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ORIGINAL ARTICLE

# The use of cold plasma technology in solving the mold problem in Kashar cheese

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**Abstract** In this study, the possibilities of using cold plasma technology in solving the mold problem, which is one of the most important problems in Kashar cheese, were investigated. For this purpose Kashar cheeses were exposed to cold plasma with different gas compositions. As a result of the study 3–4 log reduction was achieved for both *Aspergillus flavus* and *Penicillium crysogenum*. The pH and $a_w$ values of samples were decreased with cold plasma application. The b* values of samples increased while L* and a* values decreased. When all the results obtained are considered as a whole, it can be said that cold plasma technology improves the physicochemical properties of Kashar cheese and provides significant decrease in mold count of the product.

**Keywords** Cold plasma · Kashar · *Aspergillus flavus* · *Penicillium crysogenum* · Shelf-life

## Introduction

Milk and dairy products are open to microbial spoilage due to their high nutritional content (protein, lactose, fat, etc.). For this reason, it is critical to ensure food safety in milk and its products. Thermal processes are widely used for this purpose. However, it is known that thermal processes such as pasteurization and sterilization cause protein denaturation, loss of aroma, and problems in nutritional and physicochemical properties (Atik and Gumus 2021; Rocha et al. 2022). In order to minimize changes in nutritional value and sensory properties, non-thermal technologies such as high hydrostatic pressure (HPP), pulse electric field (PEF), ultraviolet light, ultrasound, and plasma (cold plasma) have been used for microbial inactivation (Shabbir et al 2021). Non-thermal food storage methods cause minimal changes in dairy products' sensory and nutritional properties, meeting consumer demand for less processed products (Akarca et al. 2022). Among the non-thermal technologies, cold atmospheric plasma is under broad consideration. It has gained much importance due to its wide application in various industries, especially for the decontamination and deactivation of enzymes for different food substrates, including milk and dairy products (Rathod et al. 2021).

Cheese is a nutritious and delicious food due to its high-quality proteins, fats, vitamins such as A, B2, B12, and minerals such as calcium and phosphorus (Feeney et al. 2021). Kashar cheese, one of the commonly fermented dairy products, is a semi-hard cheese type. (Yildirim-Mavis et al. 2022). Kashar cheese is a 'pasta filata' type of Turkish cheese. In this type of cheese, the acidified curd is boiled in hot (75–80 °C) and salty (4–5%) water and kneaded to give the desired texture and shape. It is usually ripened for 3–6 months. If conditions such as temperature and relative humidity are not well controlled during the ripening process, surface contamination could be increased. This causes fungal growth on the cheese surface, damaging the products' sensory properties and overall quality. Therefore, to reduce the commercial value loss, processes that will provide surface disinfection can be applied to prevent cheese contamination with microorganisms that cause spoilage (Ozturkoglu-Budak et al. 2021).

Molds are important spoilage organisms in dairy products, especially in low moisture products such as fermented

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milk products and cheese. Generally, contamination occurs through environmental cross-contamination. *Penicillium* and *Aspergillus* species are the most common mold species associated with spoilage in dairy products (Martin et al. 2022). Although chemical control has been the principal road of preventing mold growth on the food surface for many years (Marín et al. 2002), concerns about the use of chemicals are increasing day by day. Therefore, studies are being conducted on alternative methods to prevent mold growth.

In recent years, applications such as biological protection, new packaging and improved sanitation programs have been used to reduce mold-induced spoilage in dairy products (Martin et al. 2022). In addition, it continues to be investigated whether non-thermal decontamination methods such as ionizing radiation, cold plasma, high hydrostatic pressure, and pulsed electric field applications are alternatives to traditional methods (Raso and Barbosa-Cánovas 2003). Plasma refers to partially or fully ionized gas, mainly composed of photons, ions, free electrons, as well as atoms in their ground or excited state with a net neutral charge. Due to its unique properties, plasma, defined as the fourth state of matter, has a neutral charge (Rathod et al. 2021; Asl et al. 2022).

Plasma, which is a partially or completely ionized gas, is divided into thermal and non-thermal plasma according to its thermodynamic properties. Cold plasma technology is characterized by ionized gases containing active particles such as atoms, electrons, ions and reactive neutral species. It is a non-thermal and green process technology used as an alternative method to traditional methods and other potential applications in the food industry (Bao et al. 2021).

It is produced between electrodes using cold plasma (CAP), radio frequency (RF), dielectric barrier discharge (DBD), and microwaves (MW). Various gases such as oxygen, helium and argon are also used in this process (Pankaj et al. 2014). Reactive groups formed by cold plasma cause the death of the microorganism by damaging the cell wall and cytoplasm membrane, DNA structure and proteins of the cell (Fernandez et al. 2013). In addition, the synergistic effect of mechanisms including the production of ozone, charged particles, UV radiation, oxygen radicals and other reactive groups is also effective in the inactivation of microorganisms (Laroussi and Leipold 2004). In the literature, there is information about the use of cold plasma application in the food industry, especially to inactivate bacteria, yeast and molds. Also, cold plasma has an effect on biofilm formation and spores (Jiang et al. 2012). There are various studies on the inhibition of molds growing on the surface of foods with cold plasma application (Go et al. 2019; Mošovská et al. 2019).

In this study, It was aimed to investigate the possibilities of using cold plasma technology in solving the mold problem, which is one of the most important problems in Kashar cheese.

## Material and method

### Material

Fresh Kashar cheeses used in the study were purchased from a local market in Afyonkarahisar, Turkey. Kashar cheeses were produced from cow's milk and were cut into $8 \times 5 \times 5$ mm slices (length x width x thickness) with the help of a knife before processing. The flow diagram of the cold plasma application is shown in Fig. 1.

