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RESEARCH ARTICLE

The Effect of Atmospheric Cold Plasma (ACP) Application on the Physicochemical and Microbiological Quality of Steak Obtained from Beef *Musculus Longissimus Dorsi* Muscle

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ABSTRACT

This study examined the effect of atmospheric cold plasma (ACP) application on the microbiological and physicochemical properties of steaks obtained from beef *Musculus longissimus dorsi* muscle. In the ACP application, O_2 and Ar gases were applied to the samples separately and as a mixture for 15 and 30 minutes, respectively. While the application decreased the pH and aw of the samples, it caused an increase in TBARS values. In addition, there was a decrease in the L*, a*, and b* values of the samples in other ACP applications, except for applications using O_2 gas. Apart from this, the application was effective in the positive development of the textural properties of the samples. In steak samples obtained from the *M. longissimus dorsi* muscle, ACP application reduced the total mesophilic aerobic bacteria, total psychrophilic aerobic bacteria, total coliform, and total yeast/mold counts by an average of 2 log cfu/g.

Key words: : Cold plasma, *M. longissimus dorsi*, Meat, Quality

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Dana *Musculus Longissimus Dorsi* Kasından Elde Edilen Bifteklerin, Fizikokimyasal ve Mikrobiyolojik Kalitesi Üzerine Atmosferik Soğuk Plazma (ASP) Uygulamasının Etkisi

ÖZ

Bu çalışmada atmosferik soğuk plazma (ASP) uygulamasının sığır *Musculus longissimus dorsi* kasından elde edilen bifteklerin mikrobiyolojik ve fizikokimyasal özellikleri üzerine etkisi incelenmiştir. ASP uygulamasında O_2 ve Ar gazları ayrı ayrı ve karışım halinde, 15 ve 30 dk süreyle örneklere uygulanmıştır. Uygulama örneklerin pH ve a_w düşürürken, TBARS değerlerinde artışa neden olmuştur. Ayrıca, O_2 gazı kullanılan uygulamalar hariç diğer ASP uygulamalarında örneklerin L*, a* ve b* değerlerinde azalma meydana gelmiştir. Ek olarak uygulama örneklerin tekstürel özelliklerin olumlu gelişmesinde etkili olmuştur. *M. longissimus dorsi* kasından elde edilen biftek örneklerine ASP uygulaması toplam mezofilik aerobik bakteri, toplam psikrofilik aerobik bakteri, toplam koliform ve toplam maya/küf sayılarında ortalama 2 log kob/g oranında azalma sağlamıştır.

Anahtar Kelimeler: Soğuk plazma, *M. longissimus dorsi*, Biftek, Kalite

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INTRODUCTION

Meat and meat products are an essential food category that can provide energy and nutrition for people (Wang et al. 2023). Meat is an indispensable part of the daily diet due to the valuable nutritional components it contains. Meat consumption increases daily depending on the increasing world population and purchasing power. Meat and meat products are ideal environments for the growth and spread of microorganisms that cause meat spoilage and common foodborne pathogens. This is because they have high-quality protein content, essential amino acids, B-group vitamins, minerals, and other vital nutritional elements (Ji et al. 2023). Therefore, ensuring microbial decontamination and inactivation in meat and meat products is important for food safety. Thermal methods are commonly used for microbial inactivation. However, studies have shown that traditional thermal processes such as pasteurization, sterilization, cooking, drying, etc., can neutralize foodborne pathogens but may have a negative effect on the nutritional value and sensory qualities of the food (Jayasena et al. 2015). Meat, on the one hand, can quickly spoil due to its structure, and on the other hand, it reacts very sensitively to decontamination processes (Fröhling et al. 2012). Studies on alternative decontamination methods to heat treatment have gained momentum in recent years. Atmospheric cold plasma (ACP) is an emerging non-thermal decontamination technology (Gao et al. 2021). Plasma is literally an ionized gas defined as the fourth state of matter, along with solids, liquids, and gases (Jung et al., 2017) Cold plasma can be produced by a variety of electrical discharges, such as DC glow discharge, radio frequency (RF) discharge, dielectric barrier discharge (DBD), atmospheric pressure plasma jet (APPJ), microwave, and pulsed power discharge (Albertos et al. 2017). ACP production provides a rich mixture of reactive neutral species, energetically charged particles, UV photons, and intense transient electric fields that can interact simultaneously and synergistically on the food surface (Bauer et al. 2017). The resulting antimicrobial effects caused by reactive species such as radicals, ions, and other chemical compounds enable application for food surface decontamination (Fröhling et al. 2012).

Non-thermal cold plasma technology is a green technique for many food industries, such as inactivating microorganisms and enzymes, removing toxins, and degrading pesticides (Asl et al. 2022).

This study aimed to investigate the effect of the use of ACP, a new green technology, with different gases on the physicochemical and microbiological properties of steaks obtained from beef *M. longissimus dorsi* muscle during the storage period.

MATERIALS and METHODS

Material

The beef *Musculus longissimus dorsi* used in the study was obtained from a local slaughterhouse operating in Afyonkarahisar. The raw material was brought to Afyon Kocatepe University Food Engineering Department laboratory under the cold chain. Before the application, the meat was cut into steaks measuring 15 x 15 x 5 mm (length x width x thickness) with the help of a sterile knife.

Atmospheric Cold Plasma (ACP) Application

ACP application was made according to the method of Akarca et al. (2023). The gases used in the application were supplied from Afyonkarahisar province (Kocaşaban Gases Corp., Afyonkarahisar, Turkey). The prepared samples were exposed to ACP application with 100% O_2, 100% Ar, and 50% O_2 - 50% Ar gas mixture for 15 and 30 minutes. The application was made in a semicircular glass chamber with a radius of 28 mm. The power supply used was 25 kV, 42 kHz frequency, and the system was operated in continuous mode. Each application was carried out in two recurrences and two parallels.

