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Scientific Research

Studying the effect of cold plasma process on physical, chemical and antioxidant properties of sheep milk

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ABSTRACT

This research investigated the impact of cold plasma treatment on the characteristics of raw sheep's milk. In this study, the effect of cold plasma was considered at three voltage levels (0.5, 0.7, and 0.9 kV) and three treatment times (3, 5, and 7 minutes). The physicochemical properties of raw sheep's milk, including pH, antioxidant activity (DPPH radical scavenging activity), color indices (L, a, b), microbiological tests (total bacterial count), protein, and fat, were examined. Analysis of variance (ANOVA) showed that both voltage and treatment time significantly affected pH, with an average increase to 6.82 as voltage and time increased. Antioxidant activity was also influenced, increasing to 21.03% with increasing voltage and time. Color indices changed significantly. The lightness index (L) reached 74.58 due to voltage and treatment time, while the redness index (a) and yellowness index (b) changed to 2.15 and 10.94, respectively. Additionally, in microbiological tests, the total bacterial count decreased significantly, indicating a significant improvement in milk safety. Finally, the examination of protein and fat levels showed that with increasing voltage and treatment time, protein levels reached 3.86 g/100 mL and fat levels reached 4.53 g/100 mL. These results indicate that the use of cold plasma can be considered as an effective method for improving the quality and increasing the nutritional value of milk in the dairy industry.

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1.Introduction

Milk, as one of the most important livestock products, plays a vital role in human nutrition due to its high nutritional value and diverse biological properties. In this regard, raw sheep milk, owing to its higher dry matter content, possesses a greater energy value compared to cow milk [1]. However, the major challenge in utilizing raw milk lies in reducing its microbial load without compromising its beneficial components. Thermal processing methods, traditionally employed for this purpose, are widely used. Consequently, non-thermal processing techniques have recently garnered significant attention as innovative alternatives for milk treatment [2].

Cold plasma is an emerging technology in the food sector that, by minimizing the need for heat, can eliminate microorganisms without compromising the beneficial components of food. The strong electric currents, various ions, free radicals, and electrons generated by this technology induce significant changes in the chemical and physical properties of food products. In cold plasma treatment, input voltage and processing time are two critical factors that can influence the effectiveness of the treatment and the preservation of food quality [3]. In recent years, numerous studies have been conducted to evaluate the effects of cold plasma treatment on milk and dairy products, with findings underscoring the necessity for further investigation in this field. In this regard, Ranjbar Amani (2022) examined the effects of cold plasma on bottled milk and bacterial lethality, reporting that Bacillus stearothermophilus exhibited the highest resistance to cold plasma among the studied bacteria. It was concluded that plasma treatment within the depth of the bottle could potentially overcome the limitation of cold plasma as a surface-only treatment. Further evidence was provided by Sharma et al. (2023) [4], who studied the effects of cold plasma on the acid gelation properties of skim milk. Their findings revealed that cold plasma induced significant changes in the rheological characteristics of skim milk. A noticeable reduction in free sulfhydryl content and an increase in viscosity clearly demonstrated the potential of cold plasma to improve the quality of the final product. This study also highlighted the importance of carefully examining key parameters such as treatment duration and the type of feed gas used. Additionally, the study by Nikmehram et al. (2023), which focused on the degradation of aflatoxin M1 in milk using cold plasma, demonstrated the technology's ability to reduce hazardous toxin concentrations in milk without negatively affecting its quality. Nonetheless, this study also emphasized the need for further research regarding the impacts of cold plasma on the physicochemical properties of milk [5].

Based on these studies and similar findings, significant scientific gaps remain regarding the effects of cold plasma on raw sheep milk and the related parameters of voltage and treatment duration. Therefore, the present study aims to address these gaps by closely examining these factors and introducing novel innovations to optimize the use of cold plasma for preserving the quality and safety of raw sheep milk.

2.Materials and Methods

Materials

Sheep milk was purchased from the local market in Tabriz. Methanol, ammonia, Folin reagent, and sodium carbonate were obtained from Sigma-Aldrich.

