Research Article

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Effects of gaseous ozone treatment on the mite pest control and qualitative properties during ripening storage of pecorino cheese

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Abstract

BACKGROUND: Ripened cheeses, such as pecorino, are susceptible to mites and molds contamination on the crust area that must be removed before the product can be marketed. This study investigates the effectiveness of gaseous ozone treatment in the control of microbiological and mite growth without negatively affecting product quality.

RESULTS: Cheese samples were treated with gaseous ozone at 200 and 300 ppb for 8 h per day (overnight) for 150 days in storage rooms under controlled conditions (12 °C and 85% relative humidity). The results showed that ozone at 200 ppb limits the growth of mites starting from 25 days of storage and significantly reduced bacteria, molds, and yeasts counts starting from 75 days of storage. Concerning the physicochemical and qualitative parameters evaluated during ripening (weight loss, moisture content, dry weight, ash, fat, protein, total nitrogen, color, non-destructive firmness), no significant differences were shown between the control samples and ozone treatment at 200 ppb. Sensory analysis (consumer test) also showed no specific defects with the ozone-treated samples. It was observed that the ozone treatment at 300 ppb had limited microbiological growth and no alteration of sensory aspects but did not have the same positive impact on some aspects of overall quality, compared with ozone treatment at 200 ppb.

CONCLUSION: The use of gaseous ozone treatments during ripening of pecorino cheese can potentially offer an excellent solution for the control of mite growth, while preserving the quality and sensory characteristics of the product. For this reason, this technique could be very useful for commercial purposes.

Supporting information may be found in the online version of this article.

Keywords: dairy cheese; ozone technology; *Tyrophagus*; *Acarus*; microbiology; quality; sensory analysis

INTRODUCTION

Pecorino Foggiano is a traditional hard cheese made from sheep's milk and produced in the Foggia area (Puglia region located in south Italy, mainly named Gargano land). It has been listed among the 'Traditional Agro-Food Product' (No. 0011264, 16/02/2018) and is also identified as 'Canestrato Pugliese' among the Protected Designation of Origin pecorino cheeses. For this product, the regulation specifies that the seasoning period ranges from 2 to 10 months in weakly ventilated storage rooms.[1]

In the dairy industry, one of the biggest issues associated with the ripening of cheese is mite infestation. Mite species infesting cheese mainly belong to the order Astigmata, family Acaridae, that includes several species, such as those belonging to genera *Tyrophagus* and *Acarus*.[2] Mites are found in stored foods that are rich in fat and protein, such as cheese, ham, nuts, date fruits, oats, barley, and flour.[3-6] Consumption of mite-infested products can cause enteritis, diarrhea, and damage to the urinary tract, besides triggering allergic reactions.[7-10] The typical indication of cheese mite infestation is the presence of a fine brown powder on the cheese surface, composed of layer on layer of dead and live mites, mite excrement, and minute particles of uneaten cheese. Cheese mites normally live on the external surface of cheese but can attack the crust and penetrate inside the cheese.[11]

Cheese mite proliferation is closely linked to the development of the molds they feed on. Mites can cause significant economic losses due to the reduction of cheese weight and alterations of organoleptic properties.[12] In addition, poor hygiene and the environmental conditions in the ripening room, especially temperature and humidity, can promote mite infestation.[13,14] The remedy for mite infestation is not a simple solution. Chemical and physical methods for protecting cheese against mites have

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long been applied.[15] Though there are many decontaminating methods, as methyl bromide,[16] hydrogen phosphide,[17] gamma radiation, and ozone,[18] safety remains the primary objective in the food industry.[19] Among the ways to prevent and control cheese mite proliferation, the most efficient is the implementation of good manufacturing practices, including training of handlers and regular appropriate sanitization in storage rooms. In common practice, cheesemakers manually remove mold and mites by brushing and washing cheese surfaces. These traditional methods are expensive and ineffective; therefore, the need to find alternative methods has arisen.[20]

Ozone is considered a safe agent (US Food and Drug Administration designation 'generally recognized as safe') and seems to be a promising tool to control microbial growth owing to its high reactivity and strong oxidizing potential.[19] Ozone technology is an alternative method to improve food safety without compromising quality and endangering the environment.[21,22] Since ozone is a highly unstable molecule that auto decomposes spontaneously and rapidly into oxygen atoms, it cannot be collected, stored, or transported and must, therefore, be continuously generated *in situ*.[23] Owing to lack of information and literature on the use of ozone on mite infestation of cheese, our study is aimed to develop an alternative method based on the use of gaseous ozone to control mite infestation and mold growth on traditional Pecorino Foggiano, whilst maintaining the organoleptic and nutritional qualities during the ripening processes.

MATERIAL AND METHODS

Experimental design and ozone treatments

The ozone treatment began after 30 days of cheese ripening in a commercial storage room at 12 °C and 85% relative humidity. The ozone generator (C32-AG; Industrie De Nora Spa, Milan, Italy) used for the treatment in the laboratory was connected to the storage rooms via silicone tubes. The ozone generator consists of an air purifier device that transforms atmospheric oxygen into pure oxygen. Two storage rooms ($l \times w \times h = 8.0$ m^3 each) were treated, one with 200 ppb and the other with 300 ppb gaseous ozone with a flow rate of 1.0 L min^{-1} for 8 h per day (overnight), and another room (8.0 m^3) using normal atmospheric air was used for the control samples. An ozone probe (electrochemical sensor) to check the concentration of ozone gas in the cold rooms was used. Ten wheels of pecorino cheese with an average weight of about 2.5 kg each were placed in three storage cells. The trial was conducted for 150 days (5 months) under controlled laboratory conditions. From the arrival of the samples and approximately every 25 days, counts of live and dead mites, total bacteria, yeasts, and molds and technological, qualitative, and sensorial analyses were carried out. Three replications of each sample treatment were analyzed at each sampling time.