### Microorganism strains used in the study

Mold species belonging to *Penicillium crysogenum* (ATCC 10106), and *Aspergillus flavus* (ATCC 204304) strains were used in the study. All mold strains were obtained from the American Type Culture Collection. (ATCC, Rockville, MD, US). Stock cultures of mold strains were stored at − 20 °C. All strains were activated by cultivating in Malt Extract Agar (Merck, Germany, 105398) medium at 25 ± 0.1 °C for 72–96 h before being used in the study.

### Preparation of inoculum

For this purpose, mold species were taken from mold colonies grown in the medium with the help of a sterile loop (Orlab, Turkey). Received colonies were suspended in ringer solution (Merck, Germany, 115525) until homogeneous turbidity was formed. The density of the resulting inoculum suspension was adjusted with a densitometer (Biosan 1B, Turkey) to equal the 0.5 McFarland standard. Then, 1 mL ($10^6$–$10^7$ propagules/mL) of mold species was taken with the help of a sterile-tipped pipette (Research Plus, Eppendorf, Germany) and inoculated on the surface of Kashar cheese which was exposed to cold plasma. Following this, it was

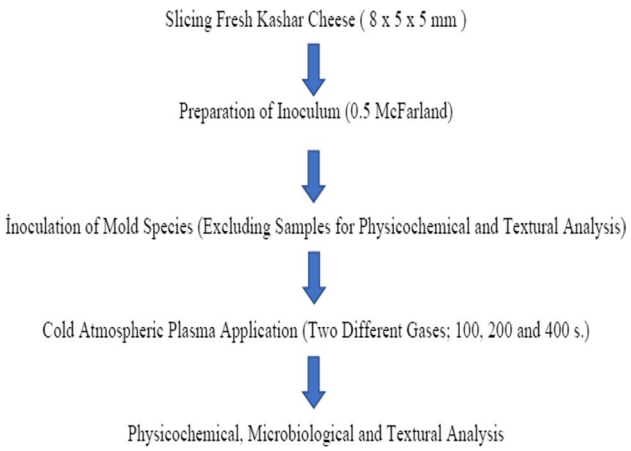


**Fig. 1** Flow Chart of the Experiment

spread homogeneously over the entire surface with the help of a sterile plastic drigalski spatula (Orlab, Turkey).

### Cold atmospheric plasma application

Cold atmospheric plasma application was made according to the points stated by Gök et al. (2019). Two different gases, Argon and Oxygen, were used in the application. The gases were supplied from Afyonkarahisar province (Kocaşaban Gazları A.Ş., Afyonkarahisar, Turkey) and mixed in certain proportions and given to the system.

The power supply used was 25 kV, 42 kHz frequency and operated in continuous mode. Plasma application was made in a semicircular glass chamber with a radius of 28 mm. The area that the treatment took place was fixed to a stainless-steel plate with a plastic ring of 46 mm diameter.

Seven 1 mm tungsten steel electrodes were used to produce the plasma, and one of the electrodes was placed horizontally in front of the other six electrodes to create plasma between the anode–cathode tips. The temperature of the cheese samples during processing was controlled by using an infrared thermometer (˂24 °C) (Coleman-Parmer, Vernon Hills, IL) (Fig. 2).

In the study, only oxygen gas plasma was applied to KM1, KM2 and KM3 coded samples, and only argon gas plasma was applied to KM4, KM5 and KM6 coded samples. Mixture plasma consisting of oxygen and argon gases at different ratios was applied to the remaining samples. Applied gases, mixing ratios, and flow rates are shown in Table 1. The exposure times of the samples to the gases were determined as 100, 200 and 400 s. Each application was made in 2 recurrences, 2 parallels, separately and on different days.

### Preparation of samples for microbiological analysis

Mold species inoculated Kashar slices were placed in sterile stomacher bags (Stomacher Lab Blender 400, London, UK) after cold plasma application. Then, 10 g of the samples in the bags were weighed on a precision balance (Laboratory Balances, Radwag PS R2.H, Poland) and taken into another sterile stomacher bag. 90 mL of sterile Ringer's solution (Merck, 115525, Germany) was added and homogenized for 2 min in a stomacher (BagMixer® 400 P-080921247). Then, the mixture was diluted with Ringer's solution by preparing serial dilutions at the desired ratios.

The same procedures were applied in the control group. In order to determine the number of molds inoculated on Kashar cheeses, samples were prepared in the same way, but plasma was not applied to these samples.

