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Research Paper / Araştırma Makalesi

Effect of Cold Plasma Treatment on Physicochemical and Microbiological Properties of Clotted Cream

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ABSTRACT

The aim of this study was to determine the effect of cold plasma treatment, which was applied by using two different gases (O_2 and Ar) and their mixtures onto sample surfaces for different intervals, on the physicochemical and microbial parameters of clotted cream during storage for up to 10 days. Cold plasma treatment caused a decrease in pH, aw, L*, and b* values of clotted cream samples and an increase in their a* and TBA values. The pH values of samples ranged from 4.68 to 6.67, and the range for a_w values was between 0.903 and 0.803. For TBA, the highest change was observed in the K2 sample with 0.479 mg malondialdehyde/kg. The color L* values of all samples were between 99.79 and 93.82, a* values between 1.82 and 1.22, and b* values between 6.22 and 4.74. Among the treatments, the treatment of O_2-Ar gas mixture (50-50%) resulted in the highest decrease in the total counts of aerobic mesophilic bacteria (TMAB), total aerobic psychrophilic bacteria, proteolytic bacteria, yeast-molds, coliform group bacteria, and *Staphylococcus aureus*. Compared to the control sample, at the end of 10 days of storage, a decrease of 2.41 for TMAB, 3.64 for total yeast-mold, 4.23 for coliform group bacteria, and 4.72 log cfu/g for *S. aureus* was achieved. Results indicated that the cold plasma treatment did not cause significant changes in the physicochemical values of clotted cream samples but reduced their microbial load.

Keywords: Cold plasma, Clotted cream, Argon, Pathogen, Quality

Soğuk Plazma Uygulamasının İnek Kaymağının Fizikokimyasal ve Mikrobiyolojik Özellikleri Üzerine Etkisi

ÖZ

Bu araştırmanın amacı, iki farklı gaz (O_2 ve Ar) ve bunların karışımının farklı sürelerde örnek yüzeyine uygulanmasıyla gerçekleştirilen soğuk plazma işleminin, kaymağın fizikokimyasal ve mikrobiyal parametrelerine etkilerinin 10 günlük depolama süresince değişiminin incelenmedir. Soğuk plazma uygulaması, kaymak örneklerinin pH, a_w, L*, b* değerlerini azaltırken, a* ve TBA değerlerini arttırmıştır. Örneklerin pH değerleri 6.67-4.68 arasında, a_w değerleri ise 0.903-0.803 arasında değişmiştir. TBA için en fazla değişim 0,479 mg malonaldehit/kg ile K2 örneğinde olmuştur. Tüm örneklerin L* değerleri 99.79-93.82, a* değerleri 1.82-1.22 ve b* değerleri 6.22-4.74 aralığında belirlenmiştir. Toplam aerobik mezofil bakteri sayısı (TMAB), toplam aerobik psikrofil bakteri sayısı, proteolitik bakteri sayısı, toplam maya-küf sayısı, koliform grubu bakteri sayısı, *Staphylococcus aureus* sayısı ve üzerinde uygulamalar arasında en fazla azalmaya gaz karışımı uygulaması neden olmuştur. Kontrol örneğine göre 10 günlük depolama sonunda, TMAB 2.41, toplam maya-küf 3.64, koliform grubu bakteri 4.23 ve *S.aureus* 4.72 log kob/g azalmıştır. Sonuçlar, soğuk

plazma uygulamasının, kaymağın fizikokimyasal özelliklerinde önemli bir değişime neden olmadığı ancak mikrobiyal yükü azalttığını göstermiştir.

Anahtar Kelimeler: Soğuk plazma, Kaymak, Argon, Patojen, Kalite

INTRODUCTION

Milk, a necessary food at all stages of human life, is an essential food product, especially for children, pregnant women, and older people, especially for balanced nutrition and improving and protecting bone health. Dairy products, as well as milk, play important roles in ensuring that people have a balanced diet and live a healthy life. Dairy products with high nutritional value contain primarily protein, fat, calcium, phosphorus, vitamin A, vitamin B12, and riboflavin [1]. Among all these components, milk fat is ranked after milk protein in importance. Milk fat, one of the leading quality criteria in the production of various dairy products, is used as a raw material in some products. The most important of these products are cream and butter [2].

As is known, due to the density difference between the phases of milk fat (0.93 g/cm^3) and serum (~ 1.036 g/cm^3), when kept for a while, the fat particles will accumulate on the surface and move upwards [3]. Over time, the layer accumulating on the surface becomes rich in fat and forms the "cream layer" structure, mainly consisting of milk fat. The cream is produced by passing this layer through different stages of the process. According to the Turkish Food Codex Cream and Clotted Cream Communiqué (2009/5), creams containing 60% milk fat are called clotted cream [4].

Clotted cream is a product with a slightly acidic taste and creamy consistency [5]. Today, clotted cream can be produced industrially or using traditional methods. The rich nutritional value and high moisture content of clotted cream are the most important factors limiting its shelf life [6]. In addition, the absence of fermentation in the process of clotted cream and its higher pH value compared to other fermented dairy products cause its shelf life to be shorter [7]. In addition, the microbiological quality of clotted cream is another factor that affects its shelf life [2].