Centesimal composition

Moisture, dry matter, and ash were determined according to the AOAC procedures.[24] In brief, 3 g of each sample were prepared into weighing pods and put in vacuum oven kept at 100 °C. The samples were dried to constant weight for 4 h under pressure of about 100 mmHg (13.3 kPa). Subsequently, the samples in the dishes were allowed to cool and weighed to determine the weight loss. For the ash determination, 3 g samples were subjected to a temperature of 550 °C for 12 h, then cooled and weighed.

Fat was determined by the Van Gulik method:[25] 3 g of each sample were finely chopped and added with 10 mL sulfuric acid and 1 mL amyl alcohol into the butyrometers. Fat is calculated as percentage (grams per 100 g) fresh weight (FW).

Total nitrogen and crude protein contents were determined by standard procedures using the Kjeldahl method:[26] to 0.5 g of each sample were added a catalyst tablet and 6 mL concentrated (95–98%) sulfuric acid. After digestion, the cooled samples were distilled with 50 mL of 30% sodium hydroxide. NaOH reacts the NH_4+ to NH_3, which boils off the sample solution. Ammonia bubbles through the standard acid solution and reacts back to ammonium salts with the weak or strong acid. The amount of nitrogen in the sample, is measured *via* titration with a solution of 0.1 mol L^{-1} hydrochloric acid. The total nitrogen and crude protein contents are expressed as the percentage (grams per 100 g) FW of cheese.

Technological analysis

The firmness of pecorino cheese was evaluated by a compression test with an Instron Universal Testing Machine (mod. 4301; Instron Inc., Canton, MS, USA). Each experimental cheese was cut into three segments from which cylindrical samples (22 mm diameter and 5.0 cm height) were obtained using a stainless-steel tube. These pieces were taken in all assays from the edge (1.0 cm of the external surface to the middle part of cheese). The samples were compressed using a compression platen (55 mm diameter) at a speed of 50 mm min^{-1}, and the compression distance was 25% of the sample height. Firmness was expressed in newtons.

The same segments of cheese used for firmness tests were also used for color assessment with a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to determine chromaticity values L^* (lightness), a^* (green to red), and b^* (blue to yellow). The hue angle H^* and chroma C^* values were calculated as reported by McGuire.[27] We also measured the color difference CIE 1976, $\Delta E^* = \left(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}\right)^{1/2}$, among the color data for each sample taken at every sampling time from the beginning of the storage. The value of ΔE^* expresses the difference between two colors numerically as follows: imperceptible ($\Delta E^* < 1$), minimal ($1 \le \Delta E^* < 2$), just perceptible ($2 \le \Delta E^* < 3$), perceptible ($3 \le \Delta E^* < 5$), strong difference ($5 \le \Delta E^* < 12$), and different color ($\Delta E^* \ge 12$).[28]

Weight loss

Before storage, at each sampling time, three cheese samples per treatment were weighed using a technical balance (Adam Equipment Co. Ltd, Milton Keynes, UK) to monitor weight loss during storage. The percentage weight loss (WL) was determined according to the following equation:

$$\mathrm{WL} = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the initial sample mass and W_t is the sample mass at time t.

Microbiological analysis

Total bacteria count (TBC) and yeasts and mold count (YMC) of samples were evaluated according to El Owni and Hamid[29] and ISO 6611[30] respectively. With a 1 cm diameter round, three subsamples were taken from each cheese wheel (2.35 cm^2). The soft cheese paste was eliminated, selecting only the rind. The rounds were homogenized with Stomacher® for 5 min in a saline buffer (0.1% sodium chloride). The homogenized product obtained was plated as is and diluted up to three decimal dilutions on 90 mm Petri dishes. Two different media were used: plate count agar to determine TBC, and yeast extract agar to determine YMC.

To determine TBC, plates were incubated at 33 °C for 48 h. Plates were incubated at 25 °C for 5 days for yeast and mold determination. Three samples were measured for each biological replicate and were reported as the number of colony-forming units (log CFU cm^{-2} cheese rind). Plates were selected for the count with the dilution showing a number of colonies between 30 and 300.

Mite investigation

The count of live and dead mites on the cheese surface of control and ozone-treated samples was determined by visual inspection using a stereomicroscope to display 1 cm^2 of cheese crust at each sampling time. If the fungal mycelium covered the cheese crust conspicuously, it was removed to check for mites below it. The area affected by the analysis was 3 cm^2 (three replicates per sample). The determination of dead mites was carried out through visual inspection and touching of the mites. They were considered dead when lack of movement was observed.

Sensory evaluation

For sensory evaluation, the control and the 200 and 300 ppb ozone-treated samples of Pecorino Foggiano cheese were evaluated at 50, 100, and 150 days. The consumer test was performed through a quantitative affective method at the sensory analysis laboratory of the University of Tuscia, according to the ISO 8589: 2007 standard.[31] The study involved 16 subjects (nine females, seven males; age range: 19–60 years; Italians) who had been asked not to eat, drink, smoke, or apply perfume 1 h before the evaluation sessions. The samples were taken from the subcrust to the central part of each cheese and cut into parallelepipeds of approximately 15.0 g and were placed in white plastic dishes. The acceptability of products by the consumer was assessed using a hedonistic scale of 1 to 5 (1: extremely bad; 2: bad; 3: normal, at the limit of consumption; 4: good; 5: excellent) for different sensory descriptors: visual aspect, flavor, taste, texture, overall liking.

Statistical analysis

All results were expressed as the means plus/minus standard error. The significant differences between the storage time and the type of treatment of cheese samples were analyzed by analysis of variance, and Tukey's test at the 5% level was calculated to compare differences between means using IBM® SPSS® Statistics. Differences at $P < 0.05$ were considered significant and are indicated with different letters.

RESULTS AND DISCUSSION

Weight loss

All the samples analyzed gradually lost weight, regardless of the type of treatment (Fig. 1). A reduction in weight of about 9–10% was observed at the end of storage (150 days): 9.8%, 9.0%, and 9.4% respectively for the control and the 200 ppb and 300 ppb ozone-treated samples. The control sample showed slightly higher percentage values throughout the storage period but did not show statistically significant differences with samples treated with ozone.