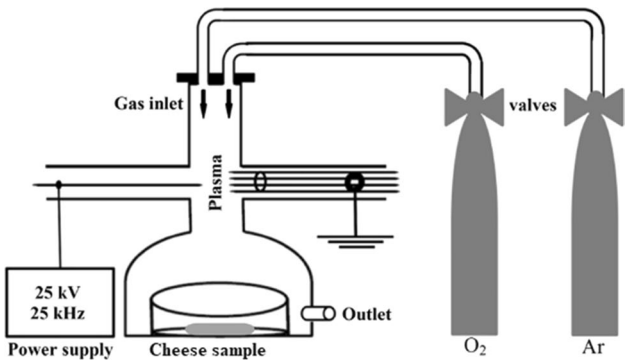


**Fig. 2** The scheme of cold atmospheric plasma treatment (Gok et al. 2019)

**Table 1** Gases used in cold plasma processing and exposure time

| Sample codes | Gas type | Exposure time (s) |
|---|---|---|
| Control | No plasma application | 0 |
| KM1 | Oxygen (1 L/min) | 100 |
| KM2 | Oxygen (1 L/min) | 200 |
| KM3 | Oxygen (1 L/min) | 400 |
| KM4 | Argon (1 L/min) | 100 |
| KM5 | Argon (1 L/min) | 200 |
| KM6 | Argon (1 L/min) | 400 |
| KM7 | Argon/oxygen (0.25/0.75 L/min) | 100 |
| KM8 | Argon/oxygen (0.25/0.75 L/min) | 200 |
| KM9 | Argon/oxygen (0.25/0.75 L/min) | 400 |
| KM10 | Argon/oxygen (0.5/0.5 L/min) | 100 |
| KM11 | Argon/oxygen (0.5/0.5 L/min) | 200 |
| KM12 | Argon/oxygen (0.5/0.5 L/min) | 400 |
| KM13 | Argon/oxygen (0.75/0.25 L/min) | 100 |
| KM14 | Argon/oxygen (0.75/0.25 L/min) | 200 |
| KM15 | Argon/oxygen (0.75/0.25 L/min) | 400 |

### Determination of mold count

The spread plate technique was used to determine the count of molds. With the help of an automatic pipette with a sterile tip, 0.1 mL was taken from all the dilutions prepared and inoculated on the surface of Dichloran Rose Bengal Chloramphenicol (DRBC) Agar (Merck, 100466, Germany) in two parallel, then incubated at 22 °C for 5–7 days in the incubator (MM Incucell 55, Germany) under aerobic conditions. At the end of the period, the colonies in the Petri dishes of 8–80 were counted separately, and the result was calculated by taking the arithmetic average (USP 2006).

### Physicochemical properties

Mold inoculum was not applied was applied to the samples used for the determination of the physicochemical analysis of the Kashar cheese samples**.**

### pH value

After weighing 10 g of Kashar cheese sample, it was mixed with 10 mL distilled water and homogenized using a homogenizator (Daihan Wisestir, HS-30 T, South Korea). The pH values of the prepared mixtures were measured with a pH meter (Hanna, HI 2215 pH/ORP meter) (AOAC 2016).

### Activity of water ($a_w$) value

The $a_w$ values of the samples were determined with a water activity analyzer (Novasina LabTouch-aw, Lachen, Switzerland) (AOAC 2016).

*Color analysis*

Color values of cheese samples were determined using a colorimeter (Minolta Chroma Meter CR-400, Osaka). The measurements of the brightness (L*), redness (a*) and yellowness (b*) values of the samples were made according to AOAC (2016).

*Texture profile analysis (TPA)*

Texture profile analysis (TPA) measurements of the samples were made with a Stable Micro Systems Texture Analyzer (Stable Micro Systems, Surrey, UK). Measurements were performed at 25 °C using Cusinga TA-XT2 texture analyzer equipped with a 25 kg load cell, cylindrical aluminum probe (aluminum cylinder probe P/50, 50 mm diameter; Stable Micro Systems LTD, Godalming, UK). Pre-test, test, and post-test speeds were set at 5, 1, and 5 mm/s, respectively. The samples were compressed to 50% of their original height with 5 s between two compressions. The hardness (N), adhesiveness (g.s), cohesiveness, springiness, chewiness (N) and gumminess (N) values of the samples were determined (Eroğlu et al. 2015).

*Statistical analysis*

The results obtained in the study were made in 2 recurrences, 2 parallels and SPSS software program V 23.0.0 was used for the variance analysis. A significant difference was determined by Duncan's multiple range tests ($P < 0.05$).

## Results and discussion

Molds are one of the microbiological factors affecting the shelf life of fresh Kashar cheese. In order to determine the effect of cold plasma application on the microbial quality of cheese, *A. flavus* and *P. crysogenum* molds were inoculated and studied. In line with the data obtained after the application 3–4 log reduction was achieved for both mold types. The change in mold count after the application is given in Table 2, and the correlation and variation results of mold development are given in Table 3.

In *A. flavus* inhibition, the application time was important ($P < 0.001$), while the gas composition was not important ($P > 0.001$). While *A. flavus* load was 5.07 log cfu/cm$^2$ in the control sample, the highest decrease (1.57 log cfu/cm$^2$) was detected in the application of 100% Oxygen for 400 s. The least decrease was found in the application of 100% Argon for 100 s with 2.64 log cfu/cm$^2$.

While the initial load was 5.06 log cfu/cm$^2$ for *P. crysogenum*, a reduction of 0.85 log cfu/cm$^2$ was achieved. The maximum inhibition was detected in the application of 100% Oxygen for 400 s and the least inhibition was detected in the application of 100% Argon for 100 s.

Cold plasma application was effective in inhibition of both *A. flavus* and *P. crysogenum*. Cold plasma application has an antifungal effect by breaking down the cell wall. In plasma application, the oxygen in the air plasma causes the formation of peroxide and lethal species such as O*, $O_2$, $O_3$. In addition, the accumulation of charged particles on the outer cell membrane, electrostatic forces could cause subsequent rupture of the cell membrane and subsequent cell death (Devi et al. 2017). DNA damage caused by UV radiation produced during plasma is also thought to be effective in microbial inactivation (Liao et al. 2017).