2-1. Cold Plasma Treatment of Raw Milk

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Cold plasma treatment was performed using a plasma device (Plasma Etch Inc., PE-50 Venus) equipped with an aluminum chamber and a horizontal electrode. Treatments were conducted at room temperature (21–25 °C) using nitrogen gas, with applied voltages of 0.5, 0.7, and 0.9 kV and exposure times of 3, 5, and 7 minutes, respectively [6].

2-2. Physical and Chemical Analysis of Milk

2-2-1. pH Measurement

The pH of the samples was measured using a calibrated pH meter, with buffer solutions of pH 4 and 7 [9].

2-2-2. Fat Content

Fat content was determined using the Gerber method [7].

2-2-3. Protein Content

Protein content in the samples was determined using the standard Kjeldahl method. Ultimately, titration was performed to quantify the ammonia content, and total protein was calculated using a nitrogen-to-protein conversion factor of 6.25 [8].
Protein (%) = Total nitrogen (%) × 6.25

2-2-4. Total Phenolic Content

Total phenolic content was measured using the Folin–Ciocalteu method. This colorimetric assay is based on a redox reaction between phenolic compounds and the Folin–Ciocalteu reagent. Standard solutions of gallic acid at concentrations of 10, 20, 50, and 80 ppm were prepared in 60% methanol. The prepared samples were reacted with the Folin reagent and 10% sodium carbonate solution. Absorbance was then measured at 765 nm using a spectrophotometer. Total phenolic content was calculated using the gallic acid standard calibration curve, based on the equation **y = 0.0087x + 0.064**, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of sample [9].

2-2-5. Antioxidant Activity

The antioxidant activity of the samples was evaluated using the DPPH radical scavenging assay. The percentage of DPPH inhibition was calculated using the following formula, and results were expressed as % discoloration, enabling comparison among samples [10]:

DPPH inhibition (%) = [(Ac – As) / Ac] × 100

Where:
Ac = Absorbance of the control (DPPH solution without antioxidant)
As = Absorbance of the sample containing antioxidant

2-3. Microbiological Tests

Microbiological analyses were conducted to assess the microbial quality of the samples. For total bacterial count, Plate Count Agar (PCA, Merck, Germany) was used. After appropriate dilution, samples were inoculated into the medium and incubated under specific temperature and time conditions. Colony-forming units were counted and results were expressed as CFU per gram of sample (CFU/g) [11].

2-4. Color Indices

Color indices of the samples were evaluated using image processing techniques. Photographs were taken under standardized lighting conditions. The digital images were analyzed

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using **ImageJ** software, which enables precise extraction of color parameters in both **RGB** and *CIELAB (Lab)** color spaces. The evaluated indices included:

- **L*** (lightness)
- **a*** (red-green axis)
- **b*** (yellow-blue axis)

Additional color parameters such as **chroma** (color intensity) and **hue angle** (color tone) were also calculated [12].

2-5. Data Analysis

Experimental design was based on a Central Composite Design (CCD), taking into account two factors: voltage intensity and exposure time. A total of 9 experimental runs were generated using Response Surface Methodology (RSM), as shown in Table 1. Regression coefficients for the effects of the factors on the desired responses, along with model fitting indices, were analyzed accordingly.

Tabel 1. Design of research experiments

Run	Factor 1 Voltage (kv) (A)	Factor 2 B:Exposure time (minutes) (B)
1	0.5	3
2	0.5	5
3	0.5	7
4	0.7	3
5	0.7	5
6	0.7	7
7	0.9	3
8	0.9	5
9	0.9	7

3.Discussion and results

3-1. Changes in Milk pH

The results indicate that both voltage and treatment duration significantly affect the pH of sheep milk, as evidenced by p-values less than 0.05 in the ANOVA table (Table 2). Since pH is a fundamental characteristic of milk and dairy product quality, variations in pH can lead to significant improvements or deteriorations in product quality.

