical characteristics of the milk as well as the speed of ozone movement in the fluid, the effective diffusion coefficient of ozone, the density of ozone gas, the diameter of ozone gas bubbles, and the vector of ozone movement in the fluid, were used as Table 1.

Table 1- Physical characteristics of milk and ozone

Property	The amount of characteristics	Unit	Reference
	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	(1)
Viscosity	2.8)^(2721.5/T[1/degC])+(0.1*4.1)-8.9(	Pa·s	(1)
Relative penetration	60		(2)
	Ozone		
Ozone density	2.14	kg/m³	(3)
The speed of movement of ozone gas in the fluid	0.003	m/s	(3)
Effective diffusion coefficient of ozone in the fluid	1.74×10^{-9}	m^2/s	(3)
Ozone gas movement vector	8.33×10^{-6}	-	(3)
Diameter of ozone bubbles	3.21	mm	(3)

[DOI: 10.22034/FSCT.21.146.42]

2.3. Equations

2.3.1. Calculating the process K-factor

During the processes of destruction of target microorganisms by thermal processes, milk acts as a complex system of different compounds. A large amount of chemical, physical and biochemical reactions occur in milk. Some of these changes are very important because they can change the characteristics of the milk and others may change the nutritional value of milk [10].

Since in plasma treatment, its non-thermal role is used to destroy microorganisms, it is believed that the incidence of temperature-affected reactions in milk will decrease. The reactions that occur in milk can be divided into five categories; Destruction of microorganisms, deactivation of enzymes, denaturation of proteins, loss of nutritional compounds, and formation of new compounds. Most of these reactions can be shown with a simple one-step irreversible reaction [10]:

$$A \rightarrow B$$

The rate of elimination and formation in such a reaction is shown by a standard reaction rate (equation 1):

$$r_A = -kC_A^n, \quad r_B = -r_A \qquad [1]$$

where *r* is the reaction rate (mol/m3.s), *k* is the reaction rate constant (m3/mol.s), and *n* is the degree of reaction. The way in which the reaction rate constant (*k*) is affected by temperature was important in determining the extent of final transformations caused by heat treatment. For this reason, equation 2 is used to show the dependence of *k* to temperature:

$$k = k_0 \exp\left(\frac{-E_a}{RT}\right) \qquad [2]$$

where, k_0 is the pre-exponential factor (m3/mol.s), E_a is the activation energy ($Jmol^{-1}$), R is the gas constant ($Jmol^{-1}K^{-1}$ 8314) and T is the absolute temperature in terms of K. The value of k_0 and E_a is a function of the type of reaction and the type of composition of the desired substance. For this reason, equations 3 to 17 have been used to calculate *k* for some of the five types of reactions mentioned in milk [10]:

Microorganism destruction

$$k_{Bacillus\ Stearothermophilus} = 101.15 \exp\left(\frac{-345.4}{8314T}\right) \qquad [3]$$

$$k_{Clostridium\ Botulinum} = 107.5 \exp\left(\frac{-351}{8314T}\right) \qquad [4]$$

$$k_{Bacillus\ Coagulans} = 151.29 \exp\left(\frac{-509}{8314T}\right) \qquad [5]$$

$$k_{Bacillus\ Cereus} = 91.92 \exp\left(\frac{-294.5}{8314T}\right) \qquad [6]$$

Enzyme deactivation

$$k_{Protease} = 15.19 \exp\left(\frac{-64}{8314T}\right) \qquad [7]$$

$$k_{Catalase} = 180.72 \exp\left(\frac{-529}{8314T}\right) \qquad [8]$$

$$k_{Peroxidase} = 222.5 \exp\left(\frac{-663}{8314T}\right) \qquad [9]$$

$$k_{Lipase} = 53.70 \exp\left(\frac{-160}{8314T}\right) \qquad [10]$$

$$k_{Phosphatase} = 95.17 \exp\left(\frac{-275}{8314T}\right) \qquad [11]$$

Protein denaturation

$$k_{AlphaLactalbumin} = 84.92 \exp\left(\frac{-296}{8314T}\right) \qquad [12]$$

$$k_{BetaLactoglobulin} = 89.43 \exp\left(\frac{-280}{8314T}\right) \qquad [13]$$

$$k_{Immunoglobulin} = 90.38 \exp\left(\frac{-275}{8314T}\right) \qquad [14]$$

Nutritional loss

[DOI: 10.22034/FSCT.21.146.42]

$$k_{\text{Thiamin}} = 29.78 \exp\left(\frac{-100.8}{8314T}\right) \quad [15]$$

$$k_{\text{Lysine}} = 8.77 \exp\left(\frac{-190}{8314T}\right) \quad [16]$$

New component formation

$$k_{Pigmentbrown} = 29.09 \exp\left(\frac{-116}{8314T}\right) \quad [17]$$

2.3.2. Calculating the process F-factor

When heat treatment is used to destroy the target microorganism in food, the time required to reach a certain degree of *k* at a certain temperature of *T* is called "thermal death time" (*F*), which according to equation 18 is obtained by multiplying *k* in *D*:

$$F_T = kD_T \quad [18]$$

The parameter *D* is calculated as equation 19:

$$t = \mathrm{D}_T log_{10}\left(\frac{N_0}{N}\right) \quad (۱۹)$$

2.3.3. Governing equations in COMSOL

The simulation was performed by COMSOL a3.5 software for a two-dimensional geometry as shown in Figure (1). Four modules of laminar bubbling, diluted species transport (for air injected between electrodes in the valve), diluted species transport (to remove bacteria or the compound under investigation in the milk), and electric field (to create plasma) were used to solve the problem.