Centesimal composition

Compared with the initial time, the moisture content decreased and the dry matter increased during ripening for all cheeses and

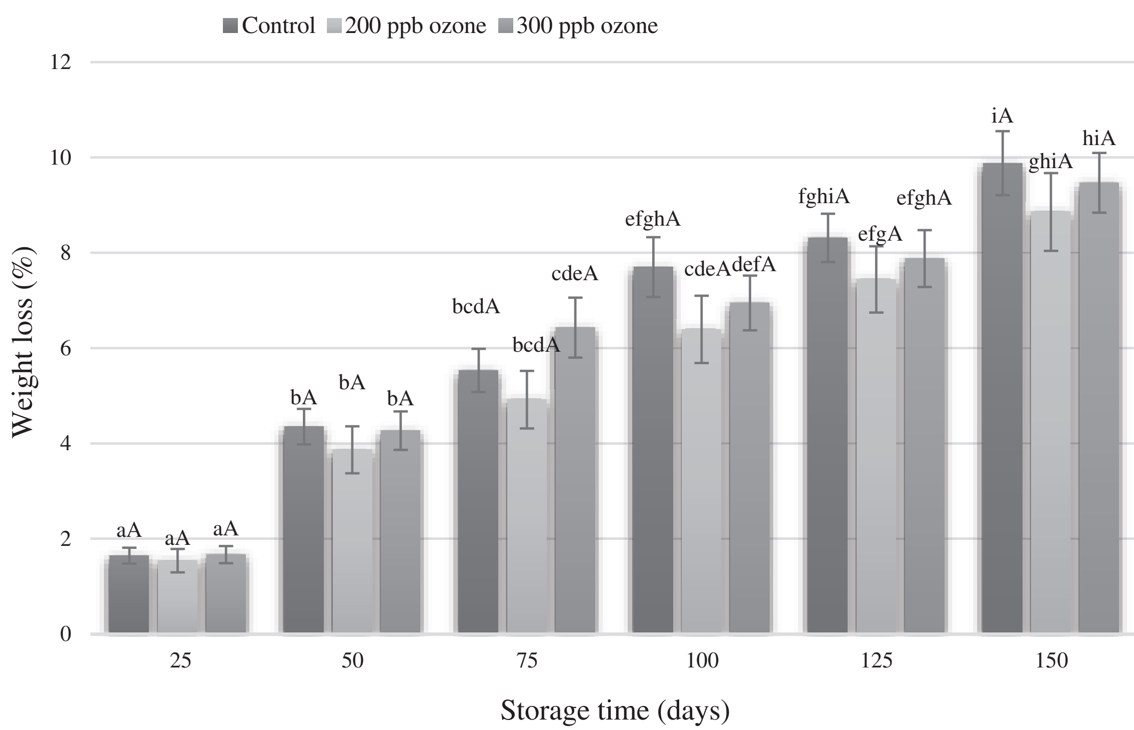

Figure 1. Weight loss in pecorino cheese at 25, 50, 75, 100, 125, and 150 days of storage at 12 °C and 85% relative humidity, in normal atmosphere (control) and gaseous ozone at 200 and 300 ppb. Different lowercase letters indicate significant differences during ripening time ($P < 0.05$). Different capital letters for each time indicate significant differences between cheeses with different treatments ($P < 0.05$).

Table 1. Centesimal composition in pecorino cheese at 0, 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone (O_3) at 200 and 300 ppb

Item	Treatment	0 days	25 days	50 days	75 days	100 days	125 days	150 days
Dry matter (%)	Control	65.2 ± 0.007 (bcdA)	65.1 ± 0.6 (abcdA)	64.2 ± 0.4 (abcAB)	64.2 ± 0.37 (abcA)	69 ± 0.38 (hiA)	66 ± 0.01 (efgA)	68.2 ± 0.06 (fghA)
	200 ppb O_3		64.6 ± 0.94 (abcA)	62.7 ± 0.61 (aA)	67 ± 0.90 (efgB)	72.5 ± 0.12 (lB)	69.5 ± 0.37 (hiB)	68.6 ± 0.61 (ghA)
	300 ppb O_3		63.1 ± 0.14 (abA)	65.5 ± 0.37 (defB)	65.4 ± 0.57 (cdeAB)	71.7 ± 0.27 (ilB)	66.2 ± 0.09 (cdeA)	72 ± 0.15 (lB)
Moisture (%)	Control	34.7 ± 0.46 (ghiA)	34.8 ± 0.6 (ghilA)	35.7 ± 0.4 (hilAB)	35.7 ± 0.37 (hilB)	30.2 ± 0.38 (bcB)	34 ± 0.01 (defB)	31.7 ± 0.06 (cdeB)
	200 ppb O_3		35.4 ± 0.94 (hilA)	37. 2 ± 0.6 (lB)	33.0 ± 0.9 (defA)	27.4 ± 0.12 (aA)	30.4 ± 0.37 (bcA)	31.3 ± 0.61 (cdeB)
	300 ppb O_3		36.8 ± 0.14 (ilA)	34.4 ± 0.37 (efgA)	34.5 ± 0.57 (fghAB)	28.2 ± 0.27 (abA)	33.7 ± 0.09 (fghB)	28 ± 0.15 (aA)
Ash (%)	Control	6.3 ± 0.09 (bA)	7.2 ± 0.06 (defB)	5.7 ± 0.05 (aA)	7.5 ± 0.2 (eB)	6.6 ± 0.13 (bcA)	7.5 ± 0.02 (efgB)	7.5 ± 0.01 (efgB)
	200 ppb O_3		6.6 ± 0.11 (bcA)	7.5 ± 0.40 (eC)	6.6 ± 0.02 bcA	6.8 ± 0.04 (cdA)	7.1 ± 0.07 (deA)	7.5 ± 0.1 (efgB)
	300 ppb O_3		6.5 ± 0.01 (bA)	6.7 ± 0.1 (bcB)	7.3 ± 0.07 (eB)	6.5 ± 0.15 (bcA)	7.5 ± 0.05 (gB)	6.5 ± 0.03 (bcA)
Fat (% FW)	Control	30 ± 0.005 (abA)	30 ± 0.003 (abA)	31 ± 0.008 (abA)	30 ± 0.003 (abA)	31 ± 0.005 (abA)	33 ± 0.005 (cB)	34 ± 0.005 (cA)
	200 ppb O_3		30 ± 0. 008 (abA)	32 ± 0.005 (bcA)	29 ± 0. 005 (aA)	31 ± 0.008 (abA)	30 ± 0.005 (abA)	32 ± 0.005 (bcA)
	300 ppb O_3		30 ± 0.005 (abA)	32 ± 0.005 (bcA)	29 ± 0.008 (aA)	30 ± 0.003 (aA)	30 ± 0.005 (abA)	32 ± 0.005 (bcA)
Nitrogen (% FW)	Control	4.15 ± 0.005 (abA)	4.14 ± 0.008 (abA)	4.18 ± 0.005 (bA)	4.17 ± 0.010 (abA)	4.18 ± 0.005 (abA)	4.16 ± 0.002 (abA)	4.17 ± 0.005 (abA)
	200 ppb O_3		4.14 ± 0.010 (aA)	4.17 ± 0.005 (abA)	4.18 ± 0.007 (abA)	4.17 ± 0.009 (abA)	4.16 ± 0.008 (abA)	4.17 ± 0.005 (abA)
	300 ppb O_3		4.15 ± 0.012 (abA)	4.15 ± 0.006 (abA)	4.16 ± 0.009 (abA)	4.17 ± 0.009 (abA)	4.16 ± 0.007 (abA)	4.17 ± 0.007 (abA)
Protein (% FW)	Control	26.47 ± 0.03 (abA)	26.45 ± 0.05 (abA)	26.72 ± 0.03 (bA)	26.66 ± 0.07 (abA)	26.7 ± 0.03 (abA)	26.58 ± 0.01 (abA)	26.63 ± 0.03(abA)
	200 ppb O_3		26.41 ± 0.04 (aA)	26.64 ± 0.03 (abA)	26.68 ± 0.04 (abA)	26.61 ± 0.05 (abA)	26.6 ± 0.05 (abA)	26.6 ± 0.03 (abA)
	300 ppb O_3		26.5 ± 0.04 (abA)	26.5 ± 0.06 (abA)	26.54 ± 0.05 (abA)	26.61 ± 0.04 (abA)	26.55 ± 0.04 (abA)	26.65 ± 0.04 (abA)