The microbiological and sensory properties of clotted cream may vary depending on the quality of the milk used and the conditions at the production stage [2]. Most of the enterprises producing clotted cream in our country are local small family enterprises, and they become vulnerable to microbial contamination due to non-compliance with hygiene and sanitation rules, lack of standard production, and failure to create hygienic conditions during packaging and storage [8]. One of the main concerns in cream production is the presence of pathogenic bacteria, particularly *Staphylococcus aureus* and *Enterococcus* species. Studies [9] have shown that methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* [10] were isolated from clotted cream samples. The presence of these bacteria requires implementing effective microbial control measures during production. Processing techniques such as pasteurization to reduce the microbial load in the cream [11], the use of natural preservatives such as nisin, a bacteriocin produced by Lactococcus lactis [12], as well as the integration of good hygienic practices and good manufacturing practices during production are recommended to minimize contamination risks [13]. Practices such as biological preservation and advanced sanitation programmes are used to prevent microbial spoilage in dairy products [14]. In addition, non-thermal minimal processes such as ionizing radiation, cold plasma, and high hydrostatic have begun to be used as an alternative to traditional methods [15]. Non-thermal techniques have emerged as promising alternatives to traditional thermal processing methods to reduce microbial loads while preserving the sensory and nutritional qualities of food products. Cold plasma technology is a promising, innovative, non-thermal method for microbial inactivation. Cold plasma produces reactive species that can damage microbial cell membranes and inactivate pathogens [16,17]. This technique has been investigated for application in various food products, including fruits and vegetables, and its potential use in dairy products is being investigated. The ability of cold plasma to decontaminate surfaces without the need for heat makes it an attractive option for preserving clotted cream quality [16].

The cold plasma has become a useful and technological method that is important for the surface sterilization of food products [18]. Plasma is defined as ionized gas or the fourth state of matter [19], which consists of reagents that contains photons, electrons, positive-negative ions, free radicals, and neutral atoms, including sufficient amounts of electrical energy to multiply chemical reactions that break covalent bonds of different types [20, 21]. Cold plasma is produced between electrodes using radio frequency (RF), dielectric barrier discharge (DBD), and microwaves (MW) using various gases such as oxygen, argon, and helium [22]. Inactivation of microorganisms on the food surface can be achieved with cold plasma [23].

Previous studies have primarily focused on the application of non-thermal processes to milk. Notably, there is a lack of research on the use of cold plasma technology in clotted cream. This study investigates the microbiological and physicochemical quality changes in clotted cream by applying cold plasma with different gases to its surface during various storage periods.

MATERIALS and METHODS

Materials

Clotted cream samples used in the research were obtained from a producer in Afyonkarahisar (Türkiye)

province. Clotted cream samples were brought to the Afyon Kocatepe University, Faculty of Engineering, Department of Food Engineering microbiology laboratory under a cold chain and kept in the refrigerator at 4°C until the analysis was completed. The gases used in the research were purchased from a company operating in Afyonkarahisar province.

Methods

Cold Plasma Treatment

The cold plasma system used in the study was generated by modifying the method used by Aktop [24]. (Figure 1). To produce the required plasma, a power source with a power of 25 kV and a frequency of 42 kHz was used. The system used two different gases (argon and oxygen) and their mixtures. The gas flow was determined as 1 L/min, and the application was made for two different periods (20 and 40 min). In the study, 7 pieces of 1 mm tungsten steel electrodes were used, and one of the electrodes was placed horizontally across the other 6 electrodes to produce plasma between the anode and cathode ends. The distance between the ends was set to 13 mm. Before the treatment, the samples obtained from the market were mixed homogeneously for 5 min with the help of a sterile mixer (Arçelik K 9250) at the lowest speed to prevent the creams from undergoing any physical changes. In this way, a homogeneous distribution of the microbial load was ensured. Then, 100 g of the samples were weighed separately into sterile Petri dishes using a sterile spatula and a Bunsen burner flame on a precision scale. The control sample was not subjected to any cold plasma treatment and was prepared by taking it from the mixture with a sterile spatula, like the other samples. All samples were adjusted to 100 g. Samples were placed in sterile Petri dishes and subjected to cold plasma treatment. The samples were prepared with a diameter of 90 mm and a height of 5 mm. The distance between the point where the plasma was formed and the sample surface was adjusted to 60 mm. Although this distance could be adjusted, it was set as standard in all samples

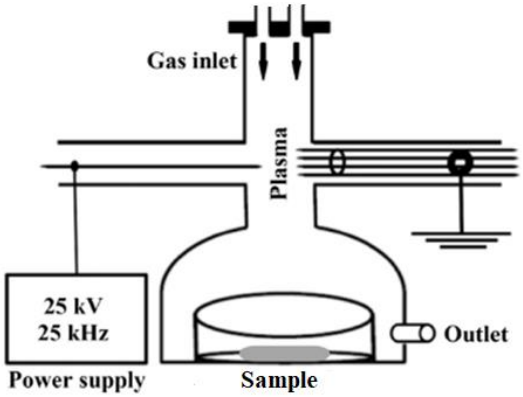

Figure 1. The cold plasma system [23]

Physicochemical Analyses

The pH values of the clotted cream samples were determined with the Cyberscan 300 model pH according to AOAC 981.12, and the a_w values were determined with Novasina LabTouch-a_w (Novasina AG, Lachen, Switzerland) according to AOAC 978.18 [25,26]. The color of the samples was measured using a colorimeter (Minolta Co., Osaka, Japan). CIE L*a*b* color space gives the lightness (L*, from 100 for white to 0 for black), redness (a*, from +120 for red to −120 for green) and yellowness (b *, from +120 for yellow to −120 for blue) values of the color [27].

2-Thiobarbituric acid (TBA) values of clotted cream samples were determined by spectrophotometric method [28]. For this purpose, absorbance values were determined with a standard spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Kyoto, Japan) against the blank sample at a wavelength of 538 nm, and TBA values were calculated by multiplying the absorbance values by 7.8.

Microbiological Analyses

In clotted cream samples, total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAPB), lipolytic bacteria, proteolytic bacteria, yeast/mold, total coliform group bacteria (TCGB), lactic acid bacteria (LAB), *Staphylococcus aureus* counts were determined using the spread plate technique [29].