Physicochemical Analysis

pH values of meat samples exposed to ACP were determined using a pH meter (Hanna, HI 2215 pH/ORP meter) while water activity (a_w) of the samples were determined using a water activity device (Novasina LabTouch-aw, Lachen, Switzerland) (AOAC 2016). TBARS value, as an indicator of lipid oxidation, was determined according to Gao et al. (2021). For this, 5 g of sample was used, and absorbance was measured at 531 nm. TBARS values were calculated from the standard curve and expressed as mg malondialdehyde (MDA)/kg meat.

Color values (L^*, a^* and b^*) of meat samples were measured using a colorimeter (Minolta Chroma Meter CR-400, Osaka) (AOAC, 2016).

Texture profile analysis (TPA) measurements of the samples were performed with Stable Micro Systems Texture Analyzer (Stable Micro Systems, Surrey, England). Measurements were performed using a 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). Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, resilience, and shear force values were determined by compressing the samples to 50% of their original height for 5 seconds between two compressions (Akarca et al. 2023), and the cutting force was determined according to Kim et al. (2014) using the TA-XT2 texture analyzer.

Microbiological Analysis

To determine the microbiological quality of the samples exposed to ACP application during the

storage period, total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAPB), total coliform group bacteria (TCGB), and total yeast mold (TYM) counts were determined.

For this purpose, 10 g of weighed meat sample was taken into a sterile stomacher bag (BagMixer® 400 P-080921247), and 90 mL of Ringer's solution (Merck, 115525, Germany) was added and homogenized for 2 minutes, then serial dilutions were prepared, and the samples were prepared for analysis (Anonymous 2001).

To determine the TAMB and TAPB numbers, 0.1 mL of sample from each of the prepared dilutions was taken with the help of a sterile pipette (Eppendorf, Research plus, Germany) and cultivated on plate count agar (Oxoid, CM0325) using the spread plate technique. The cultivated petri dishes were incubated at 30°C for 72 hours to determine the number of TAMB under aerobic conditions, and at 4°C for 5-7 days to determine the number of TAPB, and the colonies that developed at the end of the incubation were counted and the counts of TAMB and TAPB were determined (ISO 2013a; ISO 2013b, Halkman 2005). Violet Red Bile (VRB) Agar (Merck, Germany, 1.01406) medium was used for TCGB count. Petri dishes were incubated in an incubator (MM Incucell 55, Germany) for 24-48 hours at 30°C under anaerobic conditions, and the TCGB number was determined at the end of the period (ISO 1991). Potato Dextrose Agar (PDA) (Merck, Germany, 1.10130) medium was used for TYM count and the colonies were counted by incubating the cultivated petri dishes in an incubator (MM Incucell 55, Germany) at 22°C for 5-7 days under aerobic conditions. (ISO 2008).

Statistical analysis

The results obtained in the study were made in two 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

The changes in pH, aw, and TBARS values of the samples stored after ACP application during storage are given in Table 1. According to the variation analysis results, it was revealed that the effects of both sample type and storage time interactions were very highly significant on pH, aw and TBARS values ($p<0.0001$). According to the correlation analysis results, sample type and storage time had a negative correlative effect on pH and aw values and a very positive correlative effect on TBARS value.

It was determined that the lowest pH value was (5.05) in the ACP-applied samples using a mixture of 50% O_2 + 50% Ar gas for 30 minutes. Additionally, it was determined that the pH values of the samples decreased during the storage period ($p<0.05$). On the last day of storage, it was determined that the lowest pH value was 5.05 in the samples with ACP-applied, which was also made using a 50% O_2 + 50% Ar gas mixture. On the other hand, it was revealed that the highest pH value was in the control sample (5.30).

Similarly, ACP application had a decreasing effect on the a_w values of the samples ($p<0.05$). After the application, it was determined that the lowest a_w value was in the ACP application using 100% Ar gas, whereas the highest a_w value was in the control samples. Additionally, a_w values decreased in all samples during storage ($p<0.05$). Similarly, it was determined that the lowest a_w value on the 7th day of storage was in the samples performed using 100% Ar gas (Table 1).

ACP application caused the TBARS values of the samples to increase ($p<0.05$). After the application, it was determined that the highest TBARS value among the samples was 1.97 mg MA/kg in the ACP application using 100% O_2 for 30 minutes. This value was followed by samples treated with ACP using a mixture of 50% O_2 + 50% Ar gas for 30 minutes at 1.58 mg MA/kg. Again, TBARS values increased during the storage period in all samples ($p<0.05$). Although the highest TBARS value on the last day of storage was in the samples with ACP-applied, which was performed using 100% O_2 for 30 minutes, with a value of 2.98 mg MA/kg, it was determined that the increase rate in the control sample was higher compared to the ACP-applied samples (Table 1).

Both sample type and storage time interactions had very highly significant effects on the *L**, *a**, and *b** values of steak samples obtained from *M. longissimus dorsi* muscles to which ACP was applied ($p<0.0001$). In addition, the interactions between sample type and storage time showed a very negative correlative effect on the color values of the samples (Table 2).

Although using O_2 gas and the application time increased the samples' *L**, *a**, and *b** values , the use of Ar gas and the extension of the application time caused the *L**, *a**, and *b** values to decrease ($p<0.05$). At the end of the application, the highest *L**, *a**, and *b** values (46.43, 34.07, and 11.52, respectively) were detected in ACP-applied samples using 100% O_2 gas for 30 minutes, and the lowest *L**, *a**, and *b** values (39.48, 18.18, and 6.30, respectively) were detected in ACP-applied samples using 100% Ar gas for 30 minutes. Additionally, 7 days of storage had a decreasing effect on the *L**, *a**, and *b** values of all samples ($p<0.05$). At the end of storage, the lowest L*, a*, and b* values were detected in the samples with ACP-applied using 100% Ar gas for 30 min (34.85, 11.71, and 2.11, respectively). These values were followed by the ACP-applied samples using 100% Ar gas for 15 min (37.41, 16.33, and 3.50, respectively) and the control samples (37.93, 19.98, and 4.76, respectively) (Figure 1.,2., and 3.).