Note: FW: fresh weight. Different lowercase letters for the same row indicate significant differences during ripening time ($P < 0.05$). Different capital letters for the same column indicate significant differences between cheeses with different treatments ($P < 0.05$).

Table 2. Color parameters in pecorino cheese at 0, 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone (O_3) at 200 and 300 ppb

Parameter	Treatment	0 days	25 days	50 days	75 days	100 days	125 days	150 days
*L**	Control	70.6 ± 0.64 (abcdA)	73.4 ± 0.5 (defB)	70.3 ± 0.52 (abA)	71.1 ± 0.55 (abcdeAB)	73.1 ± 0.71 (bcdefA)	72.3 ± 0.38 (abcdefA)	72 ± 0.88 (abcdefA)
	200 ppb O_3		70 ± 0.23 (aA)	73.7 ± 0.52 (efB)	73.2 ± 0.5 (bcdefB)	71.7 ± 0.26 (abcdefA)	72.4 ± 0.4 (abcdefA)	71.5 ± 0.66 (cdefAB)
	300 ppb O_3		70.4 ± 0.55 (abcA)	71.2 ± 0.76 (abcdeA)	70.6 ± 0.78 (abcdA)	72.8 ± 0.51 (abcdefA)	73.2 ± 0.32 (abcdefA)	74.3 ± 0.5 (fB)
*a**	Control	−0.64 ± 0.05 (aA)	−0.12 ± 0.02 (eB)	−0.36 ± 0.06 (bcdeA)	−0.44 ± 0.05 (abcdA)	−0.25 ± 0.04 (deA)	−0.22 ± 0.03 (deA)	−0.25 ± 0.04 (deA)
	200 ppb O_3		−0.61 ± 0.03 (abA)	−0.17 ± 0.03 (eB)	−0.17 ± 0.05 (deB)	−0.28 ± 0.03 (cdeA)	−0.28 ± 0.06 (cdeA)	−0.26 ± 0.03 (deA)
	300 ppb O_3		−0.55 ± 0.03 (abcA)	−0.37 ± 0.04 (bcdeA)	−0.28 ± 0.05 (bcdeA)	−0.33 ± 0.06 (cdeA)	−0.23 ± 0.08 (deA)	−0.32 ± 0.04 (cdeA)
*b**	Control	20.86 ± 0.16 (abcdA)	22.02 ± 0.16 (dB)	21.51 ± 0.25 (cdB)	20.21 ± 0.15 (abA)	21 ± 0.17 (abcdA)	20.5 ± 0.17 (abcA)	19.81 ± 0.24 (aA)
	200 ppb O_3		21.5 ± 0.2 (bcdAB)	20.65 ± 0.11 (abcA)	20.71 ± 0.22 (abcA)	20.66 ± 0.15 (abA)	20.2 ± 0.5 (abA)	21 ± 0.18 (abcdA)
	300 ppb O_3		21.38 ± 0.1 (bcdA)	21.33 ± 0.2 (bcdAB)	20.38 ± 0.26 (bcA)	21.23 ± 0.27 (bcdA)	20.58 ± 0.28 (abcA)	20.16 ± 0.53 (babA)
*C**	Control	20.8 ± 0.16 (abcdA)	22 ± 0.16 (dB)	21.5 ± 0.24 (cdB)	20.2 ± 0.15 (abA)	21 ± 0.17 (abcdA)	20.5 ± 0.17 (abcA)	19.8 ± 0.24 (aA)
	200 ppb O_3		21.5 ± 0.2 (cdAB)	20.6 ± 0.11 (abcA)	20.7 ± 0.2 (abcA)	20.6 ± 0.15 (abcA)	20.2 ± 0.5 (abA)	21 ± 0.18 (abcdA)
	300 ppb O_3		21.4 ± 0.1 (bcdA)	21.3 ± 0.2 (bcdAB)	20.3 ± 0.26 (abcA)	21.2 ± 0.27 (bcdA)	20.5 ± 0.28 (abcA)	20.1 ± 0.52 (abA)
Hue	Control	271.7 ± 0.14 (abA)	270.3 ± 0.06 (abA)	271 ± 0.17 (abB)	271.2 ± 0.15 (abB)	270.7 ± 0.12 (bA)	270.6 ± 0.10 (abA)	270.7 ± 0.11 (abA)
	200 ppb O_3		271.6 ± 0.1 (abB)	270.4 ± 0.1 (abA)	270.4 ± 0.14 (abA)	270.8 ± 0.1 (abA)	270.8 ± 0.2 (abA)	270.7 ± 0.08 (abA)
	300 ppb O_3		271.4 ± 0.1 (abB)	271 ± 0.12 (abB)	270.8 ± 0.16 (abAB)	271 ± 0.17 (abA)	270.6 ± 0.2 (aA)	271 ± 0.12 (abA)
ΔE	Control	—	5 ± 0.47 (abB)	2.7 ± 1.06 (aA)	3 ± 0.42 (aA)	3.6 ± 0.88 (aA)	3 ± 0.81 (aA)	3 ± 0.81 (abA)
	200 ppb O_3	—	2.4 ± 0.57 (aA)	3.6 ± 0.71 (abA)	3.5 ± 0.58 (aA)	2.5 ± 0.40 (aA)	4.7 ± 1.09 (abA)	4.7 ± 1.09 (aA)
	300 ppb O_3	—	2.1 ± 0.37 (aA)	3.7 ± 0.50 (abA)	3.3 ± 0.66 (aA)	4.4 ± 0.86 (abA)	3.5 ± 0.64 (aA)	4.2 ± 0.68 (bA)