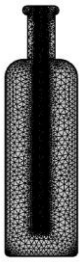

Figure 1- The two-dimensional geometry of the milk bottle inside which the plasma generator system is placed.

2.3.3.1. Laminar Bubble Flow

This module was used to simulate the movement of ozone bubbles from the plasma reactor inside the milk (equations 20 to 23):

$$\begin{aligned}\emptyset_l\rho_l\frac{\partial u_l}{\partial t} + \emptyset_l\rho_l(u_l.\nabla)u_l &= \nabla.[-pI \\ &+ \emptyset_l(\mu_l \\ &+ \mu_T)(\nabla u_l \\ &+ (\nabla u_l)^T)] \\ &+ \emptyset_l\rho_l g + F\end{aligned} \quad [20]$$

$$\rho_1\nabla.(u_l) = 0, \quad u_l = u \quad [21]$$

$$\frac{\partial \emptyset_g\rho_g}{\partial t} + \nabla.N_{\rho_g\emptyset_g} = -m_{gl}, \quad \emptyset_g\rho_g = rhogeff \quad [22]$$

$$\begin{aligned}N_{\rho_g\emptyset_g} = \emptyset_g\rho_g u_g, \quad u_g &= u_l \\ &+ u_{slip} \\ &- \mu_T\frac{\nabla\emptyset_g}{\rho_l\emptyset_g}\end{aligned} \quad [23]$$

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 the studies of Wang et al. (2020) [9] (Table 1).

2.3.3.2. Diluted species transport

Deactivation of microorganisms or enzymes, as well as nutritional loss and occurrence of chemical reactions, depend

[DOI: 10.22034/FSCT.21.146.42]

on the amount of ozone and ions formed by plasma. The rate of this reaction can be calculated according to Fick's law in the form of equations 24 and 25:

$$\frac{\partial c_i}{\partial t} + \nabla.(-D_i \nabla c_i) + u.\nabla c_i = R_i \quad [24]$$

$$N_i = -D_i \nabla c_i + u c_i \quad [25]$$

During the simulation, the value of R_i (reaction rate) was defined according to equation 26:

$$R_i = -k_{reac} c_{o_3} \quad [26]$$

2.3.3.3. Initial and boundary conditions

The outer boundary at the top of the plasma generating reactor was considered as a free surface. For the simplicity of the calculations, the surface motions of the fluid were ignored. The border of entering ozone into the milk was considered at the end of the reactor. Ozone flow rate was calculated according to equation 27:

$$n.N_1 = n.(uc_{0.j}) \quad [27]$$

Constant pressure points were also added to the ozone output boundary (p=0). The concentration of microorganisms and the operation temperature were defined according to the Design Expert.

2.4. Problem solving

COMSOL a3.5 software was used to solve four modules based on laminar flow. A device with system specifications Intel® CoreTM i5-4300U, 2.50 GHz, RAM 4 GB, and Windows 10 64-bit was used for this purpose. The relative torelance of solving the problem was 0.01 and data recording was done for ten. Normal mesh was used for geometry and fine mesh was used for reactor in 2D space according to Figure 1.

2.5. Model validation

For simulating, first, the exprimental data were simulated in COMSOL software. For this purpose, k data for microorganism deactivation were used. Then the output of software responses was checked with experimental data. After calculating R_2, and ensuring the accuracy of the simulation process, the Design Expert designed treatments that were simulated in the software.

2.6. Statistical analysis

To ensure the accuracy of the model and detect the regression coefficients and statistical significance, variance analysis was performed by ANOVA in Design Expert. Line equation, regression coefficients, and lack of fit were analyzed by R_2, *p*-value (at 0.05 level), and *Adj*-R_2 statistical parameters.

3- Results and Discussion

3.1. Simulation validation

In microbial inactivation, if the semi-logarithmic diagram of the microbial population is drawn, a linear diagram with slope *k* is obtained [11]. Process temperature can change the slope of this graph. We know that the effect of the amount 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. Also, it was determined that temperature changes can affect the amount of ozone gas produced by plasma treatment

[DOI: 10.22034/FSCT.21.146.42]

[12]. 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.