In a similar study on meat, *A. flavus* load was reduced from 5.24 log cfu/g to 2.06 log cfu/gr, and a 3-log

**Table 2** Mold count inoculated on fresh Kashar cheese after cold plasma application (log cfu/cm$^2$)

| Samples | *Aspergillus flavus* | *Penicillium crysogenum* |
|---|---|---|
| Control | $5.07 \pm 0.08^{a}$ | $5.06 \pm 0.80^{a}$ |
| KM1 | $2.27 \pm 0.05^{cde}$ | $2.08 \pm 0.16^{ef}$ |
| KM2 | $2.11 \pm 0.03^{def}$ | $1.48 \pm 0.09^{hi}$ |
| KM3 | $1.57 \pm 0.05^{j}$ | $0.85 \pm 0.11^{j}$ |
| KM4 | $2.64 \pm 0.14^{b}$ | $3.03 \pm 0.23^{b}$ |
| KM5 | $1.95 \pm 0.08^{fgh}$ | $2.47 \pm 0.08^{c}$ |
| KM6 | $1.59 \pm 0.05^{j}$ | $1.87 \pm 0.12^{fg}$ |
| KM7 | $2.34 \pm 0.04^{cd}$ | $2.50 \pm 0.14^{c}$ |
| KM8 | $2.07 \pm 0.02^{ef}$ | $2.26 \pm 0.06^{cde}$ |
| KM9 | $1.69 \pm 0.16^{ij}$ | $2.12 \pm 0.05^{def}$ |
| KM10 | $2.37 \pm 0.11^{c}$ | $2.32 \pm 0.14^{cde}$ |
| KM11 | $1.88 \pm 0.15^{fghi}$ | $1.87 \pm 0.07^{fg}$ |
| KM12 | $1.75 \pm 0.17^{ghij}$ | $1.71 \pm 0.03^{gh}$ |
| KM13 | $2.40 \pm 0.04^{c}$ | $2.38 \pm 0.06^{cd}$ |
| KM14 | $1.99 \pm 0.09^{fg}$ | $2.12 \pm 0.16^{def}$ |
| KM15 | $1.73 \pm 0.18^{hij}$ | $1.36 \pm 0.08^{i}$ |

a-j (↓): Values with the different letters in the same column for each analysis differ significantly ($P < 0.05$)

**Table 3** Correlation and variation results of the analysis applied on mold growth, pH, $a_w$, colour and TPA values of fresh Kashar cheese

| Source of variation | *Aspergillus flavus* | | *Penicillium crysogenum* | |
|---|---|---|---|---|
| | P value | r | P value | r |
| Samples (S) | < 0.0001 | −0.166 | < 0.0001 | −0.218 |
| Gas composition (G) | < 0.0001 | 0.209* | < 0.0001 | 0.348* |
| Exposure time (E) | < 0.0001 | −0.777** | < 0.0001 | −0.865** |
| E x G | < 0.0001 | – | < 0.0001 | – |
| E x S | 0.515 | – | 0.537 | – |
| G x S | 0.01 | – | < 0.0001 | – |
| E x G x S | 0.022 | – | 0.011 | – |

| Source of Variation | pH | | $a_w$ | | L*Value | | a*Value | | b* Value | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *P* value | r | *P* value | r | *P* value | r | *P* value | r | *P* value | r |
| Samples (S) | < 0.0001 | 0.286* | < 0.0001 | 0.356* | < 0.0001 | 0.195 | < 0.0001 | 0.253 | < 0.0001 | 0.124 |
| Gas composition (G) | < 0.0001 | 0.443** | < 0.0001 | 0.413** | < 0.0001 | 0.253* | < 0.0001 | −0.126 | < 0.0001 | 0.15 |
| Exposure time (E) | < 0.0001 | 0.365** | < 0.0001 | 0.290** | < 0.0001 | -0.355** | < 0.0001 | −0.422** | < 0.0001 | −0.354** |
| E x G | < 0.0001 | – | 0.093 | – | < 0.0001 | – | 0.003 | – | < 0.0001 | – |
| E x S | 0.01 | – | 0.021 | – | < 0.0001 | – | 0.045 | – | < 0.0001 | – |
| G x S | 0.035 | – | 0.01 | – | < 0.0001 | – | 0.012 | – | < 0.0001 | – |
| E x G x S | 0.043 | – | 0.04 | – | 0.01 | – | 0.035 | – | 0.02 | – |

| Source of Variation | Hardness (N) | | Adhesiveness | | Springiness (mm) | | Cohesiveness | | Gumminess (N) | | Chewiness | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P* value | r | *P* value | r | *P* value | r | *P* value | r | *P* value | r | *P* value | r |
| Samples (S) | < 0.0001 | 0.152 | < 0.0001 | 0.154 | < 0.0001 | 0.203 | < 0.0001 | 0.187 | < 0.0001 | 0.138 | < 0.0001 | 0.090 |
| Gas composition (G) | < 0.0001 | 0.153 | < 0.0001 | 0.887 | < 0.0001 | 0.887 | < 0.0001 | 0.235 | < 0.0001 | 0.193 | < 0.0001 | −0.136 |
| Exposure time (E) | < 0.0001 | -0.387** | 0.404 | 0.454** | 0.001 | 0.454** | < 0.0001 | 0.470** | < 0.0001 | −0.343** | < 0.0001 | 0.464** |
| E x G | 0.007 | – | < 0.0001 | – | 0.598 | – | 0.252 | – | 0.030 | – | 0.006 | – |
| E x S | 0.047 | – | 0.266 | – | 0.643 | – | 0.187 | – | 0.018 | – | 0.032 | – |
| G x S | 0.134 | – | 0.546 | – | 0.823 | – | 0.395 | – | 0.056 | – | 0.021 | – |
| E x G x S | 0.353 | – | 0.697 | – | 0.913 | – | 0.567 | – | 0.143 | – | 0.047 | – |

r: correlation coefficient, $P < 0.0001$: Statistically too much significant, $P < 0.01$: Statistically too significant, $P < 0.05$: Statistically significant, $P > 0.05$: Not statistically significant, ns: Not statistically significant, *: $P < 0.05$; **: $P < 0.01$

reduction was achieved (Yong et al. 2017). Ulbin-Figlewicz et al. (2015) applied cold plasma to the meat surface using helium gas and provided a 2 log reduction in the total yeast mold load. It was reported that cold plasma application on peanuts resulted in a 97.9% and 99.3% reduction in the growth of *A. parasiticus* and *A. flavus*, respectively (Devi et al. 2017). The effect of non-thermal plasma application on the inactivation of *Aspergillus* spores in black pepper was investigated, and it was reported that a 3-log reduction was achieved after 4 min of application (Tanino 2019).