The findings show a statistically significant relationship between increasing voltage and pH, meaning that as voltage increases, the pH of

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sheep milk also increases. In contrast, the effect of treatment time is also positive but less pronounced compared to voltage. The proposed statistical model was evaluated using appropriate statistical criteria:

Equation (2): pH = 5.89 – 0.48A + 0.19B

Previous studies have also demonstrated the influence of voltage and cold plasma exposure time on milk pH. For instance, Dash and Jaganmohan (2022) reported similar effects, such as an increase in pH with rising voltage during milk processing. Their study also emphasized that pH changes are influenced non-linearly by multiple factors [13]. Additionally, Abbas Said et al. (2021) reported the effect of exposure time on pH, showing that prolonged plasma treatment leads to pH alterations [14].

The current study confirms the significant effects of both voltage and exposure time on the pH of sheep milk. The increase in pH is attributed to the enhanced electrochemical reactions and generation of more alkaline compounds as voltage increases. Moreover, a longer treatment duration facilitates more extensive reactions. These findings are consistent with prior research.

The statistical model developed in this study can serve as a reliable tool for predicting pH changes during milk processing and could be applied in the development of novel techniques within the dairy industry. These results may contribute to optimizing production processes and maintaining the quality of dairy products.

Tabel 2. Regression coefficients of physicochemical properties of milk

Source	pH	Fat	Protoein	Total Polyphenol Content	DPPH	Total bacterial count
Model	**1.57	**2.05	**2.36	**10829.79	**170.50	**7.54
A	**1.36	**1.82	**2.16	**9095.61	**151.20	**7.00
B	**0.21	**0.24	**0.20	**1418.34	**19.30	**0.54
Std. Dev.	0.076	0.086	0.073	1.47	0.61	0.17
Mean	5.89	4.95	3.86	403.63	21.03	3.21
C.V. %	1.29	1.74	1.88	0.36	2.89	5.15
R^2	0.9786	0.9787	0.9868	0.9994	0.9871	0.9788
Adjusted R^2	0.9714	0.9716	0.9824	0.9984	0.9828	0.9717
Predicted R^2	0.9509	0.9534	0.9702	0.9952	0.9734	0.9516
Model accuracy	30.337	30.020	37.418	90.322	38.771	28.966
Residual	0.034	0.045	0.032	6.51	2.22	0.16
Cor Total	1.61	2.09	2.39	10836.30	172.72	7.70