Note: Different lowercase letters for the same row indicate significant differences during ripening time ($P < 0.05$). Different capital letters for the same column indicate significant differences between cheeses with different treatments ($P < 0.05$).

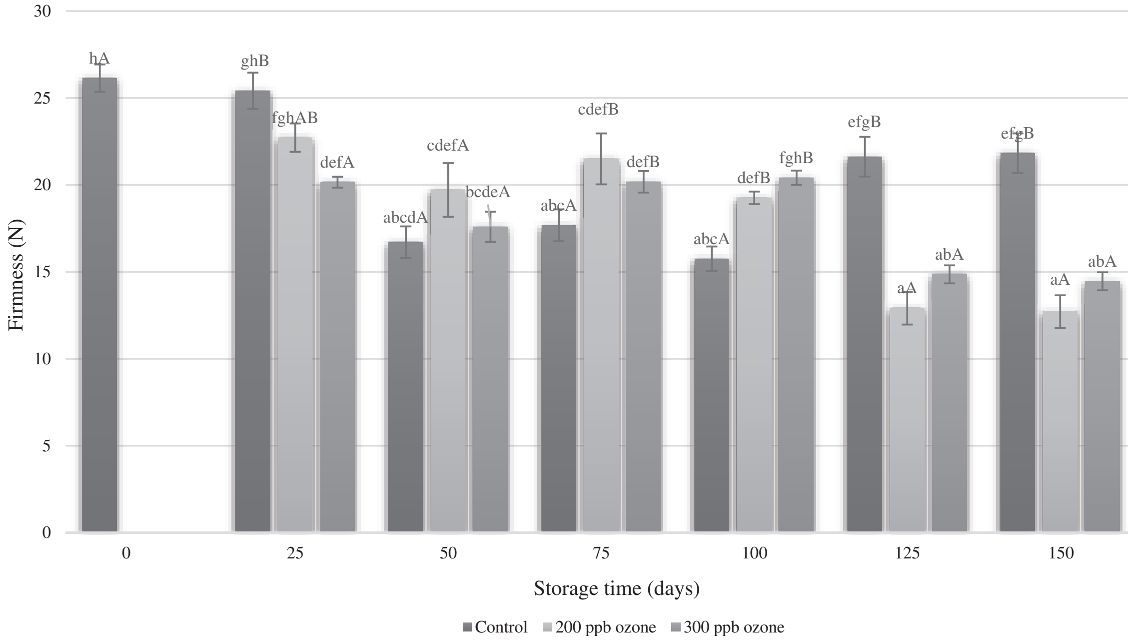

Figure 2. Firmness evolution in pecorino cheese at 0, 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone at 200 and 300 ppb. Different lowercase letters indicate significant differences during ripening time ($P < 0.05$). Different capital letters for each time indicate significant differences between cheeses with different treatments ($P < 0.05$).