The pH, $a_w$, and color values of the samples are given in Table 4, and the correlation and variation results of the results are given in Table 3.

When pH values were examined, it was determined that cold plasma application caused a decrease in pH values. While the pH of the control sample was 5.49, the lowest pH was measured as 5.12 in the application of 50% Argon / 50% Oxygen for 400 s ($P < 0.05$). The increase in $H^+$ ions during cold plasma application is thought to be effective in this decrease in pH value. Reactive species produced by plasma, mainly with acidic properties such as nitric acid ($HNO_3$) and nitrous acid ($HNO_2$), are responsible for the pH decrease.

**Table 4** pH, $a_w$ and color values (L*,a*and b*) of fresh Kashar cheese after cold plasma application

| Samples | pH | $a_w$ | L* | a* | b* |
|---|---|---|---|---|---|
| Control | $5.49 \pm 0.04^{a}$ | $0.900^{a}$ | $87.83^{a}$ | $1.67^{a}$ | $12.33^{f}$ |
| KM1 | $5.36 \pm 0.07^{b}$ | $0.894^{def}$ | $86.85^{cd}$ | $1.54^{bc}$ | $12.63^{e}$ |
| KM2 | $5.35 \pm 0.03^{b}$ | $0.891^{gh}$ | $85.13^{hı}$ | $1.30^{fg}$ | $13.21^{c}$ |
| KM3 | $5.15 \pm 0.01^{ef}$ | $0.886^{i}$ | $83.89^{k}$ | $0.93^{ı}$ | $14.54^{a}$ |
| KM4 | $5.44 \pm 0.06^{a}$ | $0.898^{b}$ | $87.15^{b}$ | $1.63^{b}$ | $12.38^{ef}$ |
| KM5 | $5.34 \pm 0.03^{b}$ | $0.895^{bcde}$ | $86.73^{de}$ | $1.52^{cd}$ | $12.47^{ef}$ |
| KM6 | $5.30 \pm 0.01^{bc}$ | $0.892^{fg}$ | $85.49^{g}$ | $1.31^{fg}$ | $12.95^{d}$ |
| KM7 | $5.37 \pm 0.03^{b}$ | $0.895^{bcde}$ | $86.96^{bc}$ | $1.55^{bc}$ | $12.39^{ef}$ |
| KM8 | $5.31 \pm 0.08^{bc}$ | $0.892^{fg}$ | $86.48^{f}$ | $1.35^{fg}$ | $12.89^{d}$ |
| KM9 | $5.25 \pm 0.02^{cd}$ | $0.889^{h}$ | $84.35^{j}$ | $1.01^{i}$ | $13.72^{b}$ |
| KM10 | $5.36 \pm 0.01^{b}$ | $0.896^{bcd}$ | $87.06^{bc}$ | $1.59^{abc}$ | $12.37^{f}$ |
| KM11 | $5.24 \pm 0.01^{cd}$ | $0.895^{bcde}$ | $89.60^{ef}$ | $1.39^{ef}$ | $12.50^{ef}$ |
| KM12 | $5.12 \pm 0.03^{f}$ | $0.893^{efg}$ | $84.91^{ı}$ | $1.18^{g}$ | $13.57^{b}$ |
| KM13 | $5.47 \pm 0.02^{a}$ | $0.897^{bc}$ | $87.11^{b}$ | $1.62^{abc}$ | $12.38^{ef}$ |
| KM14 | $5.31 \pm 0.01^{bc}$ | $0.896^{bcd}$ | $86.65^{def}$ | $1.45^{de}$ | $12.40^{ef}$ |
| KM15 | $5.20 \pm 0.04^{de}$ | $0.894^{cdef}$ | $85.27^{h}$ | $1.28^{f}$ | $13.48^{b}$ |

a–j (↓): Values with the different letters in the same column for each analysis differ significantly ($P < 0.05$)

The results were in accordance with Wang et al. (2022). It was determined that cold plasma application reduced $a_w$ in Kashar cheese ($P < 0.05$). The $a_w$ value was decreased as the plasma exposure time increased. The lowest $a_w$ value was measured as 0.886 in the application of 100% Oxygen for 400 s. $a_w$ reduction caused drying on the surface due to gas circulation. This situation was thougt to be caused by the ability of $O_2$ and Ar gases used in plasma application to take up free water molecules on the cheese surface. (Lee et al. 2020).

L* value, which is an indicator of whiteness in foods, decreased depending on both the gas concentrations used and the application time (Table 4; $P < 0.05$). It is thought that drying on the surface is responsible for the decrease in L* values. In addition, lipid oxidation can cause browning in foods, brown colored oxypolymers obtained from milk proteins are responsible for the decrease in L* value (Yong et al. 2015). Similarly, a statistically significant decrease was observed in a* value ($P < 0.001$). The lowest a* value was determined as 0.93 in the 100% Oxygen 400 s application. In a similar study, it was determined that increasing the exposure time to plasma treatment decreased the a* values (redness) of beef and pork meat samples and increased the b* values. It was stated that the reaction of hydrogen peroxide formed during plasma application with myoglobin may be effective in this change (Jayasena et al.2015). On the other hand, a significant increase was determined in the b* value. Oxidation caused by plasma treatment is responsible for the increase in b* value (Lee et al. 2012). High b* value is an indicative of non-enzymatic milk reactions, so the use of low oxygen concentrations in plasma application is recommended to prevent fat and protein oxidation (Nikmaram and Keener 2022). The highest increase was determined in the application of 100% Oxygen for 400 s and the lowest increase was determined in the application of 50% Argon / 50% Oxygen for 100 s. The application of 100% $O_2$ for 400 s was the most effective application on the color of the Kashar cheese. The results are similar to Heo et al. (2021) and Kim et al. (2015).