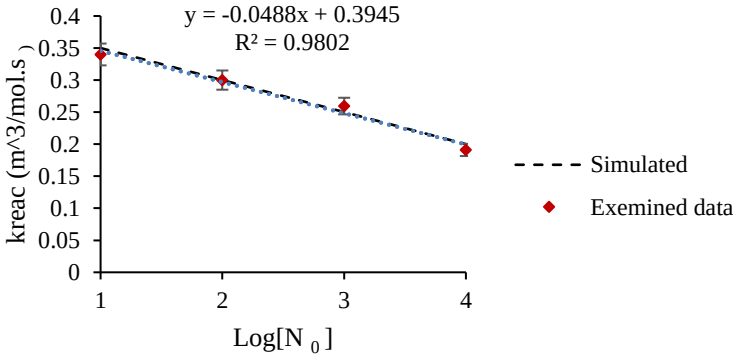

Figure 2- Fitting the simulated and experimental data

3.2. Enzyme deactivation, and protein denaturation

Figure 3 shows the inactivation time of enzymes in milk with the help of plasma treatment. This figure is related to treatment 1 and temperature 64.4 ℃ and shows that the peroxidase enzyme which during the heating process inactivated at 60℃ and 10,000s, was deactivated within 0.9 minutes under cold plasma treatment. Other enzymes, such as lipase, alkaline phosphatase, and catalase, were deactivated in a very short time of 0.5 seconds. These results show the potential ability of plasma treatment to deactivate enzymes at a much lower temperature and time than common thermal processes.

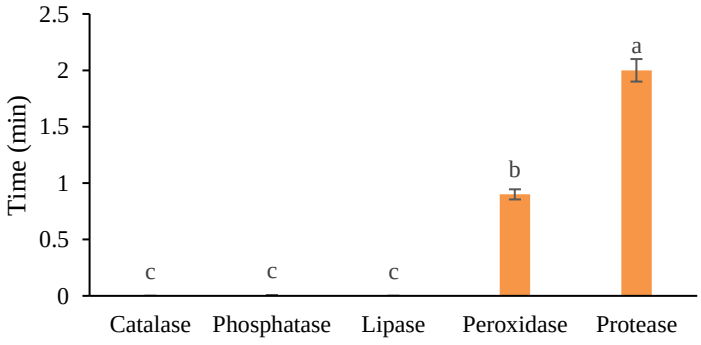

Figure 3- Inactivation time of studied enzymes in bottled milk under plasma treatment

Several studies have been conducted on the inactivation of specific destructive enzymes in various food products using plasma techniques. The inhibitory effect of plasma treatment was against tomato peroxidase [13], milk alkaline phosphatase [14], and polyphenol oxidase in freshly cut apples [15]. Among the bacteria known to produce heat-resistant extracellular proteases, the *Pseudomonas* is dominant. The fact that current industrial heat treatment is not severe enough to inactivate such proteases shows their deactivation

[DOI: 10.22034/FSCT.21.146.42]

importance in the dairy industry. The presence of these enzymes worsens the problem of sedimentation, curd gelation, and bitterness in sterile milk [14 and 15].

Mohammadpour et al. (2021) reported that during 8 days of storage the amount of activity of proteases produced by *Pseudomonas* in milk samples treated with plasma was lower than the samples without plasma treatment [16]. In addition, the longer the plasma treatment time, the lower the activity during storage. The greatest decrease in activity was observed in the 10-minute plasma treatment, which was 63.81% less than the control group at the end of storage.

It has been proven that several parameters related to plasma are involved in the effectiveness of plasma treatment against food and bacterial enzymes, including discharge type, gas composition, voltage, and treatment time [17]. Along with the plasma-related parameters, the composition of the medium used for the treatment and the structure of the enzyme are also important. The inactivation efficiency of plasma treatment against target enzymes can be reduced in complex environments such as milk. The presence of other components of milk, such as proteins and lipids, plays a protective role against the treatment and reduction of plasma power, which should be considered during plasma treatment to destroy the target enzymes.

Compared to the total number of studies dealing with the effect of plasma on food components in liquids, there are several studies on the effect of proteins and enzymes. Lysozyme was part of the study by Takai et al. (2012) who treated the enzyme in phosphate buffer using a plasma jet with helium and oxygen as process gas [18]. They observed a decrease in activity and secondary structure as well as a decrease in tryptophan fluorescence, while the molecular weight increased slightly. They also concluded that these changes are affected by UV light and not by the heat of the plasma and suggested that reactive species produced by the plasma affect lysozyme. Tammineedi et al. (2013) treated casein and whey protein in a buffer solution with plasma to reduce sensitization [19]. However, no changes were measured by SDS-PAGE or by Ci-ELISA/IgE binding analysis. In contrast, it has been reported that enzymes such as polyphenol oxidase and peroxidase have been successfully inactivated by cold plasma [13].

It has been suggested that the deactivation is due to the combined action of reactive oxygen species inherent in plasma such as hydroxyl radicals and oxidizing atomic oxygen. Certain amino acids present in enzymes lead to further structural changes and as a result loss of enzyme activity. However, degradation largely depends on the presence of accompanying substances such as carbohydrates [20].

Figure 4 shows the denaturation time of bovine serum albumin, immunoglobulin, alpha-lactalbumin, beta-lactoglobulin, and amino acid lysine in treatment one According to this graph, under the influence of non-thermal plasma treatment, the denaturation time of the studied proteins and amino acid has a significant difference at the level of 5%. In the meantime, the inactivation time of lysine amino acid was shorter than in other cases studied in this review, and β-lactalbumin protein was the longest. This shows that, like other thermal processes, the deactivation of the essential amino acid lysine occurred at a faster rate than the studied nutritional compounds. It can be assumed that since L-lysine is an

[DOI: 10.22034/FSCT.21.146.42]

essential amino acid, its destruction in this way reduces the nutritional quality of the food [21].