Table 1. pH, aw, and TBARS values of ACP-applied meat samples

Analysis	Samples	Storage Time (Day)		
		1.	4.	7.
pH	Control	5.51±0.05^{aA}	5.33±0.04^{bcB}	5.30±0.02^{aB}
	15 min % 100 O_2	5.51±0.01^{aA}	5.46±0.02^{aA}	5.20±0.05^{bB}
	15 min % 100 Ar	5.51±0.02^{aA}	5.44±0.03^{aA}	5.31±0.04^{aB}
	15 min %50 O_2 +%50 Ar	5.44±0.01^{abA}	5.41±0.02^{abA}	5.08±0.04^{cB}
	30 min % 100 O_2	5.48±0.01^{aA}	5.23±0.04^{cdB}	5.12±0.06^{bBC}
	30 min % 100 Ar	5.46±0.01^{aA}	5.31±0.09^{bcA}	5.11±0.03^{bBC}
	30 min %50 O_2 +%50 Ar	5.36±0.04^{bA}	5.15±0.02^{dB}	5.05±0.02^{cB}
	Interactions		**P Value**	**r**
	Sample (S)		<0.0001	-0.417**
	Storage Time (ST)		<0.0001	-0.805**
	S x ST		<0.0001	--
a_w	Control	0.890±0.01^{aA}	0.867±0.01^{aB}	0.860±0.01^{aC}
	15 min % 100 O_2	0.864±0.01^{bA}	0.860±0.01^{bB}	0.851±0.01^{bC}
	15 min % 100 Ar	0.863±0.01^{bcA}	0.856±0.01^{cB}	0.847±0.01^{cC}
	15 min %50 O_2 +%50 Ar	0.865±0.01^{bA}	0.853±0.01^{deB}	0.841±0.01^{dC}
	30 min % 100 O_2	0.863±0.01^{bcA}	0.855±0.01^{cdB}	0.845±0.01^{cC}
	30 min % 100 Ar	0.861±0.01^{cA}	0.849±0.01^{fB}	0.841±0.01^{dC}
	30 min %50 O_2 +%50 Ar	0.863±0.01^{bcA}	0.851±0.01^{efB}	0.845±0.01^{cC}
	Interactions		**P Value**	**r**
	Sample (S)		<0.0001	-0.489**
	Storage Time (ST)		<0.0001	-0.746**
	S x ST		<0.0001	--
TBARS	Control	0.98±0,04^{eB}	1.16±0,03^{fB}	2.48±0,08^{bA}
	15 min % 100 O_2	1.28±0,06^{cC}	1.58±0,11^{cdB}	1.90±0,05^{dA}
	15 min % 100 Ar	1.04±0,07^{deC}	1.36±0,05^{eB}	1.62±0,08^{eA}
	15 min %50 O_2 +%50 Ar	1.10±0,03^{dC}	1.52±0,05^{dB}	1.74±0,02^{dcA}
	30 min % 100 O_2	1.97±0,05^{aC}	2.28±0,06^{aB}	2.89±0,04^{aA}
	30 min % 100 Ar	1.03±0,02^{deC}	1.68±0,06^{cB}	2.12±0,14^{cA}
	30 min %50 O_2 +%50 Ar	1.58±0,05^{bC}	2.09±0,05^{bB}	2.58±0,04^{bA}
	Interactions		**P Value**	**r**
	Sample (S)		<0.0001	0,363*
	Storage Time (ST)		<0.0001	0,692**
	S x ST		<0.0001	--

a - e (↓): Values with the same capital letters in the same column for each analysis differ significantly (P< 0.05), A(→)C: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05) *P* < 0.0001: Statistically too much significant. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 2. Variation analysis of color values of ACP-applied meat samples

Interactions	L* Value		a* Value		b* Value	
	P Value	R	P Value	r	P Value	r
Sample (S)	<0.0001	-0.145	<0.0001	-0.250	<0.0001	-0.320*
Storage Time (ST)	<0.0001	-0.692**	<0.0001	-0.505**	<0.0001	-0.680**
S x ST	0.163	--	0.103	--	0.824	--