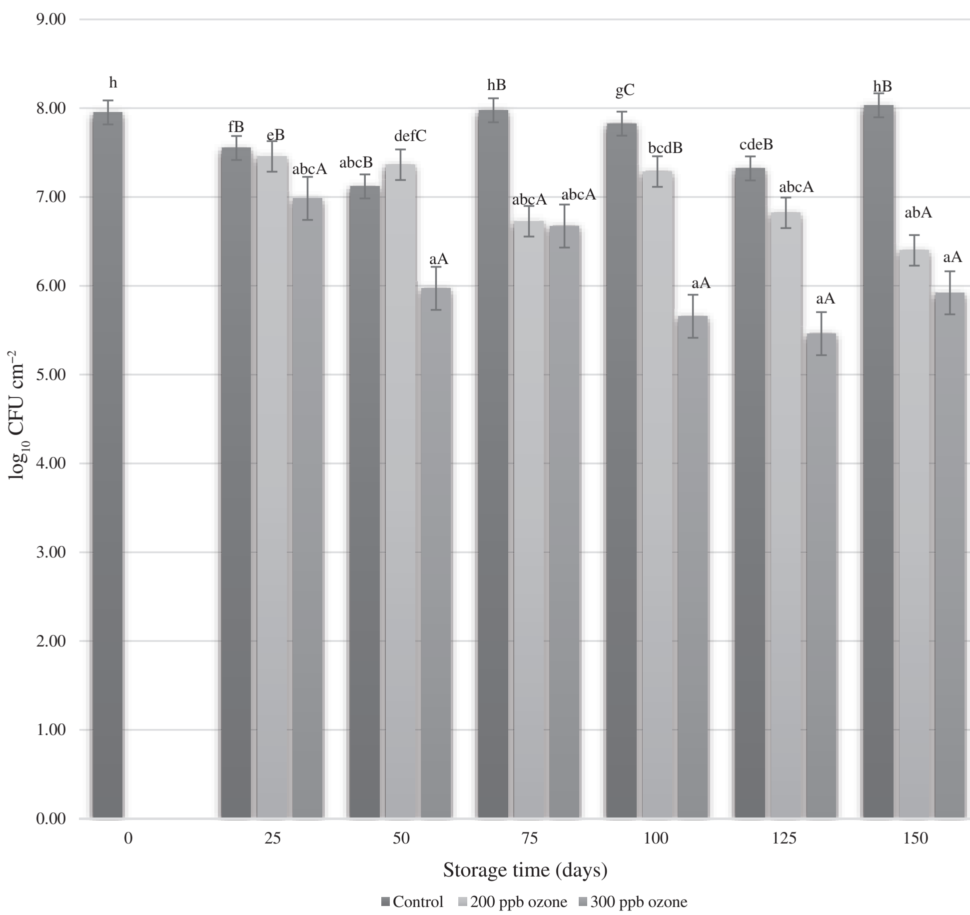

Figure 3. Total bacteria count at 0, 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone at 200 and 300 ppb. Different lowercase letters indicate significant differences during ripening time ($P < 0.05$). Different capital letters for each time indicate significant differences between cheeses with different treatments ($P < 0.05$).

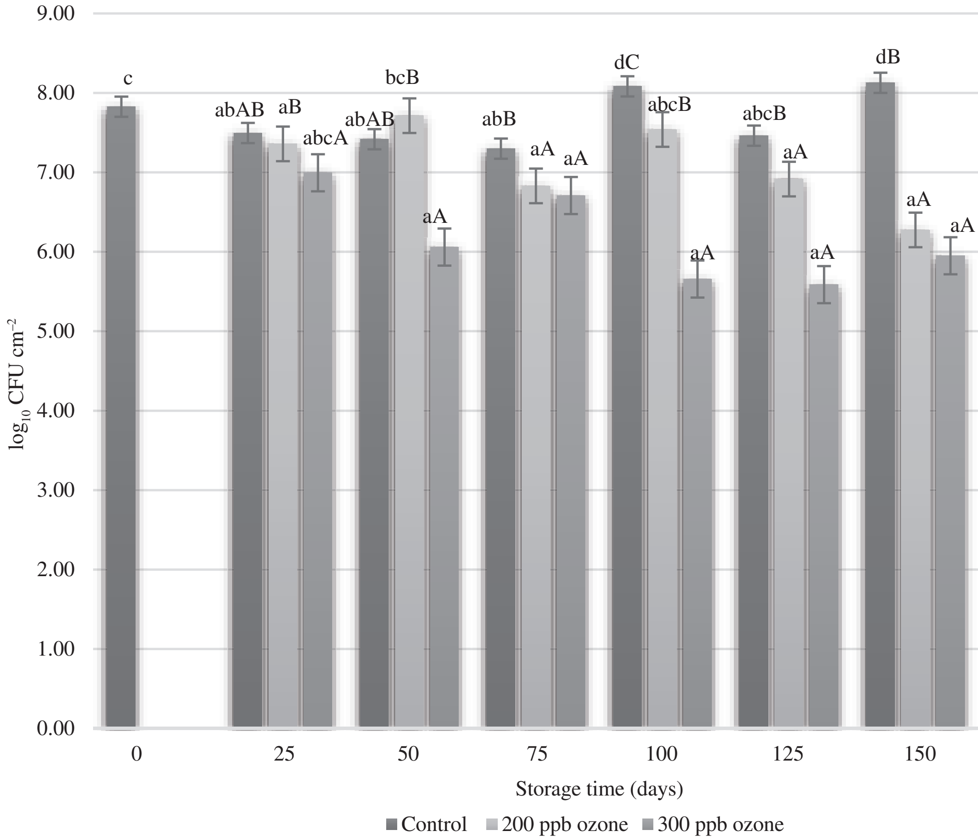

Figure 4. Yeasts and molds count at 0, 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone at 200 and 300 ppb. Different lowercase letters indicate significant differences during ripening time ($P < 0.05$). Different capital letters for each time indicate significant differences between cheeses with different treatments ($P < 0.05$).

Table 3. Mite count at 25, 50, 75, 100, 125, and 150 days of storage in normal atmosphere (control) and gaseous ozone (O_3) at 200 and 300 ppb. Different lowercase letters indicate significant differences during ripening time ($P < 0.05$). Different capital letters for each time indicate significant differences between cheeses with different treatments ($P < 0.05$)

	Mite number						
Treatment	0 days	25 days	50 days	75 days	100 days	125 days	150 days
Control	22 ± 2 (a)	19 ± 5 (cdA)	18 ± 5 (bA)	15 ± 2 (dA)	15 ± 3 (dA)	3 ± 1 (dA)	5 ± 1 (abA)
200 ppb O_3	—	1 ± 0 (cdB)	5 ± 2 (dB)	0 ± 0 (dB)	0 ± 0 (dB)	0 ± 0 (dB)	1 ± 1 (dB)
300 ppb O_3	—	2 ± 1 (aB)	0 ± 0 (aC)	0 ± 0 (aB)	0 ± 0 (aB)	0 ± 0 (aB)	0 ± 0 (aB)

showed statistically significant differences at the end of the storage.