10 g of the samples were taken under sterile conditions and transferred to sterile stomacher bags (Spa-174538, Lp Italiana, Milan, Italy), and serial dilutions up to 10^{-4} were prepared [30]. TAMB count analysis was performed using Plate Count Agar (PCA) (Merck, 1.05463, Germany). The cultivated petri dishes were incubated in an incubator (MM Incucell 55, Germany) for 48-72 hours at 30°C under aerobic conditions for TAMB counting [31, 32]. For TAPB analysis, Plate Count Agar (PCA Merck, 1.05463, Germany) medium was used, and the cultivated petri dishes were incubated at 4°C for 5-7 days. Colonies larger than 0.5 mm that grew on the medium were counted and recorded [33]. Tributyrin Agar (TBA, Merck 1.01957, Germany) medium was used to analyze lipolytic bacteria. Counts were taken after 2-3 days of incubation at 35-37°C [33]. Proteolytic bacteria analysis was performed using Plate Count Agar (PCA, Merck 1.05463, Germany) medium. After inoculation, the petri dishes were inverted and incubated at 35-37°C for 24-72 hours. After incubation, 1% HCl acid was poured into the petri dishes, waited for 1 minute, and after removing the excess acid from the petri dishes, colonies with light-colored zones around them were counted [34]. Potato Dextrose Agar (PDA Merck 1.10130, Germany) medium was used for yeast/mold count analysis, and the cultivated petri dishes were incubated in an incubator (MMM Incucell 55, Germany) for 5-7 days at 22°C under aerobic conditions [35]. Violet Red Bile Agar (VRB, Merck 1.01406, Germany) medium was used for total coliform group bacteria count, and the petri dishes were incubated in an incubator (MMM Incucell 55, Germany) for 24-48 hours at 30°C under aerobic conditions [36]. For the count of lactic acid bacteria, Man Rogasa and Sharpe Agar (MRS, Merck 1.10660, Germany) medium was used, petri dishes were placed in jars (Merck

1.16387, Germany) and incubated in an incubator (Daihan, IG50, Malaysia) under anaerobic conditions at 30°C for 24-48 hours [37]. The medium prepared using Baird-Parker Agar (BPA, Merck, 1.05406, USA) and Egg Yolk Tellurite Emulsion (Merck 1.03785, USA) was used to determine the count of *S. aureus*. The cultivated petri dishes were left to incubate for 24-48 hours in an incubator (Nüve, FN 500, Turkey) at 37°C. At the end of the period, bright black colonies with clean zones that developed on the media and had a thin white precipitation ring around the edges were marked and counted [38]. After marking the colonies, the petri dishes were subjected to a second incubation for 18 hours. At the end of the incubation, typical *Staphylococcus* colonies with white precipitation rings and bright black colonies that do not form zones were counted separately, a coagulase test was applied to 5 of each type of colony, and the count of *S. aureus* was determined by counting the colonies with positive results [39].

Statistical Analyses

A total of seven samples were studied, including six different cold plasma treatments and a control sample. Additionally, there were four different storage times within the scope of the study, and each sample was studied in two parallels. Accordingly, there were a total of fifty-six samples in the study. The analysis results obtained were statistically evaluated using the Duncan multiple comparison test using the IBM SPSS V. The 23.0 package program.

RESULTS and DISCUSSION

The changes in pH, a_w and TBA values of clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis of the effects of sample type and storage time are shown in Table 1. According to the results of the variation analysis, it was revealed that storage time and sample type and storage time x sample type interactions were very highly significant on pH and TBA values ($p<0.0001$). According to the results of the correlation analysis, storage time and sample type interactions showed negative, very highly correlational effects on the pH value. It was determined that all electrical field applications used in the study reduced the pH value ($p<0.05$). Among the samples, the lowest pH value was 6.11 in the application of using the mixture of 50% oxygen and argon gases for 40 minutes (K6), followed by values of 6.23 in the application of using 100% argon gas for 40 minutes (K4) and 6.30 in the application of using 100% oxygen gas for 40 minutes (K2) at the beginning of the storage period.

Additionally, the pH values of all samples decreased in parallel with the cold plasma application during storage ($p<0.05$). The samples whose pH values decreased the most during storage were K6, K4, and K2, respectively. Compared to the control sample, the pH value of the K6 sample decreased by 0.93 units more during storage.

Table 1. Changes in pH, a_w and TBA values of clotted cream samples during storage