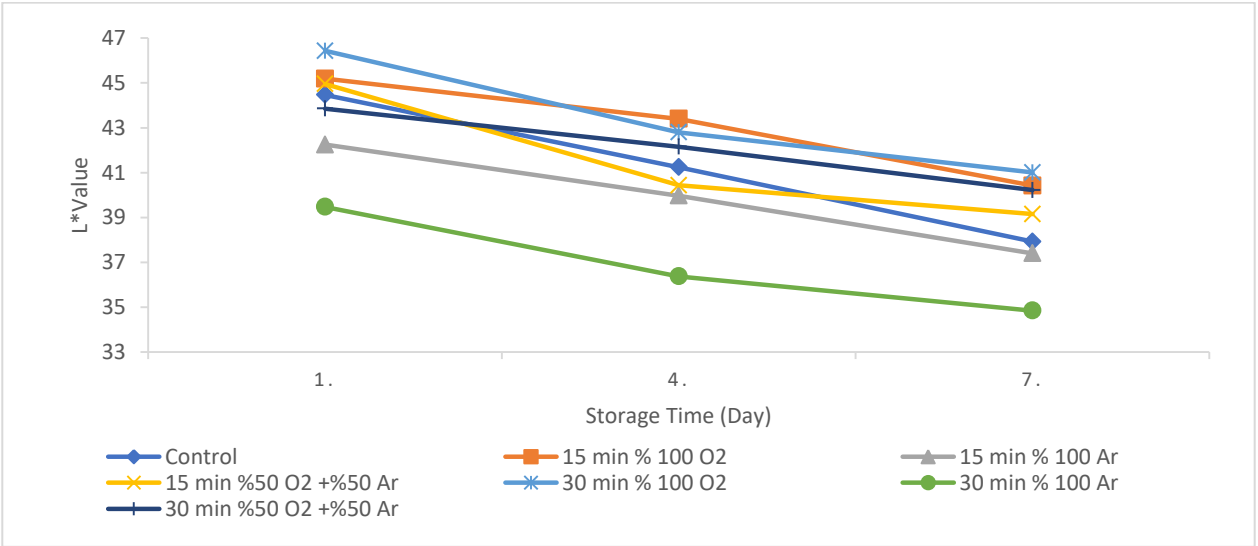

Figure 1: L* values of ACP-applied meat samples

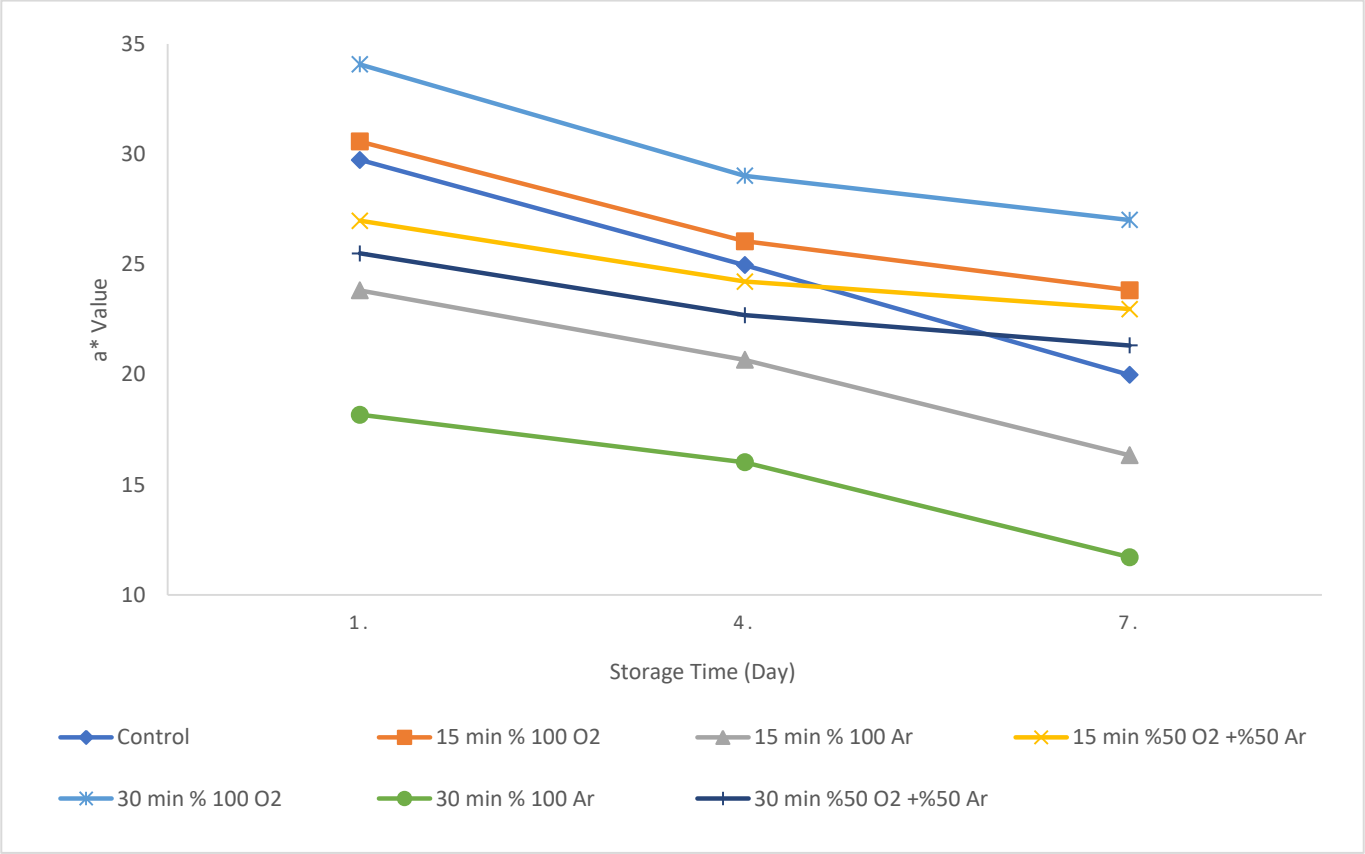

Figure 2: a* values of ACP-applied meat samples

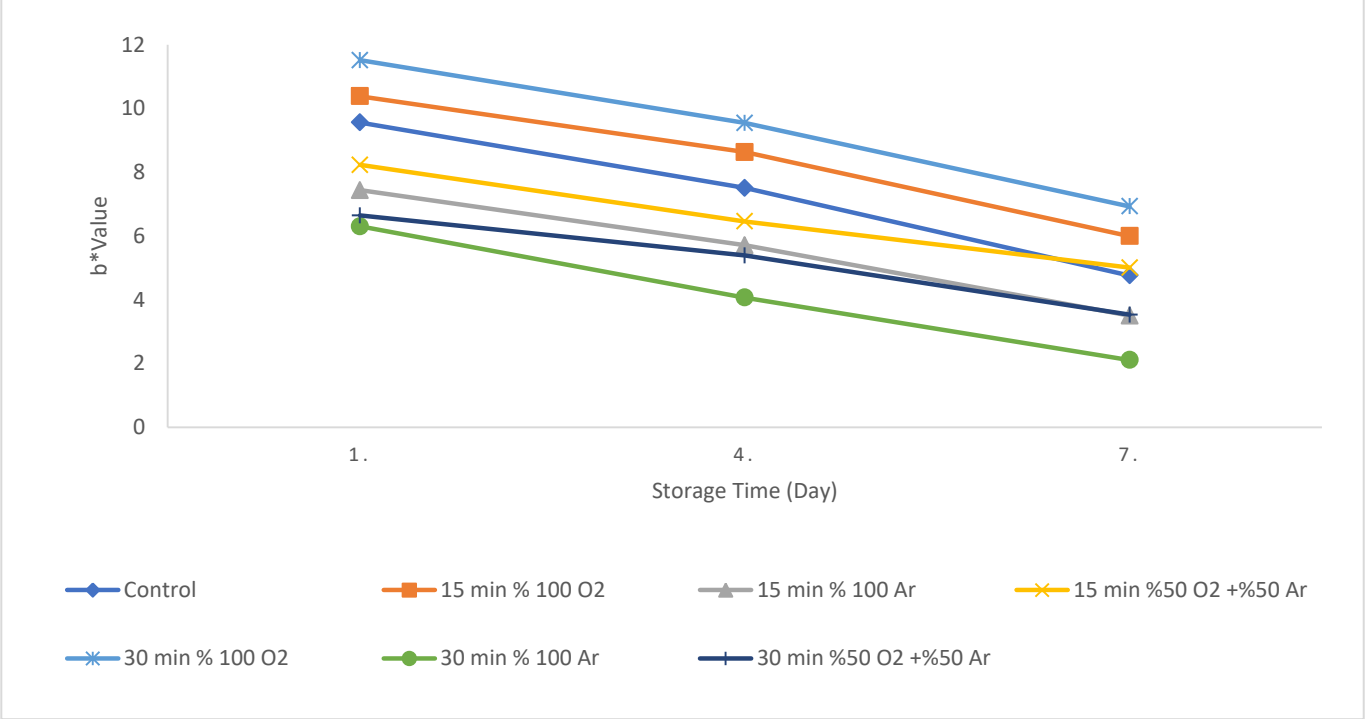

Figure 3: b* values of ACP-applied meat samples

ACP application affects the techno-functional properties of the protein depending on its natural structure (Pérez-Andrés et al. 2019). Therefore, the post-application and storage process caused the textural properties of the steak samples to change (Table 3).

Both sample type and storage time interactions had very highly significant effects on the texture values of the samples (except gumminess and chewiness) ($p<0.0001$). Only storage time interaction had very highly significant effect on gumminess and chewiness values ($p<0.0001$). According to the correlation analysis results, sample type and storage time interactions showed a positive correlative effect on hardness, gumminess, and shear force, and a very negative correlative effect on adhesiveness, and cohesiveness (Table 3).

Table 3. Texture profile analysis values of ACP-applied meat samples

Analysis	Samples	Storage Time (Day)		
		1.	**4.**	**7.**
Hardness	Control	615,10±32,83^{bC}	1804,92±90,11^{bcB}	2616,22±66,60^{aA}
	15 min % 100 O_2	671,53±55,88^{bC}	1646,39±60,71^{dB}	1944,43±57,82^{dA}
	15 min % 100 Ar	669,85±26,18^{bC}	1766,45±45,12^{cB}	1976,78±41,77^{dA}
	15 min %50 O_2 +%50 Ar	674,97±37,22^{bC}	1537,69±9,43^{dB}	1983,17±25,25^{cdA}