The effect of plasma on the endogenous enzymes of milk is similar to the action on microorganisms. Under the influence of this treatment, enzymes are deactivated through peptide oxidation reactions that change the composition of proteins and thus reduce their enzyme activity. This technology is currently used in milk and dairy products. Segat et al. (2016) evaluated the effect of atmospheric cold plasma on the activity and structure of milk alkaline phosphatase [14]. The alkaline phosphatase enzyme in solution was exposed to three separate high voltages (40, 50, and 60 kV) for a period of 15 seconds to 5 minutes of cold plasma treatment. The results showed that the plasma technology based on the discharge of the dielectric barrier can deactivate the enzyme within a few seconds. The two-color spectrum showed that the enzyme is characterized by a dominant alpha-helical structure, and the helix content tends to decrease with increasing treatment time and voltage.

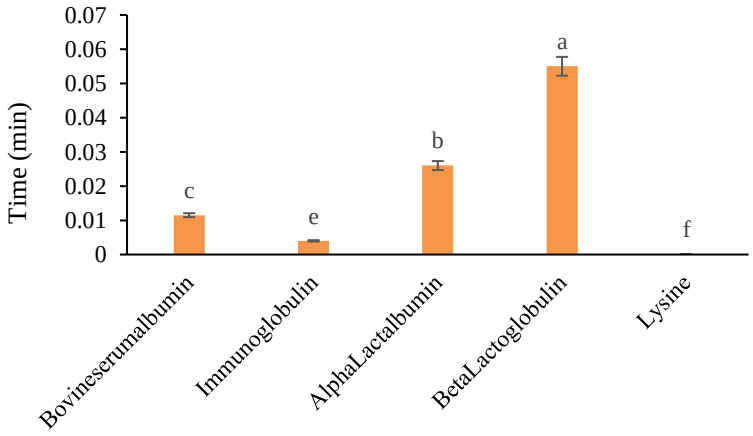

Figure 4- Inactivation time of studied proteins and lysin in bottled milk under plasma treatment

Proteins undergo a variety of chemical changes, including when lysine units are exposed to high temperatures and alkaline pH. Such changes reduce their digestibility. The reaction of reducing sugars with ε amine groups also reduces the digestibility of lysine [21].

Milk proteins (32 grams per liter to 38 grams per liter in whole milk) are an important component in milk products; Because they affect the physical, chemical, and sensory characteristics. Milk proteins are classified into two primary groups, which include casein (80%) and whey protein (20%) in whole cow's milk [22]. There are four main types of casein: α s1-, αs2, and κ-casein, on the other hand, whey proteins include α-lactalbumin (α-LA), beta-lactoglobulin (β-LG), serum albumin, and immunoglobulin [23].

Segat et al. (2015), found that DBD atmospheric cold plasma with a voltage of 70 kV for 15 minutes caused a slight oxidation of proteins, which was compared to the control sample with the amount of carbonyl groups attached to the protein. had been measured [14]. During this study, a greater amount of carbonyl groups was also observed, which can be attributed to the changes in several side chain groups of amino acids, especially with -NH and -NH2 or peptide bond fragments. There was also

[DOI: 10.22034/FSCT.21.146.42]

a decrease in SH-free groups after 30 minutes of treatment. Disulfide cross-link formation is a method to characterize protein aggregation. Disulfide cross-links usually occur during heat treatment and are related to the denaturation of whey proteins.

Reactive oxygen species produced during plasma treatment (for example, hydroxyl radical) increase cross-linking of free amino acids with sulfur-containing amino acid side chains such as cysteine, resulting in protein accumulation [24]. Manoharan et al. (2020) determined that the use of low-pressure cold plasma treatment did not change the protein content of raw milk compared to the control sample [25].

A study on non-fat dry milk did not show any significant changes in the amino acid profile [6]. They explained that atomic oxygen and hydroxyl radicals, which can be identified at 777 nm and 309 nm of the irradiated spectrum, respectively, have the highest oxidation potential by all reactive oxygen species produced in plasma and are capable of oxidizing amino acids. are. However, none of the species was detected in the optical emission spectrum.

In general, depending on the applied conditions, the effect of cold plasma may not significantly change proteins if applied under low pressure or nitrogen gas, because there are no active oxygen species. If air is used as a gas and high voltage (60 kV) and a long treatment time of more than 30 minutes, slight oxidation or significant aggregation may occur in proteins due to the higher concentration of reactive oxygen species produced. . Therefore, to minimize the protein change, the treatment conditions should be appropriate [6, 25].