Sample	pH				a_w			TBA (mg MDA/kg)			
	Storage Time (Day)										
	0.	4.	7.	10.	0.	4.	7.	10.	0.	4.	7.	10.
K0	6.67±0.056^{Aa}	6.57±0.028^{Aa}	6.35±0.070^{Ba}	6.17±0.098^{Ba}	0.903±0.005^{Aa}	0.887±0.002^{Ba}	0.871±0.001^{Ca}	0.854±0.005^{Da}	0.303±0.005^{Da}	0.401±0.004^{Cc}	0.588±0.004^{Ba}	0.630±0.001^{Ac}
K1	6.56±0.042^{Aa}	6.37±0.070^{Bb}	6.12±0.028^{Cb}	6.04±0.028^{Ca}	0.875±0.007^{Aa}	0.866±0.005^{ABb}	0.853±0.002^{BCb}	0.841±0.001^{Cab}	0.252±0.002^{Dd}	0.367±0.004^{Cd}	0.508±0.004^{Bb}	0.682±0.001^{Ab}
K2	6.30±0.070^{Aa}	5.90±0.113^{Bc}	5.37±0.028^{Ce}	5.11±0.014^{Dd}	0.841±0.002^{Aa}	0.834±0.005^{Acd}	0.831±0.005^{Ade}	0.827±0.007^{Ab}	0.264±0.002^{Dc}	0.476±0.001^{Ca}	0.592±0.004^{Ba}	0.743±0.002^{Aa}
K3	6.47±0.028^{Aa}	6.25±0.070^{Bb}	5.92±0.028^{Cc}	5.80±0.056^{Cb}	0.869±0.008^{Aa}	0.859±0.004^{ABb}	0.845±0.005^{Bbc}	0.839±0.009^{Bb}	0.251±0.002^{Dd}	0.299±0.004^{Cf}	0.378±0.004^{Be}	0.456±0.007^{Af}
K4	6.23±0.042^{Aa}	5.64±0.056^{Bd}	4.99±0.056^{Cf}	4.85±0.070^{Ce}	0.832±0.002^{Aa}	0.825±0.007^{Ade}	0.819±0.002^{ABef}	0.811±0.004^{Bc}	0.289±0.002^{Dc}	0.342±0.002^{Ce}	0.388±0.004^{Bd}	0.431±0.001^{Ag}
K5	6.42±0.028^{Aa}	6.05±0.056^{Bc}	5.72±0.014^{Cd}	5.29±0.098^{Dc}	0.852±0.002^{Aa}	0.842±0.002^{Bc}	0.837±0.002^{BCcd}	0.832±0.004^{Cb}	0.296±0.005^{Dab}	0.399±0.004^{Cc}	0.477±0.005^{Bc}	0.620±0.002^{Ad}
K6	6.11±0.056^{Aa}	5.58±0.042^{Bd}	4.81±0.014^{Cg}	4.68±0.042^{Df}	0.826±0.004^{Aa}	0.819±0.002^{ABe}	0.809±0.011^{ABf}	0.803±0.005^{Bc}	0.301±0.004^{Da}	0.455±0.004^{Cb}	0.597±0.001^{Ba}	0.608±0.004^{Ae}
Interactions			P value	r	Interactions		P value	r	Interactions		P value	r
Sample Type (S)			<0.0001	-0.534**	Sample Type (S)		0.024	-0.403**	Sample Type (S)		<0.0001	-0.069
Storage Time (T)			<0.0001	-0.622**	Storage Time (T)		0.400	-0.185	Storage Time (T)		<0.0001	0.845**
S X T			<0.0001	--	S X T		0.804	--	S X T		<0.0001	--

MDA: malondialdehyde, K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes. A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days.a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among the samples. Statistical significance: $p<0.0001$: very highly significant,, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed).

In the correlation analysis, the sample type had a negative and very highly correlational effect on the a_w value (r: -0.403) (Table 1).

The increase in the concentration of H^+ ions during cold plasma application was effective in reducing this decrease in pH values. Reactive species with mainly acidic properties, such as nitric acid (HNO_3) and nitrous acid (HNO_2) produced by the plasma, are responsible for the pH decrease [40].

It was determined that all electrical field applications used in the study had a reducing effect on the a_w value of the samples ($p<0.05$). The highest reducing effect on the a_w value was detected in the K6 (50% Ar – 50% O_2 / 40 min) sample, whereas the highest a_w value was detected in the K0 (control) sample.

The a_w values of all samples decreased during the subsequent ten-day storage ($p<0.05$). During 10 days of storage, the highest change was observed in the values of the K0 sample, in the range of 0.903-0.854, and the least change was observed in the values of the K2 sample, in the range of 0.841-0.827 (Table 1). The decrease in a_w decreased with increasing plasma exposure time ($p<0.05$). The decrease in a_w values can be attributed to the ability of O_2 and Ar gases used in the plasma process to retain free water molecules on the clotted cream surface [41].

According to the results of the correlation analysis, the storage time showed a positive and very highly correlational effect on the TBA value (Table 1). Cold plasma treatment had a decreasing effect on the TBA values of the samples ($p<0.05$). The greatest effect was revealed in the K3 sample (100% Ar / 20 min) with a value of 0.251 mg malondialdehyde/kg, and the least effect was in the K6 sample with a value of 0.301 mg malondialdehyde/kg. TBA values of all samples increased during storage ($p<0.05$). After ten days of storage, the highest increase rate was in the K2 sample with a difference of 479 mg malondialdehyde/kg, and the least increase rate was in the K4 sample with a difference of 142 mg malondialdehyde/kg. Of the two different gases used and the mixture of these gases, the most effective application was O_2 gas application. The resulting effect increased depending on the application time. A similar study [42] reported that in the cold plasma treatment applied to the surface of cheddar cheese, the TBA value, which was initially 0.132 mg malondialdehyde/kg, decreased to 0.141, 0.161 and 0.183 mg malondialdehyde/kg, respectively, after 2.5, 5 and 10 minutes of plasma application. In addition [43], it was observed that the TBA value of the 10-minute plasma application on sirloin increased with the duration of cold plasma.

The change of color values, one of the qualities that affects the admiration of foods during storage after cold plasma treatment, is shown in Figure 2. Storage time and sample type were very highly significant ($p<0.0001$) on L*, a*, and b* values. While the sample type had a negative effect on the L*, a*, and b* values, storage time had a negative, very highly correlational effect on the L* and b* values and a positive, very highly correlational effect on the a* value.