**p≤0.01, A-DBD-type plasma (Currnet), B-Exposure time

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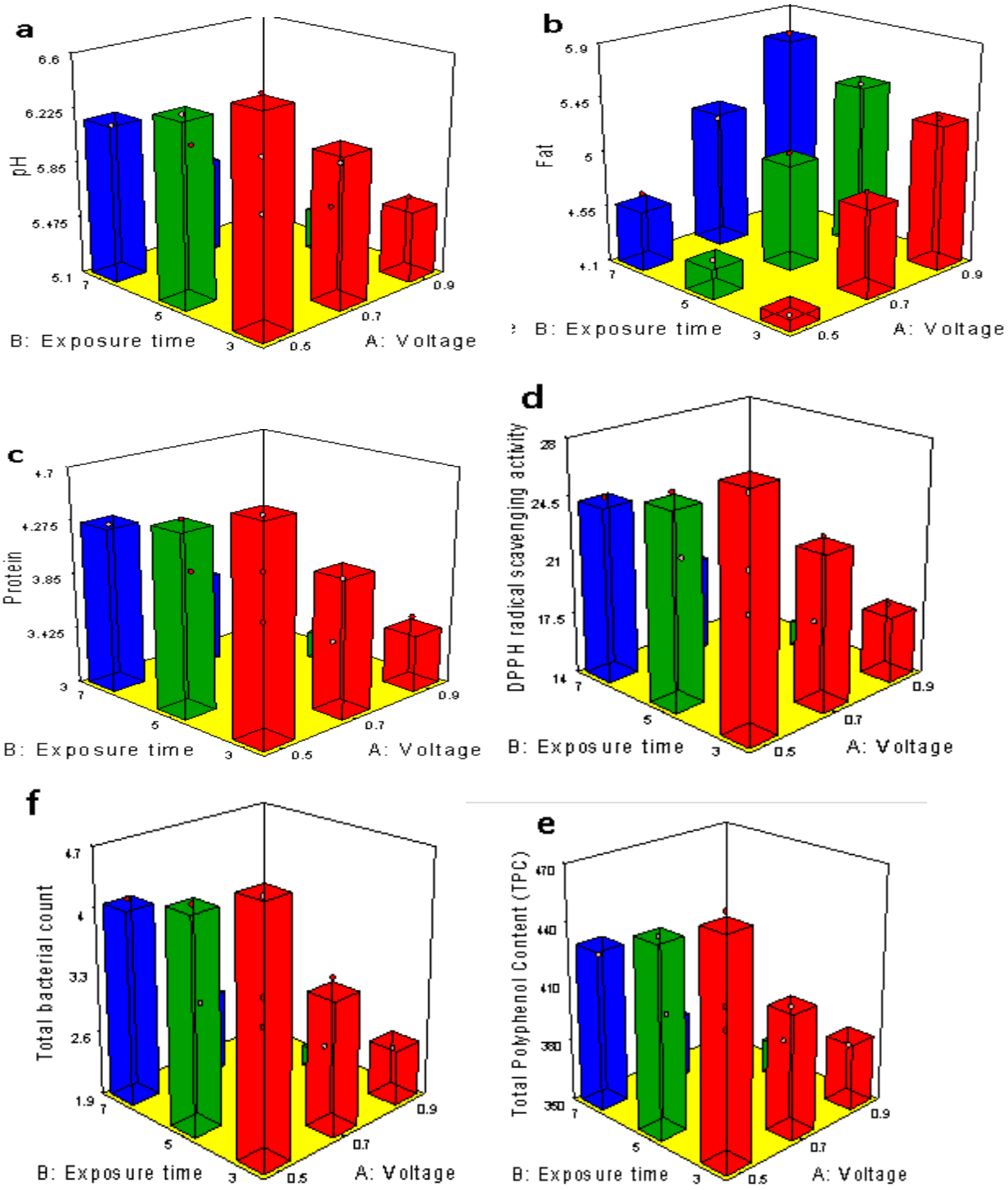

Fig 1. Physical and chemical changes of cold plasma treated milk: a: pH, b: fat, c: protein, d: DPPH, e: Total Polyphenol Content, f: Total bacterial count

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3.2. Fat Content Changes

The fat content of sheep milk treated with cold plasma was significantly influenced by two factors: voltage and treatment time, as shown in Table 2 and Figure 1-b. ANOVA results indicated that both voltage and time had a statistically significant effect on fat variation ($p < 0.05$). The model's coefficient of determination (R^2) was 0.988, and the adjusted R^2 was 0.975, demonstrating a strong agreement between the experimental data and the fitted model (Equation 3). Furthermore, the predicted R^2 was 0.938, and the model's adequate precision value was 25.01, confirming the model's high capability in predicting fat content variations in sheep milk.

This can be attributed to the significant impact of voltage and time on fat content changes, as voltage enhances the intensity of oxidative reactions, and time contributes to the continuity of these reactions, leading to alterations in milk structure and increased fat content [15, 16].

Equation (3): $\text{Fat} = 4.95 - 0.571A + 0.055B$

The observed increase in milk fat content under cold plasma treatment can be attributed to the influence of high voltage on the structure of fat globules and the disruption of their membranes, which leads to greater fat release into the milk matrix. Additionally, cold plasma induces mild chemical reactions, such as surface oxidation, which can positively affect fat composition and quality. These processes, along with improved milk homogenization and increased fat extractability, enhance the measured fat content and improve the sensory and nutritional quality of milk [15, 16].