3.3. Nutritional loss and color change

Figure 5 shows the destruction time of thiamine as a representative compound for nutrients, and the occurrence of non-enzymatic browning reactions. Studies have shown that the physicochemical characteristics of milk and dairy products are not affected by cold plasma treatment. Despite some observed color differences, many studies have reported no differences that can be detected by the human eye. Milk treated with cold plasma can have higher acidity, which may be due to the multistep reactions of active species produced by the plasma with water at the gas-water interface. Differences in the acidity of plasma-treated fluids may result from a variety of factors, including volume treated, buffer capacity, plasma source, and induction gas used. For cheese, increased lipid oxidation may occur, which may destroy flavor and affect consumer acceptance. Therefore, the application of this technology in high-fat products should be carefully evaluated. Cold plasma can be successfully used to selectively modify the protein structure and improve the performance of whey proteins. Also, this treatment was able to deactivate the alkaline phosphatase enzyme within a few seconds. Aslan (2016) showed that treatment with DBD plasma at voltages of 1.5, 3, and 5 kV with an average treatment time of ten minutes did not change the nutritional quality and physicochemical properties of milk [26]. Dash and Jaganmohan (2022) reported that the content of protein and fat in milk after treatment with plasma for the entire

[DOI: 10.22034/FSCT.21.146.42]

duration of treatment (5, 10, and 15 minutes) was not significantly different ($p<0.05$) from the control. The amount of protein and fat in the control sample was 3.41±0.001% and 3.52±0.001%, respectively. While for the 15-minute sample, the amount of protein and fat was 3.39±0.002 and 3.49±0.001%, respectively. Similar findings of protein content after cold plasma were observed in milk [27].

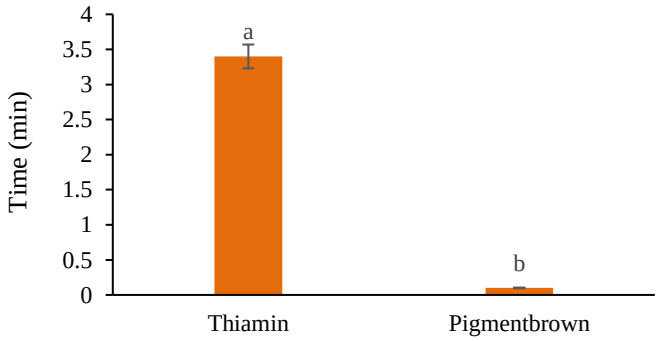

Figure 5- The time of thiamine loss and occurrence of browning reactions in milk

The color of milk is an important sensory property that not only affects the consumer's choice of one product over another but is also closely related to the quality of the resulting dairy products. The color of milk varies from yellowish white to almost white, depending on various reasons, including cow breed, feed composition, stage of lactation, time of lactation, and calving season. Color is defined by the International Illumination Commission based on a three-dimensional color space with three primary coordinates L*, a*, b*, where L* indicates lightness or darkness, a* redness or greenness, and b* yellowness or It is blue. The total color difference (ΔE) is a standard calculation measure of color changes that expresses the human visual judgment of the difference between two perceived colors [28].

As mentioned earlier, when air and oxygen are used as active gases in cold plasma, reactive oxygen species such as ozone are produced. The ratio of different active oxygen species depends on many factors, including gas composition, plasma treatment time, exposure method, and plasma source. For example, the presence of water vapor in the working gas transfers reactive oxygen species from ozone to larger peroxides that show much less lipid oxidation [29]. Gurol et al. (2012) in a research on the destruction of Escherichia coli bacteria by cold plasma, observed that milk samples did not show any significant change in color after 0, 3, 6, 9, 12, and 15 minutes of 9 kV plasma treatments and only A slight change compared to untreated milk occurred after 20 minutes [30].

Manoharan et al. (2020) showed that the values of ΔE in milk treated with plasma were 0.91 and 1.58 for treated milk with a flow rate of 6 and 3 ml/min, respectively [25]. Gurol et al. (2012) did not report any significant change in color after the treatment of raw milk plasma up to 15 minutes from the treatment time [30]. The total color difference (ΔE) for cow's milk after 9 minutes of plasma treatment with 9 kV was 0.25, while longer exposure to plasma (20 minutes) caused a slightly greater color difference with ΔE 0.52. Instead, Kim et al. (2015) did not observe a significant change in the value of ΔE after 5 and 10 minutes of milk treatment with plasma [7]. They hypothesized that the higher L* of plasma-treated milk may be related to the greater number of fat globules that can scatter light more effectively. In addition, according to the results of Popov-Ralji´c et al. (2008), the increase in b* is an indicator that determines the non-enzymatic reactions of milk color change, which is also known as Maillard reactions [31].

[DOI: 10.22034/FSCT.21.146.42]

This reaction starts by connecting the aldehyde group of lactose with the ε-amino group of lysyl residues (amino acid radical or lysine amino acid residue) from different milk proteins. It was also observed in the previous section that the time for the destruction of the amino acid lysine was very short, and considering the short time required for the initiation of browning reactions in milk, these two phenomena can be considered related.

In a study by Wu et al. (2021), the ΔE values of sample treatment with DBD plasma at 80 volts for 120 seconds were in the range between the total color changes of superheated milk (36.02) and pasteurized milk (9.13), so the color of the treated samples was considered within the accepted color range [32].

Therefore, to make a slight change in the color of dairy products, it is recommended that the treatment time be less than 5 minutes. Since higher b* (for example, more than 6) indicates non-enzymatic reactions of milk, a low concentration of oxygen in the used gas is recommended to prevent the oxidation of fat and protein, which increases the amount of yellowness.