As shown in Table 1, cheese samples had moisture and dry matter contents of about 35% and 65% respectively at the beginning of the test. After 5 months of storage, the moisture content decreased by 3.0–3.5% in the control test and in samples treated with 200 ppb ozone without significant differences between them. In the same way, a corresponding increase of dry matter was observed in these two samples (3.0–3.4%). However, at the end of storage, the moisture and dry matter contents of samples treated with 300 ppb of ozone showed significantly different values (6.7–6.8%) compared with the control and 200 ppb ozone-treated samples. The results obtained showed a not-constant trend in the ash content for all cheeses (Table 1). The ash of the cheeses treated with a higher concentration of ozone was lower than the control samples and those treated with 200 ppb at the end of storage. At the beginning of the test, the fat content was 30% and remained almost constant throughout the ripening period. In the last month of storage, the control samples showed a slightly higher fat content than ozone-treated samples did, but no significant differences were found regarding the type of treatment (Table 1). To evaluate the ozone effect on cheese quality, the total nitrogen and crude protein content were measured (Table 1). The results obtained for these components showed the same trend throughout the storage period. The total nitrogen and crude protein content in cheese did not changed in relation to either the ripening time or the type of treatment. As reported by Cavalcante *et al.*[32] the gaseous ozone oxidant activity was ineffective on fat and protein content of raw milk treated with 0.5 mg L^{-1} ozone up to 15 min.

Technological analysis

Appearance and texture are important indicators used to evaluate cheese quality and are considered primary quality attributes in forming a general opinion and preference by consumers. Color may have a fundamental role in the perception of flavor and texture of cheese food.[33]

The results in Table 2 show the effect of ozone on cheese color during the storage ripening. These results illustrate the changes in color of pecorino cheese in terms of redness a^*, yellowness b^*, and lightness L^* during 150 days of ripening. L^* values tended to increase with storage time in both treated and untreated samples. At the end of storage, control samples showed significant differences from samples treated with ozone at 300 ppb. However, samples treated with the two different ozone concentrations showed no significant differences between them.

The values of redness a^* decreased and the values of yellowness b^* were constant throughout the ripening period (Table 2). Ginzinger *et al.*[34] reported that the yellowness index indicates a prevalence of yellow in cheese and increases during ripening cheese. In our experiment, homogeneous values of chromaticity b^* were found in treated samples and in control cheeses, without significant differences depending on the type of treatment and the storage time. The trend observed contrasted with the results of other studies that showed a decrease in luminosity and an increase in b^*, as on Gouda and scamorza cheeses during ripening.[35,36] Meanwhile, another study reported that ozone treatment significantly reduced the β-carotene content of the milk, resulting in a significant increase in the lightness value and a parallel decrease in the yellowness of milk.[37] In addition, our data showed that the perceived color H^* (gradient or tonality) and C^* (intensity of the color) values remained steady during ripening of the cheese (no yellowing/browning during ripening), without significant differences for several treatments. No significant differences were found in the total color differences ΔE based either on storage time or on the type of treatment. The results showed that, in each sample analyzed, regardless of the treatment and the storage period, ΔE^* values of 5 are never exceeded, which indicates a ΔE^* color with a definition as 'perceptible' value for all samples ($3 \le \Delta E^* < 5$) (Table 2).

Texture is a property of cheese that, together with appearance, flavor, and nutritional values, affects consumer acceptance. Protein, fat, and moisture were the three foremost elements that directly influenced textural and functional properties of cheese.[38,39] To study the effect of product variability and to simulate the subjective firmness evaluation by consumers, technological tests from each cheese sample were analyzed nondestructively with the Instron apparatus. Our data showed all the samples followed the same trend up to 3 months of storage. The samples treated with ozone showed a significant decrease starting from 125 days, and these samples were found to have a more fragmented texture than the control samples (Fig. 2). Research on pecorino cheese showed that long storage could lead to rapid loss of firmness. *Miraglia et al.* showed that casein hydrolysis caused a decrease in firmness and that lipases increased the degree of lipolysis, resulting in a simultaneous decrease of fat content.[40] The study of Sert and Mercan[41] on milk and whey protein concentrates showed that ozone treatment significantly decreases the values of the textural properties, probably due to the aggregation of milk proteins because of the destabilization of protein structure by ozone. The results of texture values on butter production showed that ozone treatment increased the textural properties of samples caused by fat aggregation, which resulted in a higher viscosity than the untreated sample.[42] Other researchers reported that treatment with gaseous ozone did not change the textural properties of dairy fermented products.[43]

Microbiological analysis

The results of our study revealed that the number of CFU cm^{-2} of bacteria, yeasts, and molds decreased in the samples treated with gaseous ozone at 200 and 300 ppb compared with the control test (Figs 3, 4). Namely, for both TBC and YMC, a significant reduction in microbial growth was observed in the ozone-treated samples starting from day 25 for the 300 ppb sample and day 75 for

Table 4. Consumers' liking mean values for the control and the ozone (O_3)-treated samples (200 and 300 ppb) at 50, 100, and 150 days

Sensory descriptor	Treatment	50 days	100 days	150 days
Visual aspect	Control	4.2 ± 0.4 (aA)	4.3 ± 0.5 (aA)	4.0 ± 0.6 (aA)
	200 ppb O_3	3.8 ± 0.2 (aA)	4.3 ± 0.3 (aA)	4.7 ± 0.3 (aA)
	300 ppb O_3	3.8 ± 0.2 (aA)	4.0 ± 0 (aA)	4.0 ± 0.6 (aA)
Flavor	Control	3.8 ± 0.6 (aA)	4.3 ± 0.3 (aA)	3.7 ± 0.3 (aA)
	200 ppb O_3	4.2 ± 0.4 (aA)	3.8 ± 0.6 (aA)	3.7 ± 0.7 (aA)
	300 ppb O_3	4.6 ± 0.2 (aA)	4.0 ± 0.4 (aA)	4.0 ± 0 (aA)
Taste	Control	3.8 ± 0.6 (aA)	4.5 ± 0.5 (aA)	4.3 ± 0.3 (aA)
	200 ppb O_3	3.4 ± 0.2 (aA)	3.8 ± 0.8 (aA)	3.7 ± 0.3 (aA)
	300 ppb O_3	4.2 ± 0.2 (aA)	3.8 ± 0.5 (aA)	4.0 ± 0 (aA)
Texture	Control	4.2 ± 0.4 (aA)	3.5 ± 0.9 (aA)	4.7 ± 0.3(aA)
	200 ppb O_3	4.0 ± 0.4 (aA)	3.5 ± 0.5 (aA)	4.7 ± 0.3 (aA)
	300 ppb O_3	4.0 ± 0.3 (aA)	3.8 ± 0.3 (aA)	4.3 ± 0.3 (aA)
Overall liking	Control	4.2 ± 0.4 (aA)	4.8 ± 0.3 (aA)	4.3 ± 0.3 (aA)
	200 ppb O_3	4.0 ± 0 (aA)	3.8 ± 0.5 (aA)	4.3 ± 0.3 (aA)
	300 ppb O_3	4.0 ± 0.3 (aA)	3.8 ± 0.3 (aA)	4.0 ± 0 (aA)