TPA results of the samples are given in Table 5, correlation and variation results are given in Table 3. The gas composition used and the application time caused a statistically significant change on hardness ($P < 0.05$). Hardness value was increased. Decreased $a_w$ is one of the main reasons for the increase in the hardness value of the product. Depending on the drying and hardening of the product, a decrease was detected in the adhesiveness values. The hardness value was increased in almond slices treated with cold plasma using Ar gas. It was stated that the reason for this increase might be related to the reaction between the moisture content of the almond species and the plasma types and then the conversion of moisture to other compounds (Shirani et al. 2020). As the cold plasma application time increased, the decrease in the adhesiveness value increased. It was determined that the application of 75% Argon/25% Oxygen for 100 s had the same springiness characteristics as the control, while 100% Oxygen for 400 s had the highest springiness value. Both the application time and the gas composition used were effective for the cohesiveness, gumminess and chewiness values ($P < 0.001$). It was determined that there was an increase in all three values as the application time increased. The highest chewiness value was determined in the application of 100% Oxygen for 400 s. Treatment of Kashar cheese for 400 s using 100% $O_2$ gas in cold plasma was the most effective application in improving the textural properties of Kashar cheese. In addition, studies show that nonthermal processes improve the sensory properties of kashar cheese (Rocha et al. 2022).

## Conclusion

Molds are the most critical spoilage factor in cheeses with low aw value, such as Kashar cheese. Molds contaminated by cross-contamination during post-production stages can develop rapidly in the product. Cold plasma application was carried out in order to prevent mold growth in Kashar cheese. It was determined that cold plasma application with different gas compositions was effective in the inactivation of *A. flavus* and *P. crysogenum*. A reduction of 3–4 logs was achieved for both mold types. On the other hand, the applied process also had an effect on the

**Table 5** TPA values of fresh Kashar cheese after cold plasma application

| Samples | Hardness (N) | Adhesiveness | Springiness (mm) | Cohesiveness | Gumminess (N) | Chewiness (N x m) |
|---|---|---|---|---|---|---|
| Control | 56.57 $^{g}$ | −0.515$^{a}$ | 0.550$^{e}$ | 0.48 $^{k}$ | 27.15 $^{m}$ | 0.0149$^{j}$ |
| KM1 | 59.35$^{d}$ | −0.648$^{de}$ | 0.590$^{cde}$ | 0.62 $^{cd}$ | 36.50 $^{fg}$ | 0.0215$^{ef}$ |
| KM2 | 63.31$^{c}$ | −0.756 $^{h}$ | 0.640$^{bc}$ | 0.64$^{bc}$ | 40.20 $^{cd}$ | 0.0257$^{c}$ |
| KM3 | 66.32$^{a}$ | −0.816$^{ı}$ | 0.710$^{a}$ | 0.70$^{a}$ | 46.42$^{a}$ | 0.0329$^{a}$ |
| KM4 | 58.03$^{f}$ | −0.575$^{b}$ | 0.550$^{e}$ | 0.50$^{jk}$ | 31.45 $^{k}$ | 0.0159$^{ıj}$ |
| KM5 | 59.33$^{d}$ | −0.615$^{bcd}$ | 0.590$^{cde}$ | 0.53$^{hı}$ | 29.02 $^{l}$ | 0.0186$^{gh}$ |
| KM6 | 62.93 $^{cd}$ | −0.682 $^{fg}$ | 0.620 $^{cd}$ | 0.54$^{ghı}$ | 33,98$^{ıj}$ | 0.0210$^{f}$ |
| KM7 | 59.53$^{e}$ | −0.610$^{bcd}$ | 0.575$^{de}$ | 0.59$^{de}$ | 35.12$^{ghı}$ | 0.0202 $^{fg}$ |
| KM8 | 63.02 $^{cd}$ | −0.673$^{ef}$ | 0.620 $^{cd}$ | 0.62 $^{cd}$ | 38.76$^{de}$ | 0.0240 $^{cd}$ |
| KM9 | 66.02$^{a}$ | −0.744$^{gh}$ | 0.675$^{b}$ | 0.66$^{b}$ | 43.24$^{b}$ | 0.0292$^{b}$ |
| KM10 | 59.18$^{e}$ | −0.595$^{bc}$ | 0.565$^{e}$ | 0.56 $^{fg}$ | 33.14$^{j}$ | 0.0187$^{gh}$ |
| KM11 | 62.58 $^{cd}$ | −0.634$^{cde}$ | 0.600$^{cde}$ | 0.58$^{ef}$ | 36.30$^{fgh}$ | 0.0217$^{ef}$ |
| KM12 | 65.74$^{ab}$ | −0.705 $^{fg}$ | 0.625 $^{cd}$ | 0.63$^{c}$ | 41.09$^{c}$ | 0.0257$^{c}$ |
| KM13 | 58.97$^{e}$ | −0.583$^{bc}$ | 0.555$^{e}$ | 0.52$^{ıj}$ | 30.66$^{kl}$ | 0.0170$^{hı}$ |
| KM14 | 62.28$^{d}$ | −0.623$^{cde}$ | 0.595$^{cde}$ | 0.55$^{fgh}$ | 34.56$^{hıj}$ | 0.0205 $^{fg}$ |
| KM15 | 65.14$^{b}$ | −0.698 $^{fg}$ | 0.620 $^{cd}$ | 0.58$^{ef}$ | 37.78$^{ef}$ | 0.0234$^{de}$ |