Similar studies have shown that the use of various technologies, including cold plasma, can positively influence the quality of milk fat. In particular, increased voltage in electrical processes can improve fat quality and enhance the sensory attributes of milk [17]. Other studies have also confirmed that both factors—voltage and time—significantly affect fat composition in milk [15, 16].

3.3. Protein Content Changes

Protein content variations in sheep milk subjected to cold plasma treatment were significantly affected by voltage (DBD-type plasma current) and exposure time, as illustrated in Figure 1-c. The ANOVA results (Table 2) indicate that both voltage and treatment duration have a significant impact on milk protein content.

The obtained results show that voltage has an inverse effect on protein levels. Specifically, the voltage coefficient (A) in the derived model equation is -0.59, indicating that increasing voltage leads to a decrease in protein content. This reduction may be associated with protein structure degradation due to the intensity of cold plasma treatment [18]. Likewise, the exposure time also negatively affects protein content, as reflected by a coefficient of 0.201. This suggests that prolonged exposure can result in a further decrease in milk protein levels. The proposed model has been evaluated based on relevant statistical indicators (Equation 4).

Similar studies have confirmed the impact of voltage and time on protein structure and composition in milk [16, 19], concluding that increased voltage in cold plasma techniques may lead to protein reduction in dairy products due to the breakdown of protein molecular structures. In other words, cold plasma may negatively affect milk protein content, which generally occurs for several reasons. One primary reason is structural modification of proteins: cold plasma can break chemical bonds such as hydrogen and disulfide bonds, resulting in protein denaturation.

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Moreover, reactive oxygen species (ROS) generated by cold plasma can oxidize amino acids and degrade polypeptide chains within proteins. Additionally, reduced protein solubility caused by these processes may contribute to lower measurable protein content in milk solutions.

Collectively, these factors indicate that increasing the duration or intensity of cold plasma exposure may lead to further reductions in milk protein content [16, 19]. However, some studies have also suggested that longer exposure times in certain dairy processing techniques may help preserve protein composition [18]. These findings are consistent with the results of the present study. **Equation (4):** Protein = 3.86 − 0.59A − 0.201B

3.4.Changes in Total Polyphenol Content (TPC)

The results obtained from the changes in total polyphenol content in sheep milk treated with cold plasma (Figure 1-d) show that both voltage and treatment time significantly affect the total polyphenol content. ANOVA analysis (Table 2) indicates that both factors, voltage and time, have a significant impact on the polyphenol content. According to the derived equation (Equation 5), the effect of voltage with a coefficient of -6.56 indicates a decrease in the total polyphenol content. This may be due to the effect of cold plasma on the degradation of polyphenolic compounds in the milk. In contrast, the treatment time (B) with a coefficient of 15.49 contributes to an increase in the total polyphenol content. This increase may be due to the release of more polyphenolic compounds over time under the influence of cold plasma, which leads to a higher total polyphenol content in the milk [20]. The statistical model used has been evaluated with acceptable indicators (Equation 5). The R^2 value of 0.98 and the adjusted R^2 value of 0.97 show that the model fits well with the experimental data. Additionally, the predicted R^2 value of 0.94 and the model accuracy of 25.73 indicate the high predictive power of the model for changes in total polyphenol content under the influence of cold plasma. Previous studies also confirm that the use of high voltage in cold plasma techniques can reduce the total polyphenol content [20]. Previous research has shown that high voltage may lead to the breakdown and degradation of polyphenolic compounds. The intensity of cold plasma voltage can significantly impact the reduction of polyphenolic compounds in the milk. Polyphenolic compounds, due to their specific chemical structure, are sensitive to oxidation and degradation in the presence of reactive oxygen species (ROS). Cold plasma, by generating these reactive oxygen species at high voltages, can accelerate oxidative reactions, leading to the degradation of the polyphenolic compounds' structure. Additionally, increasing the voltage intensity results in more energy being produced in the cold plasma system, which can break polyphenolic chains and cause a substantial reduction of these compounds in the milk [20]. Therefore, the higher the voltage intensity in cold plasma, the greater the likelihood of reducing polyphenolic compounds, as more chemical reactions occur, negatively affecting the stability of these compounds. Yang et al. (2024) concluded that increasing the duration of cold plasma treatment with a specific voltage, due to the release of more polyphenolic compounds from the food matrix, could lead to an increase in the total polyphenol content [21]. **Equation (5)**
Total Polyphenol Content (TPC) = 42.22 - 6.56A + 15.49B