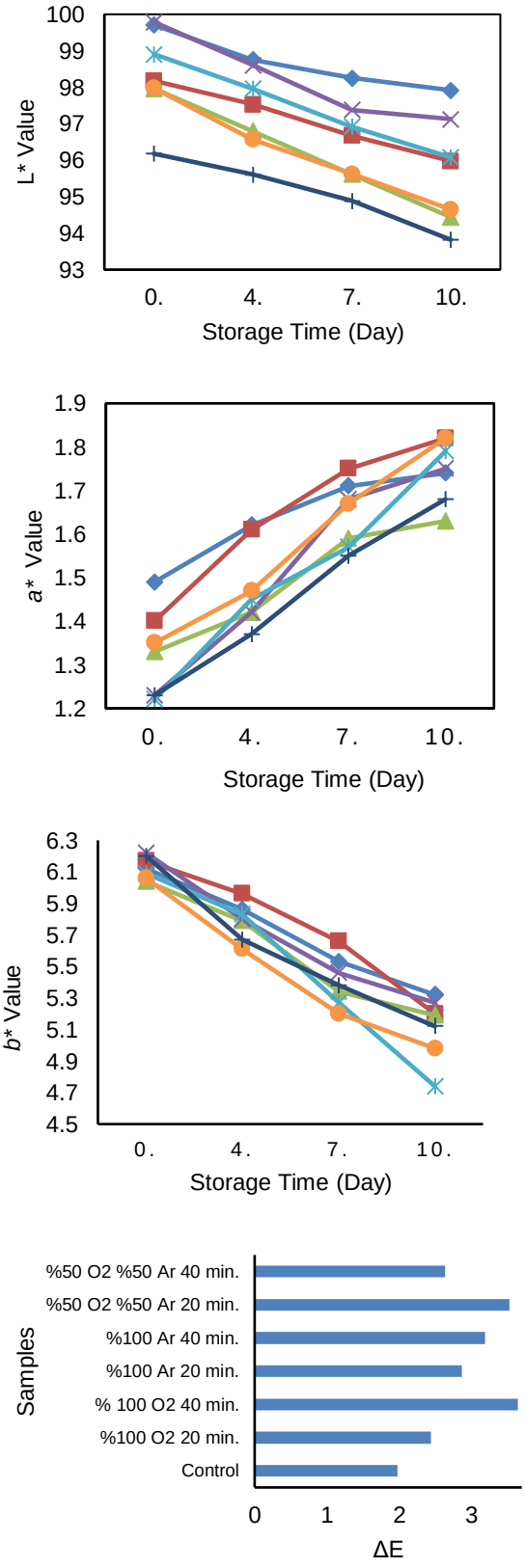

Figure 2. Changes in color (*L**, *a**, *b** and ΔE) values of samples during storage (K0: Control (◆), K1: 100% O_2 20 min (■), K2:100% O_2 40 min (×), K3: 100% Ar 20 min (▲) K4:100% Ar 40 min (✱), K5:50% O_2 and 50% Ar 20 min (●) and K6: 50% O_2 and 50% Ar 410 min (+))

L* value, an indicator of brightness in foods, decreased with cold plasma application ($p<0.05$). The highest L* value was measured as 99.71 in the control sample, and the lowest L* value was 96.18 in the K6 sample. Among the two different gases used during the application, the L* value decreased the most in the K6 sample. During 10 days of storage, the maximum change was observed between the range of 97.97-94.44 in the K2 sample, and the least change was observed between the range of 99.71-97.92 in the K0 sample. It is thought that drying on the surface is effective in decreasing L* values. Additionally, lipid oxidation can cause browning in foods, and brown oxypolymers obtained from milk proteins are responsible for the decrease in the L* value [42].

When the change in a* values was examined, it was seen that there was a decrease in a* value due to cold plasma application ($p<0.05$). During 10 days of storage, there was an increase in the a* value. Of the two different gases used and the mixture of these gases, the most effective one was determined to be Ar gas, and the resulting effect increased depending on the application time. Among the two different gases used during the application, the a* value increased the most in the K4 sample. During 10 days of storage, the highest change was observed in the K4 sample values in the range of 1.79-1.22, and the least change was observed in the K0 sample values in the range of 1.74-1.49.

While cold plasma application did not cause a significant change in the b* value ($p>0.05$), a decrease in the b* value was detected during storage ($p<0.05$). Among the two different gases used during the application, the highest effect on the b* value was detected on the K4 sample. After storage, the maximum change in b* values was determined in the K4 sample with a change of 1.35 units, and the minimum change was determined in the K0 sample with a change of 0.80 units.

When the total color change ΔE values of the samples (Figure 2) were examined, it was determined that cold plasma application was effective on the color of the samples. The highest color changes were observed in the samples K2 (100% O_2 40 min) and K5 (50% O_2 and 50% Ar 20 min). The color change was minimal in the control sample (K0).

The change in TAMB and TAPB counts of clotted cream samples after cold plasma application during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 2. It was determined that the storage time and sample type, and storage time x sample type interactions were very highly significant on TAMB and TAPB counts ($p<0.0001$). It was determined that storage time had a positive, very highly correlational effect on the counts of TAMB and TAPB, and the sample type interaction had a negative, highly correlation for TAMB and a positive, very highly correlation for TAPB (Table 2). Cold plasma treatment had a reducing effect on the initial TAMB counts of the samples ($p<0.05$). It was determined that the application using 100% Ar gas for 40 minutes (K4) was the one that reduced the TAMB count the most. In addition, although the TAMB counts of all samples increased during storage, electrical field applications had a slowing effect on this increase ($p<0.05$). The K6 sample was determined to have the lowest TAMB count among the samples on the last day of storage (5.14 log cfu/g) (Table 2). Ulbin-Figlewicz et al. [44] reported that the total count of microorganisms, which was initially 5.45 log cfu/cm^2, decreased to 3.13 log cfu/cm^2 by using argon gas in a 10-minute plasma application.