	30 min % 100 O_2	804,76±49,34^{aC}	1899,25±30,96^{abB}	2097,30±12,85^{cA}
	30 min % 100 Ar	879,54±34,18^{aC}	1933,28±45,30^{aB}	2322,78±64,25^{bA}
	30 min %50 O_2 +%50 Ar	834,33±45,26^{aC}	1923,68±25,91^{abB}	2102,30±47,96^{cA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		0,062
	Storage Time (ST)	<0.0001		0.928**
	S x ST	<0.0001		--
Adhesiveness	Control	-15,89±0,81^{eA}	-56,93±2,18^{dB}	-74,04±0,23^{fC}
	15 min % 100 O_2	-12,61±1,47^{dA}	-35,90±0,77^{cB}	-56,65±2,21^{eC}
	15 min % 100 Ar	-9,90±1,07^{bcA}	-23,66±0,69^{bB}	-38,24±1,27^{dC}
	15 min %50 O_2 +%50 Ar	-10,85±0,30^{cdA}	-26,18±1,20^{bB}	-32,93±0,86^{dC}
	30 min % 100 O_2	-8,89±0,33^{bA}	-12,53±1,67^{aB}	-28,75±0,79^{cC}
	30 min % 100 Ar	-6,90±0,04^{aA}	-10,05±1,25^{aB}	-19,87±0,76^{aC}
	30 min %50 O_2 +%50 Ar	-8,23±0,29^{abA}	-11,33±0,96^{aB}	-24,95±0,42^{bC}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0.234
	Storage Time (ST)	<0.0001		-0.900**
	S x ST	<0.0001		--
Springiness	Control	14,52±0,33^{aA}	9,20±0,22^{bB}	6,35±0,19^{dC}
	15 min % 100 O_2	13,53±0,16^{bA}	11,62±0,21^{aB}	7,38±0,09^{acdC}
	15 min % 100 Ar	12,92±0,13^{cA}	11,25±0,18^{aB}	7,13±0,10^{abC}
	15 min %50 O_2 +%50 Ar	13,06±0,07^{cA}	11,40±0,19^{aB}	7,21±0,15^{abC}
	30 min % 100 O_2	11,25±0,13^{eA}	9,36±0,17^{bB}	6,93±0,16^{bcC}
	30 min % 100 Ar	9,98±0,14f^{A}	8,84±0,27^{bB}	6,67±0,11^{cdC}
	30 min %50 O_2 +%50 Ar	11,79±0,24^{dA}	9,29±0,51^{bB}	6,71±0,23^{cdC}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		0.610**
	Storage Time (ST)	<0.0001		-0.649**
	S x ST	<0.0001		--