4- Conclusion

It is clear that plasma treatment significantly reduces the time and temperature required for the milk sterilization or pasteurization process. In this way, the preservation of nutritional compounds and the occurrence of chemical reactions that lead to the appearance of taste and color in milk are reduced. The peroxidase enzyme, which needs more than 10,000 seconds to inactivate at 60°C, was inactivated in 0.9 minutes with the help of plasma treatment. Other enzymes such as lipase, alkaline phosphatase, and catalase were deactivated in a very short time of 0.5 seconds. These results show the potential ability of plasma treatment to inactivate enzymes at a much lower temperature and time than common thermal processes. Under the influence of non-thermal plasma treatment, the denaturation time of the studied proteins and amino acids has a significant difference at the 5% level. In the meantime, the inactivation time of lysine amino acid was shorter than in other cases studied in this review, and β-lactalbumin protein was the longest. Considering the application of cold plasma in the food industry, the economic cost associated with the use of the new technology compared to heat treatment should be evaluated. The cost mainly depends on the equipment investment, the energy cost of this treatment, and the overall production costs. The machines related to this technology should be cheap, perform treatment continuously at high speed and with minimal maintenance, and work with all kinds of gases. Therefore, avoiding the use of expensive noble gases due to low operating margins is of primary importance. Ideally, plasma sources capable of ionizing air in large gaps would be suitable. In addition, plasma sources operating in place of radio frequency energy sources can lead to cost management. Non-thermal methods usually have higher costs than thermal processes, but these costs are expected to decrease with more efforts to commercialize them. In addition, the benefits related to sensory properties and product quality can be greater. It is clear that plasma treatment significantly reduces the time and temperature required for the

[DOI: 10.22034/FSCT.21.146.42]

milk sterilization or pasteurization process. In this way, the preservation of nutritional compounds and the occurrence of chemical reactions that lead to the appearance of taste and color in milk are reduced. The peroxidase enzyme, which needs more than 10,000 seconds to inactivate at 60°C, was inactivated in 0.9 minutes with the help of plasma treatment. Other enzymes such as lipase, alkaline phosphatase, and catalase were deactivated in a very short time of 0.5 seconds. These results show the potential ability of plasma treatment to inactivate enzymes at a much lower temperature and time than common thermal processes. Under the influence of non-thermal plasma treatment, the denaturation time of the studied proteins and amino acids has a significant difference at the 5% level. In the meantime, the inactivation time of lysine amino acid was shorter than in other cases studied in this review, and β-lactalbumin protein was the longest. Considering the application of cold plasma in the food industry, the economic cost associated with the use of the new technology compared to heat treatment should be evaluated. The cost mainly depends on the equipment investment, the energy cost of this treatment, and the overall production costs. The machines related to this technology should be cheap, perform treatment continuously at high speed and with minimal maintenance, and work with all kinds of gases. Therefore, avoiding the use of expensive noble gases due to low operating margins is of primary importance. Ideally, plasma sources capable of ionizing air in large gaps would be suitable. In addition, plasma sources operating in place of radio frequency energy sources can lead to cost management. Non-thermal methods usually have higher costs than thermal processes, but these costs are expected to decrease with more efforts to commercialize them. In addition, the benefits related to sensory properties and product quality can be greater.

5- Acknowledgement

We would like to thank the research assistant of Sari Agricultural Sciences and Natural Resources University for the financial support of this research in the form of project number 02-1401-20.

6- References

[1] Nikmaram, N., & Keener, K. M. (2022). The effects of cold plasma technology on physical, nutritional, and sensory properties of milk and milk products. *LWT, 154*, 112729.

[2] Misnal, M. F. I., Redzuan, N., Zainal, M. N. F., Ahmad, N., Ibrahim, R. K. R., & Agun, L. (2022). Cold Plasma: A Potential Alternative for Rice Grain Postharvest Treatment Management in Malaysia. *Rice Science, 29*(1), 1-15.

[3] Xiang, Q., Liu, X., Liu, S., Ma, Y., Xu, C., & Bai, Y. (2019). Effect of plasma-activated water on microbial quality and physicochemical characteristics of mung bean sprouts. *Innovative Food Science & Emerging Technologies, 52*, 49-56. doi:10.1016/j.ifset.2018.11.012

[4] Balthazar, C. F., Pimentel, T. C., Ferrao, L. L., Almada, C. N., Santillo, A., Albenzio, M., et al. (2017). Sheep milk: Physicochemical characteristics and relevance for functional food development. *Comprehensive Reviews in Food Science and Food Safety, 16*(2), 247–262.

[5] Dhanashekar, R., Akkinepalli, S., & Nellutla, A. (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. *Germs, 2*(3), 101–109.

[DOI: 10.22034/FSCT.21.146.42]

[6] Chen, D., Peng, P., Zhou, N., Cheng, Y., Min, M., Ma, Y., et al. (2019). Evaluation of Cronobacter sakazakii inactivation and physicochemical property changes of non-fat dry milk powder by cold atmospheric plasma. *Food Chemistry*, *290*, 270–276. https://doi.org/10.1016/j.foodchem.2019.03.149

[7] Kim, H. H. Y., & Jimenez-Flores, R. (1995). Heat-induced interactions between the proteins of milk-fat globule-membrane and skim milk. *Journal of Dairy Science*, *78*(1), 24–35.