Table 2. Change in TAMB and TAPB counts of clotted cream samples during storage (log cfu/g)

Sample	TAMB				TAPB			
	Storage Time(Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	3.27 ± 0.098^{Dab}	5.01 ± 0.212^{Ca}	6.97 ± 0.084^{Ba}	7.55 ± 0.353^{Aa}	3.10 ± 0.056^{Dab}	4.89 ± 0.014^{Ca}	6.11 ± 0.042^{Ba}	7.35 ± 0.056^{Aa}
K1	3.34 ± 0.028^{Da}	4.88 ± 0.056^{Cab}	5.32 ± 0.028^{Bc}	6.11 ± 0.014^{Ac}	3.20 ± 0.084^{Da}	4.67 ± 0.028^{Cb}	5.92 ± 0.042^{Bb}	6.45 ± 0.042^{Ab}
K2	3.29 ± 0.063^{Da}	4.81 ± 0.028^{Cabc}	5.27 ± 0.07^{Bcd}	6.02 ± 0.282^{Ac}	3.01 ± 0.028^{Dbc}	4.44 ± 0.056^{Cc}	5.79 ± 0.014^{Bc}	6.09 ± 0.084^{Ac}
K3	3.25 ± 0.042^{Dab}	4.67 ± 0.028^{Cabc}	6.01 ± 0.028^{Bb}	6.82 ± 0.014^{Ab}	2.80 ± 0.042^{Dd}	3.96 ± 0.028^{Cd}	5.38 ± 0.042^{Bd}	5.75 ± 0.028^{Ad}
K4	3.14 ± 0.042^{Cb}	4.40 ± 0.565^{Bbcd}	4.83 ± 0.042^{Be}	5.67 ± 0.028^{Ad}	2.97 ± 0.014^{Dc}	3.81 ± 0.049^{Ce}	5.24 ± 0.028^{Be}	5.57 ± 0.014^{Ae}
K5	3.20 ± 0.056^{Dab}	4.29 ± 0.042^{Ccd}	5.19 ± 0.028^{Bd}	5.97 ± 0.07^{Acd}	2.81 ± 0.014^{Dd}	4.11 ± 0.028^{Cd}	4.67 ± 0.042^{Bf}	5.04 ± 0.028^{Af}
K6	3.27 ± 0.028^{Dab}	4.11 ± 0.014^{Cd}	4.84 ± 0.028^{Be}	5.14 ± 0.042^{Ae}	2.79 ± 0.028^{Cd}	4.10 ± 0.141^{Bd}	4.29 ± 0.084^{Bg}	4.73 ± 0.028^{Ag}

Interactions	P value	r	Interactions	P value	r
Sample Type (S)	<0.0001	-0.299*	Sample Type (S)	<0.0001	0.368**
Storage Time (T)	<0.0001	0.883**	Storage Time (T)	<0.0001	0.881**
S X T	<0.0001	--	S X T	<0.0001	--

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days. a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among samples. Statistical significance: $p<0.0001$: very highly significant;, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

Cold plasma also had a reducing effect on the TAPB count. The highest effect was detected in the K6 sample. After 10 days of storage, the highest TAPB count was determined as 7.35 log cfu/g in the K0 sample, and the lowest TAPB count was 4.73 log cfu/g in the K6 sample.

The changes in the lipolytic and proteolytic bacterial counts of the clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 3. According to variation analysis, sample type, storage time, and sample type x storage time were very highly significant on the counts of both lipolytic bacteria and proteolytic bacteria ($p<0.0001$). Although the type of sample showed a negative correlational effect, storage time showed a positive correlation effect (Table 3).

Table 3. Change in lipolytic and proteolytic bacteria counts of clotted cream samples during storage (log cfu/g)

Sample	Lipolytic Bacteria				Proteolytic Bacteria			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	3.07±0.098^{Da}	4.78±0.056^{Ca}	5.52±0.014^{Ba}	6.25±0.042^{Aa}	2.28±0.056^{Db}	3.80±0.028^{Ca}	5.22±0.028^{Ba}	6.52±0.028^{Aa}
K1	3.05±0.056^{Da}	4.21±0.042^{Cc}	4.77±0.028^{Bc}	5.33±0.028^{Ab}	2.45±0.042^{Da}	3.62±0.056^{Cb}	4.97±0.028^{Bb}	5.23±0.042^{Ab}
K2	3.02±0.028^{Da}	4.42±0.014^{Cb}	4.86±0.056^{Bb}	5.42±0.028^{Ab}	2.38±0.056^{Dab}	3.56±0.014^{Cb}	4.86±0.014^{Bc}	5.11±0.028^{Ab}
K3	3.11±0.014^{Da}	4.41±0.028^{Cb}	4.75±0.042^{Bc}	5.15±0.056^{Ac}	2.11±0.042^{Dc}	3.31±0.042^{Cc}	3.72±0.014^{Bd}	4.68±0.084^{Ac}
K4	3.15±0.042^{Da}	4.36±0.07^{Cb}	4.69±0.028^{Bc}	5.11±0.042^{Ac}	2.14±0.042^{Dc}	3.22±0.028^{Cc}	3.67±0.028^{Bd}	4.51±0.014^{Ad}
K5	3.14±0.042^{Da}	4.29±0.028^{Cc}	4.47±0.028^{Bd}	4.86±0.042^{Ad}	2.47±0.014^{Da}	3.12±0.056^{Cd}	3.48±0.084^{Be}	4.21±0.014^{Ae}
K6	3.09±0.042^{Da}	4.12±0.028^{Cc}	4.39±0.042^{Bd}	4.62±0.056^{Ae}	2.29±0.028^{Db}	3.08±0.028^{Cd}	3.28±0.056^{Bf}	3.96±0.014^{Af}
Interactions		P value	r	Interactions		P value	r	
Sample Type (S)		<0.0001	-0.252	Sample Type (S)		<0.0001	-0.382**	
Storage Time (T)		<0.0001	0.896*	Storage Time (T)		<0.0001	0.860**	
S X T		<0.0001	--	S X T		<0.0001	--	

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly (p<0.05) among storage days. a - f (↓):Values with different lowercase letters in the same column differ significantly (p<0.05) among samples. Statistical significance: p<0.0001: very highly significant;, p<0.01: highly significant, p<0.05: significant, p>0.05: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

The application of cold plasma did not cause a significant change in the count of lipolytic bacteria in the clotted cream samples (p>0.05) at the first day of storage. However, it had a decreasing effect on the rate of increase in the count of lipolytic bacteria during storage. In particular, the highest effect was determined in the K6 sample. At the end of the 10-day storage period, the highest count of lipolytic bacteria was detected in control sample (6.25 log cfu/g) and the lowest in K6 sample (4.62 log cfu/g).