…Continues Table 3. Texture profile analysis values of ACP-applied meat samples

Analysis	Samples	Storage Time (Day)		
		1.	4.	7.
Cohesiveness	Control	0,925±0,01^{abA}	0,825±0,02^{aB}	0,695±0,02^{cC}
	15 min % 100 O_2	0,950±0,01^{aA}	0,865±0,02^{aB}	0,810±0,01^{aC}
	15 min % 100 Ar	0,915±0,01^{bA}	0,840±0,01^{aB}	0,780±0,01^{abC}
	15 min %50 O_2 +%50 Ar	0,930±0,01^{abA}	0,865±0,02^{aB}	0,790±0,01^{abC}
	30 min % 100 O_2	0,870±0,01^{cA}	0,835±0,02^{aAB}	0,785±0,04^{abB}
	30 min % 100 Ar	0,820±0,01^{dA}	0,775±0,02^{bAB}	0,740±0,01^{bcB}
	30 min %50 O_2 +%50 Ar	0,845±0,01^{cdA}	0,820±0,02^{abA}	0,755±0,01^{bB}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0,255
	Storage Time (ST)	<0.0001		-0.802**
	S x ST	0.001		--
Gumminess	Control	569,08±34,72^{cC}	1490,01±112,63^{abB}	1817,56±9,21^{aA}
	15 min % 100 O_2	638,35±62,58^{abcB}	1424,77±87,43^{abA}	1575,40±74,33^{bcA}
	15 min % 100 Ar	612,82±19,22^{cB}	1484,14±69,88^{abA}	1542,18±60,54^{cA}
	15 min %50 O_2 +%50 Ar	627,46±25,07^{bcC}	1330,20±40,78^{bB}	1566,88±47,99^{bcA}
	30 min % 100 O_2	699,79±31,54^{abB}	1586,20±67,14^{aA}	1646,61±84,24^{bcA}
	30 min % 100 Ar	720,98±15,59^{aC}	1497,81±5,89^{aB}	1719,31±80,39^{abA}
	30 min %50 O_2 +%50 Ar	704,85±32,34^{abB}	1577,23±5,94^{aA}	1587,74±80,80^{bcA}
	Interactions	**P Value**		**r**
	Sample (S)	0.001		0.047
	Storage Time (ST)	<0.0001		0.910**
	S x ST	0.007		--
Chewiness	Control	8269,03±697,37^{abC}	13703,25±710,16^{cdA}	11551,53±405,51^{aB}
	15 min % 100 O_2	8631,56±738,47^{aC}	16554,08±724,30^{aA}	11623,30±414,91^{aB}
	15 min % 100 Ar	7919,42±166,09^{abC}	16690,82±434,63^{aA}	11000,72±290,19^{aB}
	15 min %50 O_2 +%50 Ar	8193,81±283,12^{abC}	15167,12±211,15^{bA}	11301,53±113,61^{aB}
	30 min % 100 O_2	7874,08±261,03^{abC}	14841,24±349,93^{bcA}	11426,11±852,03^{aB}
	30 min % 100 Ar	7197,84±48,62^{bC}	13241,46±454,58^{dA}	11472,15±354,27^{aB}
	30 min %50 O_2 +%50 Ar	8306,29±211,94^{aC}	14658,90±758,80^{bcA}	10671,14±913,11^{aB}
	Interactions	**P Value**		**r**
	Sample (S)	0.001		-0.088
	Storage Time (ST)	<0.0001		0.448**
	S x ST	0.001		--

…Continues Table 3. Texture profile analysis values of ACP-applied meat samples

Analysis	Samples	Storage Time (Day)		
		1.	4.	7.
Resilience	Control	0,80±0,01^{cA}	0,77±0,01^{dA}	0,62±0,02^{eB}
	15 min % 100 O_2	0,84±0,01^{bcA}	0,80±0,01^{cdA}	0,69±0,03^{dB}
	15 min % 100 Ar	0,86±0,01^{bA}	0,82±0,02^{bcA}	0,73±0,03^{cdB}
	15 min %50 O_2 +%50 Ar	0,84±0,01^{bcA}	0,80±0,01^{cdAB}	0,76±0,01^{bcB}
	30 min % 100 O_2	0,91±0,01^{aA}	0,86±0,01^{abAB}	0,82±0,04^{abB}
	30 min % 100 Ar	0,93±0,03^{aA}	0,90±0,03^{aAB}	0,85±0,02^{aB}
	30 min %50 O_2 +%50 Ar	0,87±0,01^{bA}	0,83±0,01^{bcB}	0,78±0,01^{bcC}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		0.569**
	Storage Time (ST)	<0.0001		-0.648**
	S x ST	<0.0001		--
Shear Force	Control	179,34±2,85^{eC}	242,57±8,19^{bB}	488,70±4,81^{bA}
	15 min % 100 O_2	189,05±4,80^{dC}	255,56±13,93^{bB}	470,69±5,49^{cA}
	15 min % 100 Ar	201,75±5,07^{cC}	257,07±4,15^{bB}	467,78±6,45^{cA}
	15 min %50 O_2 +%50 Ar	195,61±2,74^{cdC}	248,97±7,45^{bB}	471,76±11,72^{cA}
	30 min % 100 O_2	232,42±4,73^{bC}	301,78±8,98^{aB}	496,09±6,04^{abA}
	30 min % 100 Ar	245,94±3,06^{aC}	319,27±22,43^{aB}	506,76±6,29^{aA}
	30 min %50 O_2 +%50 Ar	238,23±3,93^{abC}	292,25±11,37^{aB}	501,29±4,17^{abA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		0.155
	Storage Time (ST)	<0.0001		0.935**
	S x ST	0.006		--

a - e (↓): Values with the same capital letters in the same column for each analysis differ significantly (P< 0.05), A(→)C: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05) *P* < 0.0001: Statistically too much significant. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

ACP application caused the hardness value of the samples to increase ($p<0.05$). Among the samples, the highest increase in hardness value was detected in the ACP-applied samples using 100% Ar gas for 30 minutes with 879.54 N. This sample was followed by ACP-applied samples using 50% O_2 and 50% Ar gas for 30 minutes (834.33 N), and 100% O_2 gas for 30 minutes (804.76 N). Similarly, there was an increase in the hardness values of all samples during 7 days of storage ($p<0.05$).

Although the adhesiveness, springiness, and cohesiveness values of the samples increased with ACP application ($p<0.05$), they decreased during storage (Table 3; $p<0.05$).

ACP application and storage time caused an increase on the gumminess value ($p<0.05$). On the last day of storage, the highest gumminess value was detected in the control samples with 1817.56 N, and the lowest value was detected in ACP-applied samples using 100% Ar gas for 15 minutes with 1542.18 N.