[8] Farrell, H. M., Jr., Wickham, E. D., Unruh, J. J., Qi, P. X., & Hoagland, P. D. (2001). Secondary structural studies of bovine caseins temperature dependence of β-casein structure as analyzed by circular dichroism and FTIR spectroscopy and correlation with micellization. *Food Hydrocolloids*, *15*, 341–354.

[9] Wang, Y., Wang, Z., Yang, H., & Zhu, X. (2020). Gas phase surface discharge plasma model for yeast inactivation in water. *Journal of Food Engineering*, *286*, 110117. doi:https://doi.org/10.1016/j.jfoodeng.2020.110117

[10] De Jong, P. (2008). Thermal processing of milk. *Advanced dairy science and technology*, 1-34.

[11] Ibarz, A., & Barbosa-Cánovas, G. V. (2002). *Unit operations in food engineering*: CRC press.

[12] Ranjbar Nedamani, A. (2022). Finding Effective Plasma Process Factors on Yeast Deactivation by Numerical Simulation and RSM. *Iranian Food Science and Technology Research Journal*, -. doi:10.22067/ifstrj.2022.75558.1152

[13] Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of Cold Plasma on Food Quality: A Review. *Foods*, *7*(1). doi:10.3390/foods7010004

[14] Segat, A., Misra, N., Cullen, P. J., & Innocente, N. (2016). Effect of atmospheric pressure cold plasma (ACP) on activity and structure of alkaline phosphatase. Food and Bioproducts Processing, 98, 181e188.

[15] Tappi, S., Berardinelli, A., Ragni, L., Dalla Rosa, M., Guarnieri, A., & Rocculi, P. (2014). Atmospheric gas plasma treatment of fresh-cut apples. Innovative Food Science & Emerging Technologies, 21, 114e122.

[16] Mohammadpour, H., Zarei, M., Cullen, P. J., Valtchev, P., Schindeler, A., & Dehghani, F. (2021). Potential application of non-thermal atmospheric plasma in reducing the activity of Pseudomonas-secreted proteases in milk. *International Dairy Journal*, *120*, 105078.

[17] Fernandez, A., Shearer, N., Wilson, D., & Thompson, A. (2012). Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of Salmonella enterica serovar Typhimurium. International Journal of Food Microbiology, 152, 175e180.

[18] Takai, E., Kitano, K., Kuwabara, J., & Shiraki, K. (2012). Protein inactivation by low-temperature atmospheric pressure plasma in aqueous solution. *Plasma processes and polymers*, *9*(1), 77-82.

[19] Tammineedi, C. V., Choudhary, R., Perez-Alvarado, G. C., & Watson, D. G. (2013). Determining the effect of UV-C, high intensity ultrasound and nonthermal atmospheric plasma treatments on reducing the allergenicity of α-casein and whey proteins. *LWT-Food Science and Technology*, *54*(1), 35-41.

[20] Surowsky, B., Schlüter, O., & Knorr, D. (2014). Interactions of Non-Thermal Atmospheric Pressure Plasma with Solid and Liquid Food Systems: A Review. *Food Engineering Reviews*, *7*(2), 82-108. https://doi.org/10.1007/s12393-014-9088-5

[21] Fennema, O. R. (2008). *Food chemistry* (Vol. 76). CRC Press.

[22] Davoodi, S. H., Shahbazi, R., Esmaeili, S., Sohrabvandi, S., Mortazavian, A. M., Jazayeri, S., et al. (2016). Health-related aspects of milk proteins. *Iranian Journal of Pharmaceutical Research*, *15*(3), 573–591. https://doi.org/10.22037/ijpr.2016.1897

[DOI: 10.22034/FSCT.21.146.42]

[23] Sharma, S., & Singh, R.k. (2020). Cold plasma treatment of dairy proteins in relation to functionality enhancement. In *Trends in Food Science and Technology* (Vol. 102, pp. 30–36). Elsevier Ltd. https://doi.org/10.1016/j.tifs.2020.05.013

[24] Meinlschmidt, P., Ueberham, E., Lehmann, J., Reineke, K., Schlüter, O., Schweiggert-Weisz, U., et al. (2016). The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Science & Emerging Technologies*, *38*, 374–383. https://doi.org/10.1016/j.ifset.2016.06.007

[25] Manoharan, D., Stephen, J., & Radhakrishnan, M. (2020). Study on low-pressure plasma system for continuous decontamination of milk and its quality evaluation. *Journal of Food Processing and Preservation*, *45*(2). https://doi.org/10.1111/jfpp.15138

[26] Aslan, Y. (2016). "The Effect of Dielectric Barrier Discharge Plasma Treatment on the Microorganisms Found in Raw Cow's Milk." Türkiye Tarımsal Araştırmalar Dergisi **3**(2): 169-173.

[27] Dash, S., & Jaganmohan, R. (2022). Stability And Shelf-Life Of Plasma Bubbling Treated Cow Milk. *bioRxiv*.