r: correlation coefficient, $P<0.0001$: Statistically too much significant, $P<0.01$: Statistically too significant, $P<0.05$: Statistically significant, $P>0.05$: Not statistically significant, ns: Not statistically significant, *: $P<0.05$; **: $P<0.01$

a–m (↓): Values with the different letters in the same column for each analysis differ significantly ($P<0.05$)

physicochemical properties. A decrease was observed in pH and aw values. At the same time, while L*, a* values decreased, b* values increased. Depending on the gas composition used and the application time, cold plasma application improved the textural properties of Kashar cheese. In line with these data, cold plasma application can be used to extend the shelf life of Kashar cheese. Furthermore, studies could be conducted with different gas compositions and times to determine the process parameters that will cause a minimum change in physicochemical properties.

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**Conflict of Interest** The writing of all sections in the manuscript belongs to us.We declare that we have no conflict of interest.

**Ethical approval** We declare that the writing of the letter does not violate any code of ethics.

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## References

Akarca G, Ozkan M, Özcan T (2022) The impact of combination of solution plasma processing and pulsed electric field on the viability of probiotic bacteria, microbial growth, and structure of yoghurt drink. J Food Process Preserv 46(54):1–15. https://doi.org/10.1111/jfpp.16616

AOAC (2016). Association of official analytical chemist, official methods of Analysis. 20th edn. Washington

Asl PJ, Rajulapati V, Gavahian M, Kapusta I, Putnik P, Khaneghah AM, Marszałek K (2022) Non-thermal plasma technique for preservation of fresh foods: a review. Food Control 134:108560. https://doi.org/10.1016/j.foodcont.2021.108560

Atik A, Gumus T (2021) The effect of different doses of UV-C treatment on microbiological quality of bovine milk. LWT- Food Sci Technol 136:110322. https://doi.org/10.1016/j.lwt.2020.110322

Bao T, Hao X, Sishir MRI, Karim N, Chen W (2021) Cold plasma: an emerging pretreatment technology for the drying of jujube slices. Food Chem 337:127783. https://doi.org/10.1016/j.foodchem.2020.127783

Devi Y, Thirumdas R, Sarangapani C, Deshmukh RR, Annapure US (2017) Influence of cold plasma on fungal growth and aflatoxins production on groundnuts. Food Control 77:187–191. https://doi.org/10.1016/j.foodcont.2017.02.019

Eroglu A, Dogan M, Toker OS, Yilmaz MT (2015) Classification of Kashar cheeses based on their chemical, color and instrumental textural characteristics using principal component and hierarchical cluster analysis. Int J Food Prop 18:909–921. https://doi.org/10.1080/10942912.2013.864673

Feeney EL, Lamichhane P, Sheehan JJ (2021) The cheese matrix: understanding the impact of cheese structure on aspects of cardiovascular health - a food science and a human nutrition perspective. Int J Dairy Technol 74(4):656–670. https://doi.org/10.1111/1471-0307.12755

Fernandez A, Noriega E, Thompson A (2013) Inactivation of *Salmonella enterica* serovar typhimurium on fresh produce by cold atmospheric gas plasma technology. Food Microbiol 33:24–29. https://doi.org/10.1016/j.fm.2012.08.007

Go SM, Park MR, Kim HS, Cho WS, Leon RD (2019) Antifungal effect of non-thermal atmospheric plasma and its application for control of postharvest *Fusarium oxysporum* decay of paprika. Food Control 98:245–252. https://doi.org/10.1016/j.foodcont.2018.11.028

Gök V, Aktop S, Özkan M, Tomar O (2019) The effects of atmospheric cold plasma on inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* and some quality characteristics of pastırma—a dry-cured beef product. Innov Food Sci Emerg Technol 56:102188. https://doi.org/10.1016/j.ifset.2019.102188

Heo YS, Yim DG, Baek KH, Kang T, Lee YE, Kim J, Choe W, Jo C (2021) Effect of inkjet-printed flexible dielectric barrier discharge plasma on reduction of pathogen and quality changes on sliced cheese. LWT - Food Sci Technol 143:111128. https://doi.org/10.1016/j.lwt.2021.111128

Jayasena DD, Kim HJ, Yong HI, Park S, Kim K, Choe W, Jo C (2015) Flexible thin-layer dielectric barrier discharge plasma treatment of pork butt and beef loin: effects on pathogen inactivation and meat-quality attributes. Food Microbiol 46:51–57. https://doi.org/10.1016/j.fm.2014.07.009

Jiang C, Schaudinn C, Jaramillo DE, Webster P, Costerton JW (2012). In vitro antimicrobial effect of a cold plasma jet against *Enterococcus faecalis* biofilms. ISRN Dentistry 295376. https://doi.org/10.5402/2012/295736

Kim HJ, Yong HI, Park S, Kim K, Choe W, Jo C (2015) Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. Food Control 47:451–456. https://doi.org/10.1016/j.foodcont.2014.07.053

Laroussi M, Leipold F (2004) Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. Int J Mass Spectrom 233:81–86. https://doi.org/10.1016/j.ijms.2003.11.016

Lee HJ, Jung S, Jung H, Park S, Choe W, Ham JS, Jo C (2012) Evaluation of a dielectric barrier discharge plasma system for inactivating pathogens on cheese slices. J Anim Sci Technol 54:191–198. https://doi.org/10.5187/JAST.2012.54.3.191