3.5.DPPH Antioxidant Activity

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The results obtained from the evaluation of the antioxidant activity of cold plasma-treated sheep milk, measured by the percentage of DPPH radical scavenging, show (Figure 1-e) that both voltage and treatment time have significant effects on antioxidant activity. ANOVA analysis (Table 2) indicates that voltage, with a p-value less than 0.0001, significantly increases antioxidant activity. Additionally, treatment time, with a p-value of 0.004, also has a significant effect on this activity. According to the derived equation (Equation 6), voltage with a coefficient of 4.96 has the greatest effect on increasing antioxidant activity. This suggests that increasing the voltage of cold plasma leads to an increase in the milk's antioxidant capacity to neutralize DPPH free radicals. This result may be due to the activation of antioxidant compounds and their increased ability to combat free radicals. In contrast, treatment time (B) with a coefficient of 0.12 also significantly impacts the increase in antioxidant activity, but its effect is much less than that of voltage. Previous studies also confirm that cold plasma can enhance the antioxidant capacity of food products [3]. Lee et al. (2020) reported that the use of cold plasma increased the antioxidant activity in milk [22], which aligns with the results of the present study. Furthermore, Hish et al. (2024) stated that increasing cold plasma voltage enhances antioxidant activity by stimulating and activating antioxidant compounds [23]. The findings of this study indicate that increasing cold plasma voltage significantly increases antioxidant activity in sheep milk, and treatment time also has a noteworthy effect. To explain this finding, it can be said that the increase in cold plasma voltage and its impact on enhancing the antioxidant activity of milk can be attributed to chemical changes and the production of reactive oxygen species (ROS). At higher voltages, cold plasma generates more energy, leading to the production of greater amounts of reactive oxygen and nitrogen species, which can react with compounds in the milk and strengthen its antioxidant structure. Additionally, high voltage can lead to the release of polyphenolic compounds and other natural antioxidants present in the milk that were previously bound or inactive. Moreover, cold plasma might affect the antioxidant enzymes in milk, enhancing their activity, which contributes to strengthening the antioxidant capacity. This finding is consistent with results related to the reduction of polyphenolic compounds, as the increased reactivity of these compounds in the presence of cold plasma not only leads to the degradation of some sensitive compounds but also enhances their activity in neutralizing free radicals [20].

Equation (6)

DPPH Radical Scavenging Activity = 21.03 + 4.96A + 0.12B

3.6. Microbial Count

The analysis of the microbiological characteristics of cold plasma-treated sheep milk indicates that both voltage and treatment time significantly affect the reduction in total bacterial count (Figure 1-f). ANOVA results (Table 2) show that voltage, with a p-value less than 0.0001, and treatment time, with a p-value of 0.012, have a significant effect on the microbiological properties of sheep milk. According to the derived equation (Equation 7), voltage with a coefficient of 1.15 has a positive and significant effect on the reduction of bacterial count. In other words, increasing the voltage of cold plasma leads to a reduction in the total bacterial count, which may be due to physical and chemical changes in the bacterial structure [24] and their inability to continue growth and multiplication [1]. On the other hand, treatment time (B), with a coefficient of -0.135, has a negative effect on bacterial count, meaning that