[28] Browder, R. (2018). *Nutraceutrical Snack Prepared From Sprouted Rough Rice And Green Gram And Its Physichocemical Properties, In Vitro Glycemic Index, And Sensory Attributes*. https://scholarworks.uark.edu/fdscuht/4.

[29] Feizollahi, E., Misra, N. N., & Roopesh, M. S. (2021). Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications. *Critical reviews in food science and nutrition*, *61*(4), 666-689.

[30] Gurol, C., Ekinci, F., Aslan, N., & Korachi, M. (2012). Low temperature plasma for decontamination of E. coli in milk. *International journal of food microbiology*, *157*(1), 1-5.

[31] Popov-Ralji´c, J. V., Laki´c, N. S., Lali˘ci´c-Petronijevi´c, J. G., Bara´c, M. B., & Sikimi´c, V. M. (2008). Color changes of UHT milk during storage. *Sensors*, *8*(9), 5961–5974. https://doi.org/10.3390/s8095961

[32] Wu, X., Luo, Y., Zhao, F., & Mu, G. (2021). Influence of dielectric barrier discharge cold plasma on physicochemical property of milk for sterilization. *Plasma Processes and Polymers*, *18*(1), 1900219.

[DOI: 10.22034/FSCT.21.146.42]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

محاسبه عددی میزان دناتوره شدن آنزیم‌ها، پروتئین‌های تغذیه‌ای و بروز واکنش‌های قهوه‌ای شدن در شیر بطری‌شده تحت تاثیر تیمار پلاسمای سرد

آزاده رنجبر ندامانی[1*]

۱- استادیار گروه مکانیک بیوسیستم- دانشگاه علوم کشاورزی و منابع طبیعی ساری- مازندران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۲/۱/۱۱

تاریخ پذیرش: ۱٤۰۲/۱۱/۱٤

کلمات کلیدی:

واکنش‌های قهوه‌ای شدن،

تیمار پلاسما،

غیرفعال‌سازی آنزیمی،

پروتئین‌های تغذیه‌ای

DOI: 10.22034/FSCT.21.146.42

* مسئول مکاتبات:

a.ranjbar@sanru.ac.ir

چکیده

هدف از این پژوهش، مطالعه اثر تیمار پلاسمای سرد بر دناتوراسیوان آنزیم‌ها، پروتئین‌های تغذیه‌ای و بروز واکنش‌های قهوه‌ای شدن در شیر خام بطری شده است. یک سیستم پلاسمای تخلیه سطح برای این منظور استفاده شد. راکتور این سیستم یک استوانه کوارتزی با قطر ۱ سانتی‌متر و ارتفاع ۲۵ سانتی‌متر بود. از یک پوشش استیل با ضخامت ۱ میلی‌متر و ارتفاع ۲۵ سانتی‌متر در سطح داخلی راکتور و به عنوان الکترود تخلیه ولتاژ بالا استفاده شد. مایع درون بطری (شیر) نیز به عنوان الکترود خنثی در نظر گرفته شد. تخلیه برق با فرکانس و ولتاژ مورد مطالعه به الکترود انجام شد. در این مطالعه زمان غیرفعال‌شدن کاتالاز، فسفاتاز قلیایی، لیپاز، پراکسیداز، آنزیم‌های پروتئازی، و همچنین زمان دناتوره شدن آلبومین سرمی، ایمنوگلوبولین‌ها، آلفا لاکتالبومین، بتالاکتوگلوبولین، لیزین و در نهایت میزان تخریب ویتامین تیامین مورد بررسی قرار گرفت. شبیه‌سازی توسط نرم افزار کامسول ورژن a۳.۵ برای یک هندسه دوبعدی انجام شد. نتایج نشان دادند زمان غیرفعال‌سازی کاتالاز، فسفاتاز و لیپاز بسیار اندک بود در حالیکه آنزیم‌های پروتئازی و پراکسیداز طولانی‌ترین زمان غیرفعال‌شدن را نشان دادند. با این حال زمان غیرفعال‌شدن نهایی تمام آنزیم‌ها در مقایسه با تیمارهای حرارتی رایج در صنایع لبنی بسیار اندک بود. پراکسیداز در ۰/۹ دقیقه و پروتئاز در ۲ دقیقه بعد از آغاز تیمار غیر فعال شدند. زمان غیر فعال‌سازی سایر آنزیم‌ها ۰/۵ ثانیه بود. همچنین، زمان دناتوراسیون پروتئین‌ها و اسیدهای آمینه به شکل معناداری متفاوت بود ($p<۰/۰۵$) زمان غیرفعال شدن اسید آمینه لیزین کمتر از سایر موارد مورد مطالعه بود و بتالاکتوگلوبولین بالاترین زمان دناتوراسیون را داشت. زمان آغاز واکنش قهوه‌ای شدن تحت تیمار پلاسما ۳/٤ دقیقه بود. به طور کلی می‌توان نتیجه گرفت که شرایط تیمار پلاسمایی که مورد مطالعه قرار گرفت، اثر منفی بر پروتئین‌ها و رنگ شیر نداشت.

[DOI: 10.22034/FSCT.21.146.42]