Note: Different lowercase letters for the same row indicate significant differences during ripening time ($P < 0.05$). Different capital letters for the same column indicate significant differences between cheeses with different treatments ($P < 0.05$).

the 200 ppb sample up to the end of the storage period. Bacterial colony growth in the control sample was observed to remain fairly constant during ripening (Fig. 3), whereas it tended to increase significantly over time for the yeast and mold control sample (Fig. 4). Our results agree with what has been observed in other studies on dairy products, including Alexopoulos *et al*.[43] in which the treatment with 2.5–3 ppm ozone reduced microbial growth a few days after the treatment or in the prolonged exposure to ozone treatment.[44] Several studies on the use of ozone treatments to reduce microbial contamination include the study by Segat *et al*.[45] on Mozzarella stored in cold bags with ozonated water and research by Cavalcante *et al*.[46] on Minas Frescal cheese sanitized with ozonated water.

Mite investigation

The number of mites exposed to different ozone concentrations are reported in Table 3. The results showed that ozone treatment at 200 and 300 ppb dramatically reduced the number of mites on the treated samples compared with the control. Regarding the data trend, no significant difference was observed during ripening between the results of the ozone treatment samples, whereas a notable difference was verified between the control and the treated samples. In the period from 25 to 50 days and beyond through to the end of storage, the mites found on the cheese's superficial sides are reduced to a few units (200 ppb ozone) and/or were totally eliminated (300 ppb ozone) in the two ozone-treated samples. Apart from the wide spectrum of microbial control, ozone can potentially kill the mites, while excess ozone auto decomposes quickly to produce oxygen and thus leaves no harmful residues in the food.

The literature reports a small number of studies concerning the use of innovative methods for the control of spoilage on cheeses. Among these, one study highlights different chemical and physical methods that were able to control the *Acarus farris* pest in Cabrales cheese.[15] Another work on Cheddar cheese seasoned in caves suggests an experiment using coatings or nets on the superficial sides of cheese (xanthan gum, carrageenan, etc.) to prevent pest infestation by *Tyrophagus putrescentiae* under different environmental conditions (10, 15, and 20 °C and 75 ± 2 and 85 ± 2% relative humidity).[47] Additional research explored the use of controlled atmospheres for the management of arthropods infesting cured hams.[48] Concerning ozone treatment, a specific review investigates its effectiveness on multiple contaminants (bacteria, yeasts, fungi, insects, and mites) on different foods.[49]

Sensory evaluation

For Pecorino Foggiano, a ripening period between 2 and 3 months in the storage rooms is an appropriate threshold to start the marketing of the product. For this reason, we decided to begin sensory testing after 50 days from the start of cheese storage (80 days from cheese production). The mean values of the consumer test for the pecorino are shown in Table 4. Regarding the overall results, untreated and treated products were accepted by consumers, with mean values ranging from 3.8 to 4.8. The preferred sample in respect to the overall score was the control at 100 days, followed by the control and 200 ppb ozone-treated sample at 150 days. The results obtained for all sensory descriptors (visual aspect, flavor, taste, texture, overall liking) showed no significantly different values at each storage time, regardless of the treatment type. Therefore, the use of ozone technology on sensorial quality of pecorino cheese did not manifest in changes in the acceptability of the final product. According to the study of Alexopoulos *et al*.[43] there were no significant differences in sensorial attributes of untreated and ozone-treated (2.5–3 ppm) freshly filled yoghurt cups for 0, 10, 30, and 60 s with regard to flavor, texture color and overall acceptability. In contrast, significant differences were found on feta cheese samples treated with 2.5–3 ppm for 10, 20, and 30 min concerning the flavor and overall acceptance. Kurtz *et al*.[50] reported that no flavor defects or adverse effects were observed on the organoleptic properties of powdered milk products treated with high concentrations of ozone (2.0 ppm). The literature shows very few studies on ozone treatments in cheeses for sensory-associated analysis. Moreover, in these studies, ozone treatments were carried out with much higher concentrations than those used in our experimental trial.

CONCLUSION

The use of gaseous ozone treatment during ripening of Pecorino Foggiano cheese can offer an excellent solution for the control of mite growth, and this technique could be useful for commercial purposes of other ripened cheese products that face similar issues. The contents of bacteria, yeast, and mold – the latter notably being responsible for commercial downgrading of the product – are limited by ozone treatments during ripening. As the control, the overall quality (physicochemical and sensory properties) of pecorino cheese treated with ozone at 200 ppb is found to be well preserved, but this was not observed in the sample treated with 300 ppb ozone.

From an industrial perspective, the application of ozone during cheese ripening is an eco-friendly and sustainable technology that contributes to reducing the usual 'washing' operations to be carried out by hand and/or with suitable tools. Overall, the control of mite growth with ozone can therefore provide (1) an improvement in food sanitation and safety company conditions and (2) a significant economic benefit with regard to the value of the product, due to the possible containment of weight loss during cheese ripening, and for the dairy supply-chain procedures, due to the reduced necessity of the work force.

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CONFLICT OF INTEREST

We confirm that no conflict of interest exists for any of the authors.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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