Cold plasma treatment on proteolytic bacteria had a reducing effect on the count of bacteria. It had a slowing effect on the increase in the count of proteolytic bacteria during storage. At the end of storage, the highest change in the count of proteolytic bacteria was detected in the K0 sample with 4.24 log cfu/g, and the least change was detected in the K6 sample with 1.67 log cfu/g.

The changes in the TYM (Total Yeast/Mold) and LAB (Lactic Acid Bacteria) counts of the clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 4.

Table 4. Change in yeast-mold and lactic acid bacteria counts of clotted cream samples during storage (log cfu/g)

Sample	Yeast-Mold				Lactic Acid Bacteria			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	2.93±0.042^{Da}	4.23±0.014^{Ca}	5.67±0.042^{Ba}	7.78±0.113^{Aa}	2.13±0.042^{Dcd}	4.60±0.056^{Ca}	5.90±0.070^{Ba}	6.57±0.042^{Aa}
K1	2.42±0.028^{Dd}	3.55±0.07^{Cd}	4.23±0.042^{Bcd}	5.05±0.07^{Ac}	2.21±0.028^{Dbc}	4.22±0.028^{Cb}	5.22±0.028^{Bb}	5.80±0.042^{Ab}
K2	2.64±0.056^{Dc}	3.97±0.028^{Cb}	4.66±0.056^{Bb}	5.41±0.014^{Ab}	2.06±0.028^{Dd}	4.11±0.028^{Cc}	5.01±0.028^{Bc}	5.25±0.070^{Ad}
K3	2.86±0.042^{Dab}	3.65±0.056^{Ccd}	4.11±0.028^{Bd}	4.75±0.028^{Ad}	2.54±0.028^{Da}	3.41±0.028^{Ce}	4.97±0.028^{Bc}	5.11±0.042^{Ae}
K4	2.83±0.042^{Dab}	3.72±0.028^{Cc}	4.29±0.127^{Bc}	5.07±0.028^{Ac}	2.46±0.070^{Da}	3.32±0.014^{Cf}	4.72±0.042^{Bd}	5.17±0.028^{Ade}
K5	2.75±0.07^{Db}	3.40±0.056^{Ce}	3.77±0.028^{Be}	4.61±0.014^{Ae}	2.27±0.070^{Db}	4.01±0.028^{Cd}	4.22±0.028^{Be}	4.51±0.028^{Af}
K6	2.61±0.021^{Dc}	3.29±0.056^{Ce}	3.53±0.042^{Bf}	4.14±0.028^{Af}	2.18±0.028^{Dbc}	3.96±0.014^{Cd}	4.09±0.014^{Bf}	5.43±0.028^{Ac}
Interactions		P value	r	Interactions		P value	r	
Sample Type (S)		<0.0001	-0.367**	Sample Type (S)		<0.0001	-0.226	
Storage Time (T)		<0.0001	0.812**	Storage Time (T)		<0.0001	0.903**	
S X T		<0.0001	--	S X T		<0.0001	--	

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly (p<0.05) among sample days. a - f (↓):Values with different lowercase letters in the same column differ significantly (p<0.05) among samples. Statistical significance: p<0.0001: very highly significant;, p<0.01: highly significant, p<0.05: significant, p>0.05: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

It was revealed that sample type, storage time, and storage time x sample type interactions were very highly significant on both TYM and LAB counts (p<0.0001). In addition, while the sample type showed a negative, correlational effect on the counts of TYM and LAB, storage time showed a positive, very highly correlational effect (Table 4). Cold plasma treatment reduced the count of TYM. After 10 days of storage, lower TYM counts were detected in the cold plasma applied samples compared to the control group. The highest TYM was detected in the K0 sample with 7.78 log cfu/g, and the lowest TYM was detected in the K6 sample with 4.14 log cfu/g. Ulbin-Figlewicz et al. [44] first reported that the total count of yeast and mold on the meat surface, which was initially 4.43 log cfu/cm^2, decreased to 3.28, 2.31 and 3.37 log cfu/cm2, respectively, depending on the type of argon, helium and nitrogen gases in a 10-minute plasma application. In their study [45], on cold plasma application in mold species, inoculated on kashar cheese, found that there was a 3-4 log reduction in all mold species. In the present study, cold plasma treatment had an increasing effect on the LAB count, except for the K2 sample (p<0.05) at the first day of storage. The count of LAB increased in all samples during storage, but the increase rate was lower in the samples treated with cold plasma. The lowest effect on the count of LAB was detected on the K5 sample. During 10 days of storage, the most change was observed in the range of 6.57-2.13 log cfu/g in the

K0 sample, while the least change was observed in the range of 4.51-2.27 log cfu/g in K5 sample.