Lee SY, Park HH, Min SC (2020) Pulsed light plasma treatment for the inactivation of *Aspergillus flavus spores, Bacillus pumilus* spores, and *Escherichia coli* O157:H7 in red pepper flakes. Food Control 118:107401. https://doi.org/10.1016/j.foodcont.2020.107401

Liao X, Liu D, Xiang Q, Ahn J, Chen S, Ye X, Ding T (2017) Inactivation mechanisms of non-thermal plasma on microbes: a review. Food Control 75:83–91. https://doi.org/10.1016/j.foodcont.2016.12.021

Marín S, Guynot ME, Neira P, Bernadó M, Sanchis V, Ramos AJ (2002) Risk assessment of the use of sub-optimal levels of weak-acid preservatives in the control of mould growth on bakery products. Int J Food Microbiol 79:203e211. https://doi.org/10.1016/S0168-1605(02)00088-0

Martin NH, Snyder A, Wiedmann M (2022). Spoilage mold in dairy products. In: Mc. Sweeney PLH, Mc. Namara JP (eds.), Encyclopedia of dairy sciences, pp 607–610. Academic Press.

Mošovská S, Medvecká V, Gregová M, Tomeková J, Valík L, Mikulajová A, Anna Zahoranová A (2019) Plasma inactivation of *Aspergillus flavus* on hazelnut surface in a diffuse barrier discharge using different working gases. Food Control 104:256–261. https://doi.org/10.1016/j.foodcont.2019.05.003

Nikmaram N, Keener KM (2022) The effects of cold plasma technology on physical, nutritional, and sensory properties of milk and milk products. LWT-Food Sci Technol 154:112729. https://doi.org/10.1016/j.lwt.2021.112729

Ozturkoglu-Budak S, Akal CH, Bereli N, Cimen D, Akgonullu S (2021) Use of antimicrobial proteins of donkey milk as preservative agents in Kashar cheese production. Int Dairy J 120:105090. https://doi.org/10.1016/j.idairyj.2021.105090

Pankaj SK, Bueno-Ferrer C, Misra NN, Milosavljević V, O'Donnell CP, Bourke P, Cullen PJ (2014) Applications of cold plasma technology in food packaging. Trends Food Sci Technol 35:5–17. https://doi.org/10.1016/j.tifs.2013.10.009

Raso J, Barbosa-Cánovas GV (2003) Nonthermal preservation of foods using combined processing techniques. Crit Rev Food Sci Nutr 43:265–285. https://doi.org/10.1080/10408690390826527

Rathod NB, Kahar SP, Ranveer RC, Annapure US (2021) Cold plasma an emerging nonthermal technology for milk and milk products: a review. Int J Dairy Technol 74(4):615–626. https://doi.org/10.1111/1471-0307.12771

Rocha C, Magnani M, de Paiva Anciens Ramos GL, Bezerril FF, Freitas MQ, Cruz AG, Pimentel TC (2022) Emerging technologies in food processing: impacts on sensory characteristics and consumer perception. Curr Opin Food Sci 47:100. https://doi.org/10.1016/j.cofs.2022.100892

Shabbir MA, Ahmed H, Maan AA, Rehman A, Afraz MT, Iqbal MW, Khan IM, Amır RM, Ashraf W, Khan MR, Adıl RM (2021) Effect of non-thermal processing techniques on pathogenic and spoilage microorganisms of milk and milk products. Food Sci Technol Campinas 41(2):279–294. https://doi.org/10.1590/fst.05820

Shirani K, Shadidi F, Mortazavi SA (2020) Investigation of decontamination effect of argon cold plasma on physicochemical and sensory properties of almond slices. Int J Food Microbiol 335:108892. https://doi.org/10.1016/j.ijfoodmicro.2020.108892

Tanino T, Arisaka T, Iguchi Y, Matsui M, Ohshima T (2019) Inactivation of *Aspergillus* sp. spores on whole black peppers by nonthermal plasma and quality evaluation of the treated peppers. Food Control 97:94–99. https://doi.org/10.1016/j.foodcont.2018.10.023

Ulbin-Figlewicz N, Brychcy E, Jarmoluk A (2015) Effect of low-pressure cold plasma on surface microflora of meat and quality attributes. J Food Sci Technol 52:1228–1232. https://doi.org/10.1007/s13197-013-1108-6

USP (2006). The United States Pharmacopeia <1227> Validation of Microbial Recovery from Pharmacopeial Articles. USP 29. United States Pharmacopeial Convention. pp 3053–3055.

Wang S, Liu Y, Zhang Y, Lü X, Zhao L, Song Y, Zhang L, Jiang H, Zhang J, Ge W (2022) Processing sheep milk by cold plasma technology: Impacts on the microbial inactivation, physicochemical characteristics, and protein structure. LWT - Food Sci Technol 153:112573. https://doi.org/10.1016/j.lwt.2021.112573

Yildirim-Mavis C, Ozmen D, Yakisik E, Toker OS, Palabiyik I, Kaner O (2022) Evaluation of kashar cheese meltability by tack and large amplitude oscillatory shear (LAOS) tests. Int Dairy J 127:105242. https://doi.org/10.1016/j.idairyj.2021.105242

Yong HI, Kim HJ, Park S, Kim K, Choe W, Yoo SJ, Jo C (2015) Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin layer dielectric barrier discharge plasma. Food Res Int 69:57–63. https://doi.org/10.1016/j.foodres.2014.12.008

Yong HI, Lee H, Park S, Park J, Choe W, Jung S, Jo C (2017) Flexible thin-layer plasma inactivation of bacteria and mold survival in beef jerky packaging and its effects on the meat's physicochemical properties. Meat Sci 123:151–156. https://doi.org/10.1016/j.meatsci.2016.09.016