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as treatment time increases, a smaller reduction in bacterial numbers is observed. The statistical model has been evaluated with significant R^2 and adjusted R^2 values. An R^2 value of 0.99 and an adjusted R^2 value of 0.98 show that the model fits the experimental data well. Additionally, a predicted R^2 value of 0.96 and a model accuracy of 28.64 confirm the high predictive power of the model. The results of this study are consistent with previous research [1], which has shown that cold plasma treatment can significantly reduce bacterial count in dairy products, reinforcing the findings of this research. Furthermore, Wang et al. (2022) concluded in another study that increasing voltage in cold plasma enhances the antibacterial properties of food products [2]. These results indicate that cold plasma voltage can significantly reduce bacterial count, and treatment time is also an effective factor in this process. The high voltage of cold plasma leads to a significant reduction in bacterial count by directly affecting the bacterial cell wall through physical and chemical effects. Physically, the strong electric field generated by high voltage can cause ruptures or degradation of bacterial cell walls and membranes, leading to the leakage of cellular contents and bacterial death. Additionally, the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during cold plasma treatment results in the oxidation of bacterial lipids, proteins, and DNA, causing irreparable damage to vital structures. Furthermore, longer contact time of milk with cold plasma increases the duration of action of these reactive species, providing more opportunities for destructive reactions. Moreover, cold plasma may affect bacterial defense mechanisms and reduce their resistance [2]. These findings suggest that cold plasma, with controlled voltage and exposure time, can be used as an effective technique for reducing the microbial load of milk and improving its antibacterial properties, thereby enhancing its quality and safety.

Equation (7)

$$\text{Total Bacterial Count} = 3.21 + 1.15A - 0.135B$$

3.7.Color Changes in Milk

This study comprehensively examined the impact of cold plasma on three main color indices of sheep milk, including redness, lightness, and yellowness (Table 3). The redness index (a*) showed that both voltage and treatment time had a significant effect ($p < 0.0001$). With increasing voltage, the redness index decreased, indicating a reduction in the redness of the milk. This reduction is likely related to changes in the molecular structure of pigments in milk influenced by cold plasma [25, 26]. Treatment time also negatively affected the redness index, but to a lesser extent than voltage. Similar results were observed in studies by Coutinho et al. (2018) and Nikmaram et al. (2022), which confirmed that cold plasma reduces the redness of dairy products [6].

For the lightness index (L*), both voltage and treatment time significantly increased the lightness of the milk ($p < 0.0001$). This increase suggests that cold plasma improves the optical properties and appearance of milk. Voltage had a more substantial effect than treatment time, and the findings of this study align with those of Wang et al. (2022) and Özer et al. (2021), who reported the positive impact of cold plasma on the lightness of dairy products [27].

Finally, the yellowness index (b*) also indicated that both voltage and treatment time significantly increased the yellowness of the milk ($p < 0.0001$). With increasing voltage, the intensity of yellowness increased [28, 29]. Compared to lightness, voltage had a greater impact on yellowness. The results of this study are consistent with those of Onur et al. (2019) and Niknam et al. (2022), who reported the positive

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impact of cold plasma on color changes in dairy products [30]. The increase in yellowness in milk may be due to changes in protein and lipid compositions as a result of oxidation reactions induced by cold plasma. Cold plasma, by generating reactive oxygen species (ROS) and reactive nitrogen species (RNS), can lead to fat oxidation and the formation of byproducts such as aldehydes and ketones, which have a yellowish color. Additionally, oxidation reactions can affect the structure and stability of carotenoids and other natural pigments in milk, leading to an increase in yellow color. Furthermore, the impact of cold plasma on the structure of milk proteins, especially casein, may result in color changes due to the aggregation or structural alteration of protein-associated pigments [28, 29].