Although the chewiness value increased in the first 7 days of storage, it decreased in later periods ($p<0.05$). It was revealed that the lowest difference between the first and last days of storage was 2364.85 N in the application using 50% O_2 and 50% Ar gas for 30 minutes, and the highest difference was 4274.31 N in the ACP-applied samples using 50% O_2 and 50% Ar gas for 15 minutes.

ACP application increased resilience and shear force values ($p<0.05$). It was revealed that the highest resilience and shear force values at the beginning of the application were 0.93 and 245.93, respectively, in the ACP-applied samples using 50% O_2 and 50% Ar gas for 30 minutes. Although the cutting force values of the steak samples decreased during the 7-day storage period, it was determined that there was an increase in the shear force values ($p<0.05$). At the end of storage, the lowest resilience value was determined as 0.62 in the control, and the highest resilience value was determined as 0.85 in the ACP-applied samples using 50% O_2 and 50% Ar gas for 30 minutes. Similarly, on the 7th storage day, the highest shear force was determined to be 506.76 in the samples using 50% O_2 and Ar gas mixtures for 30 minutes.

Sample type, storage time, and sample type x storage time interactions had very highly significant effects on all microbiological analysis results ($p<0.0001$). Sample type interaction had a negative correlative effect, while storage time interaction had a very positive correlative effect ($p<0.0001$) on the microbiological analysis results (Table 4).

ACP application showed a reducing effect on the counts of total aerophilic mesophilic bacteria (TAMB), total aerophilic psychrophilic bacteria (TAPB), total coliform group bacteria (TCGB), and total yeast & mold (TYM) ($p<0.01$). At the beginning of the application, it was determined that the applications using 50% O_2 and Ar gas mixtures for 30 minutes had the lowest TAMB, TAPB, TCGB, and TYM counts among the samples. TAMB, TAPB, TCGB and TYM counts increased in all steak samples obtained from beef *M. longissimus dorsi* muscles during the storage period ($p<0.05$). All microbiological analysis results on the last day of storage determined that the lowest microorganism counts were in the samples where 50% O_2 and Ar gas mixtures were used for 30 minutes, and the highest microorganism counts were in the control group samples (Table 3, Table 4).

Table 4. Microbiological analysis results of ACP-applied meat samples

Analysis	Samples	Storage Time (Day)		
		1.	4.	7.
TAMB	Control	4.48±0,04^{aC}	5.77±0,019^{aB}	7.11±0,03^{aA}
	15 min % 100 O_2	4.30±0,04^{bB}	4.64±0,17^{bB}	5.14±0,04^{bA}
	15 min % 100 Ar	4.24±0,03^{bC}	4.50±0,08^{bcB}	4.98±0,08^{cA}
	15 min %50 O_2 +%50 Ar	4.11±0,02^{cC}	4.32±0,01^{cdB}	4.61±0,09^{dA}
	30 min % 100 O_2	3.98±0,05^{dB}	4.13±0,06^{deB}	4.41±0,04^{eA}
	30 min % 100 Ar	3.90±0,08^{deB}	4.06±0,06^{deB}	4.29±0,04^{efA}
	30 min %50 O_2 +%50 Ar	3.79±0,03^{eB}	3.90±0,05^{eAB}	4.12±0,11^{fA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0,693**
	Storage Time (ST)	<0.0001		0.462**
	S x ST	<0.0001		--
TAPB	Control	4.15±0,04^{aC}	4.71±0,08^{aB}	6.07±0,07^{aA}
	15 min % 100 O_2	4.07±0,02^{abB}	4.33±0,07^{bB}	4.90±0,12^{bA}
	15 min % 100 Ar	4.05±0,03^{cC}	4.27±0,08^{bB}	4.77±0,06^{bcA}
	15 min %50 O_2 +%50 Ar	3.95±0,06^{dC}	4.19±0,06^{bB}	4.67±0,04^{cA}
	30 min % 100 O_2	3.60±0,03^{eC}	3.90±0,03^{cB}	4.40±0,07^{dA}
	30 min % 100 Ar	3.54±0,04^{efC}	3.79±0,04^{cdB}	4.12±0,06^{eA}
	30 min %50 O_2 +%50 Ar	3.49±0,01^{fC}	3.70±0,09^{dB}	3.93±0,04^{fA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0.678**
	Storage Time (ST)	<0.0001		0.614**
	S x ST	<0.0001		--
TCGB	Control	3,19±0,02^{aC}	4,06±0,04^{aB}	5,08±0,08^{aA}
	15 min % 100 O_2	3,08±0,02^{bC}	3,68±0,04^{bB}	4,15±0,01^{bA}
	15 min % 100 Ar	3,03±0,02^{bC}	3,57±0,04^{cB}	4,05±0,01^{cA}
	15 min %50 O_2 +%50 Ar	2,96±0,01^{cC}	3,52±0,02^{cB}	4,02±0,01^{cA}
	30 min % 100 O_2	2,86±0,02^{dC}	3,24±0,05^{dB}	3,88±0,04^{dA}
	30 min % 100 Ar	2,73±0,01^{eC}	3,21±0,04^{deB}	3,72±0,02^{eA}
	30 min %50 O_2 +%50 Ar	2,54±0,03^{fC}	3,14±0,04^{eB}	3,64±0,03^{fA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0,503**
	Storage Time (ST)	<0.0001		0.819**
	S x ST	<0.0001		--
TYM	Control	3,63±0,03^{aC}	4,86±0,02^{aB}	6,75±0,04^{aA}
	15 min % 100 O_2	3,60±0,03^{abC}	4,47±0,06^{bB}	5,32±0,02^{bA}
	15 min % 100 Ar	3,56±0,02^{abcC}	4,37±0,02^{cB}	5,11±0,04^{cA}
	15 min %50 O_2 +%50 Ar	3,55±0,01^{bcC}	3,90±0,02^{dB}	4,48±0,03^{dA}
	30 min % 100 O_2	3,49±0,02^{cdC}	3,81±0,03^{eB}	4,35±0,04^{eA}
	30 min % 100 Ar	3,45±0,02^{dC}	3,58±0,04^{fB}	4,30±0,04^{efA}
	30 min %50 O_2 +%50 Ar	3,45±0,04^{dC}	3,55±0,03^{fB}	4,25±0,03^{fA}
	Interactions	**P Value**		**r**
	Sample (S)	<0.0001		-0.522**
	Storage Time (ST)	<0.0001		0.716**
	S x ST	<0.0001		--