The changes in the counts of coliform group bacteria and *S. aureus* during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 5. According to the variation analysis, after the cold plasma treatment applied to the surface of the clotted cream, sample type, storage time and storage time x sample type interactions were very highly significant on both the counts of coliform group bacteria and *S. aureus* ($p<0.0001$). Sample type had a negative, very highly correlational effect ($p<0.01$) and storage time had a positive, very highly correlational effect on both the coliform group bacteria and *S. aureus* counts (Tablo 5). Cold plasma treatment caused a decrease in the counts of coliform group bacteria ($p<0.05$). At the end of storage, the highest count of coliform group bacteria was detected in the K0 (5.45 log cfu/g) sample and the lowest count of coliform group bacteria was detected in the K6 (1.22 log cfu/g) sample. Cold plasma treatment applied to clotted cream samples was effective on *S. aureus* ($p<0.05$). The most effective was the 40-minute application (K6) in which 50% O_2 and 50% Ar gas mixtures were used. At the end of storage, the highest count of *S. aureus* was detected in sample K0 as 5.54 log cfu/g, and the lowest count of *S. aureus* was detected in sample K6 as 0.82 log cfu/g. In a similar study [46], reported that in a 10-minute plasma treatment using helium and argon gases, the counts of *S. aureus* inoculated into the agar medium decreased to a highest of 2.02 and 0.96 log cfu/g, respectively.

Table 5. Change in total coliform group bacteria and *S.aureus* counts of clotted cream during storage (log cfu/g)

Sample	Total Coliform Group Bacteria				*S. aureus*			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	1.79 ± 0.028^{Da}	3.59 ± 0.028^{Ca}	4.18 ± 0.028^{Ba}	5.45 ± 0.028^{Aa}	1.54 ± 0.014^{Da}	2.81 ± 0.056^{Ca}	4.11 ± 0.028^{Ba}	5.54 ± 0.063^{Aa}
K1	1.53 ± 0.056^{Cb}	1.88 ± 0.07^{Bb}	2.02 ± 0.014^{Bb}	2.24 ± 0.084^{Ad}	0.46 ± 0.028^{Dd}	0.64 ± 0.042^{Cc}	0.98 ± 0.014^{Bd}	1.12 ± 0.028^{Ac}
K2	1.49 ± 0.042^{Bb}	1.28 ± 0.042^{Cc}	1.57 ± 0.07^{Bc}	1.82 ± 0.014^{Ae}	0.27 ± 0.014^{De}	0.58 ± 0.056^{Cc}	0.76 ± 0.014^{Be}	0.92 ± 0.070^{Ad}
K3	1.57 ± 0.084^{Bb}	1.75 ± 0.028^{ABb}	2.30 ± 0.42^{Ab}	2.36 ± 0.042^{Ac}	0.68 ± 0.028^{Db}	1.02 ± 0.042^{Cb}	1.54 ± 0.028^{Bc}	1.97 ± 0.028^{Ab}
K4	1.50 ± 0.141^{Db}	2.00 ± 0.07^{Cb}	2.42 ± 0.07^{Bb}	2.72 ± 0.014^{Ab}	0.55 ± 0.07^{Dc}	1.10 ± 0.028^{Cb}	1.77 ± 0.028^{Bb}	2.04 ± 0.042^{Ab}
K5	0.84 ± 0.056^{Bc}	1.2 ± 0.282^{ABc}	1.37 ± 0.098^{Acd}	1.62 ± 0.042^{Af}	0.31 ± 0.028^{Da}	0.47 ± 0.028^{Cd}	0.77 ± 0.028^{Be}	0.91 ± 0.014^{Ad}
K6	0.44 ± 0.028^{Dd}	0.67 ± 0.042^{Cd}	1.09 ± 0.014^{Bd}	1.22 ± 0.056^{Ag}	0.23 ± 0.042^{Da}	0.39 ± 0.028^{Cd}	0.69 ± 0.028^{Bf}	0.82 ± 0.028^{Ad}

Interactions	P value	r	Interactions	P value	r
Sample Type (S)	<0.0001	-0.647**	Sample Type (S)	<0.0001	-0.520**
Storage Time (T)	<0.0001	0.422**	Storage Time (T)	<0.0001	0.429**
S X T	<0.0001	--	S X T	<0.0001	--

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days. a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among samples. Statistical significance: $p<0.0001$: very highly significant;, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

In cold plasma treatment, the type of gas used and the exposure time are effective in microbial inactivation [47]. Reactive oxygen (ROS) and reactive nitrogen (RNS) species formed during treatment play roles in inactivation [48]. ROS produced during plasma production causes strong oxidative stress. Cells are damaged by enzyme inactivation, lipid peroxidation, and DNA fragmentation. RNS is toxic and can cause cell death by damaging DNA. In general, when the results of microbiological analysis are evaluated, the cold plasma technique, in which a 50% O_2 + 50% Ar gas mixture is applied for 40 min, is sufficient for microbial inactivation.

CONCLUSION

In this research, the effects of the cold plasma technique on the clotted cream surface by applying two different gases (O_2 and Ar) and mixtures of these gases for different periods and their effects on the physicochemical and microbial parameters of the clotted cream were examined during the storage period (10 days).

In order to increase the shelf life of the clotted cream and improve its quality properties, the cold plasma technique was used with different gas and time applications. It has been observed that cold plasma application significantly reduces the microbial activity of clotted cream. In particular, cold plasma treatment using a 50% O_2 – 50% Ar gas mixture for 40 minutes (K6) was the most effective application. In line with this result, it can be recommended to use the cold plasma technique in the food industry for foods with high microbial activity. When applying the cold plasma technique to foods with high amounts of fat, preliminary studies should be conducted to determine the gas and time that will not accelerate lipid oxidation. It can be used by choosing the appropriate gases and times determined as a result of these preliminary studies. For this reason, studies can be conducted with different gas compositions and durations to determine the process parameters that will cause a minimum change in physicochemical properties. Furthermore, it has been observed as a very suitable technique for foods with high microbial activity or foods whose microbial activity increases rapidly.

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