Tabel 3. Regression coefficients of Color properties of milk

Source	L*	A*	B*
Model	**808.21	**259.37	**98.23
A	**668.24	**236.00	**88.24
B	**139.97	**23.36	**9.98
Std. Dev.	2.56	0.80	0.54
Mean	74.58	2.00	10.94
C.V. %	3.43	39.80	4.98
R-Squared	0.9537	0.9856	0.9822
Adj R-Squared	0.9382	0.9808	0.9762
Pred R-Squared	0.9130	0.9710	0.9563
Model accuracy	20.834	35.918	32.581
Residual	39.25	3.79	1.78
Cor Total	847.46	263.16	100.01

**p≤0.01, A-DBD-type plasma (Currnet), B-Exposure time

4.Conclusion

The results of this study demonstrate the significant impact of two factors—voltage and treatment time—with cold plasma on the quality characteristics of sheep milk. These two factors, as independent variables, significantly affected dependent variables such as pH, antioxidant activity, color indices (L, a, b), fat, and protein content. Specifically, increasing the voltage (up to 2 kV) and treatment time (up to 4 minutes) resulted in an increase in pH level and antioxidant activity (with p-values of 0.0001). These findings confirm that increasing voltage and time creates better conditions for improving the quality characteristics of milk.

Additionally, the results showed that cold plasma treatment increased the protein content (up to 3.86 g/100 mL) and fat content (up to 4.53 g/100 mL) in milk, making it an effective method for enhancing the quality of dairy products in the industry. Microbiological tests also indicated a significant reduction in total bacterial count, which could contribute to improving the safety and shelf life of milk.

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Overall, the findings of this study suggest that the use of cold plasma, with optimal voltage and treatment time settings, could be an innovative strategy for enhancing the quality and increasing the nutritional value of milk in the dairy industry. These results could serve as the basis for further research into the application of cold plasma technology in other food products.

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بررسی تأثیر فرآیند پلاسمای سرد بر ویژگی‌های فیزیکی و شیمیایی و آنتی اکسیدانی شیر گوسفندی

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چکیده

این پژوهش به بررسی تأثیر فرآیند پلاسمای سرد بر ویژگی‌های شیر خام گوسفند شیر پرداخته است. در این مطالعه، اثر پلاسمای سرد از روی شدت ولتاژ در سه سطح (۰/۵، ۰/۷ و ۰/۹ کیلوولت) و اعمال ولتاژ در سه سطح (۳، ۵ و ۷ دقیقه) در نظر گرفته شد. خصوصیات فیزیکی و شیمیایی شیر خام گوسفند شامل pH، میزان چربی و پروتئین، فنل کل، فعالیت آنتی‌اکسیدانی(درصد رنگبری رادیکال DPPH)، آزمون‌های میکروبی(تعداد کل باکتری‌ها) و شاخص‌های رنگی (L*، a*، b*) بررسی شدند. تحلیل واریانس (ANOVA) نشان داد که ولتاژ و زمان به‌طور معناداری بر pH تأثیر گذاشتند، به‌طوری‌که با افزایش ولتاژ و زمان، pH شیر به طور متوسط به ۶/۸۲ افزایش یافت. همچنین، فعالیت آنتی‌اکسیدانی نیز تحت تأثیر قرار گرفت و با افزایش ولتاژ و زمان، به ۲۱/۰۳ درصد افزایش یافت. شاخص‌های رنگی نیز به طور معناداری تغییر کردند. شاخص روشنایی (L) در اثر ولتاژ و زمان درمان به ۷۴/۵۸ رسید، در حالی که شاخص قرمزی (a*) و زردی (b*) نیز به ترتیب به ۲/۱۵ و ۱۰/۹۴ تغییر یافتند. بررسی پروتئین و چربی نشان داد که با افزایش ولتاژ و زمان درمان، مقادیر پروتئین به ۳.۸۶ و چربی به ۴.۵۳ g/100 mL رسیدند و همچنین، در آزمون‌های میکروبی، تعداد کل باکتری‌ها کاهش معناداری پیدا کرد و این نشان‌دهنده بهبود قابل توجهی در ایمنی شیر بود . در نهایت، این نتایج نشان می‌دهند که استفاده از پلاسمای سرد می‌تواند به عنوان یک روش مؤثر در بهبود کیفیت و افزایش ارزش تغذیه‌ای و عمر ماندگاری شیر در صنعت لبنیات مورد توجه قرار گیرد.

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