TAMB: Total aerobic mesophilic bacteria; TAMP: Total aerobic psychrophilic bacteria
TCGB: Total coliform group bacteria, TYM: Total yeast mold
a - f (↓): Values with the same capital letters in the same column for each analysis differ significantly (P< 0.05), A(→)C: Values with the same capital letters in the same column for each analysis differ significantly (P<0.05) *P* < 0.0001: Statistically too much significant. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

ACP application showed a decreasing effect on the pH values of the samples (p<0.05). It was determined that the lowest pH value was (5.05) in the ACP-applied samples using a mixture of 50% O_2 + 50% Ar gas for 30 minutes. Additionally, it was determined that the pH values of the samples decreased during the storage period (p<0.05). On the last day of storage, it was determined that the lowest pH value was 5.05 in the samples with ACP-applied, which was also made using a 50% O_2 + 50% Ar gas mixture. On the other hand, it was revealed that the highest pH value was in the control sample (5.30).

The significantly higher drop in pH after ACP application can be attributed to acidogenic molecules such as NOx normally produced in air plasmas (Gao et al. 2021).

The decrease in a_w value is because the O_2 and Ar gases used in the application retain the free water molecules on the sample. (Lee et al. 2020). In addition, it is thought that the drying occurring on the

surface due to gas circulation during application may also be effective in the decrease in the a_w value.

Reactive oxygen and nitrogen species (RONS) produced by ACP are free to interact with intramuscular fat-containing surface tissue. Reactive oxygen species interact specifically with the unsaturated fatty acid fraction and can, therefore, cause lipid peroxidation. Secondary oxidation products can initiate conformational changes in myoglobin, causing both increased oxidation and brown discoloration (Bauer et al. 2017). Malondialdehyde (MDA) is a polyunsaturated fatty acid oxidation product and is considered an important marker of lipid oxidation (Gonz´alez-Gonz´alez et al. 2021). Therefore, an increase in TBARS values, which is an indicator of oxidation, is expected after the application of ACP.

The color of red meat is an important factor in meat quality and appeal. The reason for this is that the color of meat is perceived by the consumer as an indicator of freshness and quality. Therefore, the consumer's purchasing decision is directly affected (Fröhling et al. 2012).

Since the use of O_2 gas increases the formation of oxymyoglobin, which combines with myoglobin, gives the meat its red color and makes the meat appear brighter and redder, the *L**, *a**, and *b** values of the samples using O_2 gas were found to be higher. On the other hand, using Ar gas during application caused a decrease in the *L**, *a**, and *b** values, as it caused metmyoglobin formation due to the decrease in contact with O_2 on the surface (Jayasena et al.), and drying due to gas contact on the surface (Kim et al. 2013). Reactive species formed during the ACP application can enter into oxidative reactions with pigment compounds in the food product and change the color of the product (Lee et al. 2022).

Decreasing a_w, is one of the main reasons for the increase in hardness value. The airflow created by gas circulation during application causes drying on the surface. Therefore, both a_w decreased and hardness value increased. In the samples, adhesiveness, springiness and cohesiveness values decreased due to the decreasing a_w value and the increase in hardness.

ACP application reduced the water retention capacity of proteins in the *M. longissimus dorsi* muscle, resulting in a decrease in aw in the samples (Pérez-Andrés et al. 2019). It is thought that drying and decreasing moisture content on the surface may be the reason for the increase in shear force values.

Reactive species present in plasma have oxidative effects on the external surfaces of microbial cells (Asl et al. 2022). ACP application can generate specific types of ROS, such as oxygen atoms, ozone, metastable oxygen molecules, peroxide, superoxide, and hydroxyl radicals, all bactericidal (Kim et al. 2015). The gas mixture containing air or O_2 used in the ACP system produces (i) reactive species (RNS and ROS) that play an important inactivation role by attacking the microbial cell wall, leading to cell rupture and oxidation of peptidoglycan or lipopolysaccharides and (ii) intracellular components (Laroque et al. 2022).

In addition, ACP reactive species against fungal cells cause deformation of the micelle tip, cell apoptosis, cellular protein damage, disintegration, and release, causing loss of function and death of cells (Misra et al. 2019).

CONCLUSION

This research investigated the effect of ACP application with different gases and mixtures on the physicochemical and microbiological properties of beef steaks obtained from the *M. longissimus dorsi* muscle during the storage period. While ACP application decreased the pH and a_w of the samples, it caused an increase in TBARS values. In addition, there was a decrease in the *L**, *a**, and *b** values of the samples in other ACP applications, except for applications using O_2 gas. Besides, the application was effective in the positive development of the textural properties of the samples. There was a decrease in the counts of TAMB, TAPB, TCGB, and TYM in all samples where ACP was applied. Additionally, it was determined that there was a smaller increase (on average 2-3 logs) in the TAMB, TAPB, TCGB, and TYM counts of these samples compared to the control samples during the storage period.

Meat is a valuable food product in terms of nutrition due to its protein content, high biological value, and richness in essential amino acids, B-group vitamins, and various minerals. The high a_w value and rich nutritional content of meat are effective in providing a suitable environment for microbial growth. Therefore, microbial decontamination and inactivation of meat is mandatory for food safety.

Research findings revealed that ACP application can be used to extend the shelf life and increase the physicochemical quality of steaks obtained from beef *M. longissimus dorsi* muscles. These results will lead to future studies on non-thermal techniques considered to be applied in meat preservation.

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Ethical approval: "This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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