

ORIGINAL ARTICLE

Improving beef burger quality during cold storage: effect of *Aloe vera* gel, packaging, and temperature

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Abstract

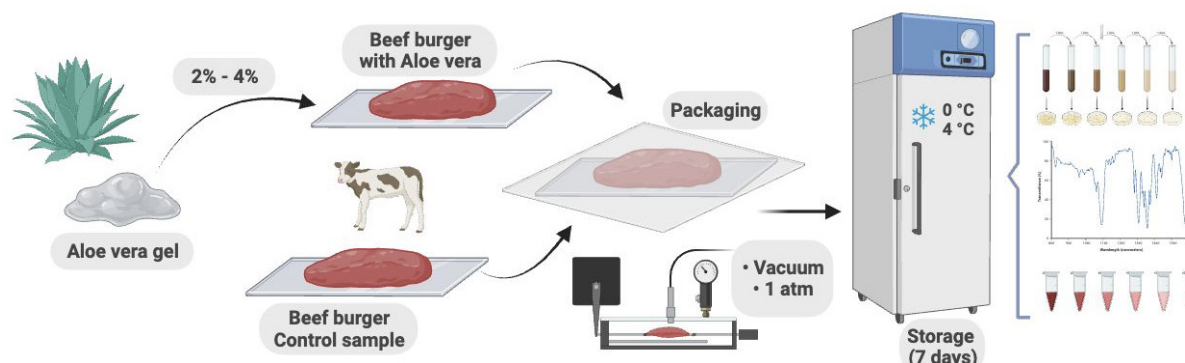
Lipids, one of the main macronutrients in beef burgers, can undergo oxidative reactions, negatively affecting product quality. Recently, *Aloe vera* (*Aloe barbadensis* Miller), a natural antioxidant, has gained attention as a functional food ingredient in the food industry due to its potential to promote food preservation and human health. For this reason, the present study aimed to investigate the feasibility of using *Aloe vera* to improve the quality of beef burgers and extend their shelf life during refrigerated storage. Additionally, the study examined the influence of the packaging system (atmospheric and vacuum) and storage temperature (0 °C and 4 °C). The pH value increased from the fifth day of beef burger storage ($p > 0.05$). The concentration of *Aloe vera* gel used in this study (2% or 4%) reduced lipid and pigment oxidation, particularly when combined with the lower storage temperature (0 °C). Regarding the metmyoglobin content, which affects the color of the meat, the best results were observed in samples stored in vacuum packaging with 2% or 4% *Aloe vera* gel at 4 °C (treatments 3 and 4), as well as in samples in conventional packaging (1 atm) with 4% *Aloe vera* gel stored at 4 °C (treatment 8). The growth of aerobic mesophilic bacteria was reduced when *Aloe vera*, vacuum packaging, and storage at 0 °C were combined (treatments 1 and 2). Regarding meat color, using 2% *Aloe vera* gel helped maintain the quality of this sensory attribute, which is crucial for product acceptance. In conclusion, using 2% *Aloe vera* gel, particularly in combination with vacuum packaging and storage at 0 °C, maintains the quality, oxidative stability, and safety of beef burgers ($p < 0.05$) for a longer period. This combination also preserved the attractive color of the burgers, thus making it a promising approach for extending the shelf life of meat products.

Keywords: Meat product; Beef burger; *Aloe vera*; Shelf life; Lipid oxidation; Physicochemical properties.



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Graphical Abstract



Highlights

- Addition of *Aloe vera* gel reduces lipid and pigment oxidation in beef burgers particularly when combined with refrigerated storage at 0 °C
- Vacuum packaging and a temperature of 4 °C, in conjunction with *Aloe vera* gel, minimize metmyoglobin formation
- The lowest counts of mesophilic aerobic bacteria were found in samples treated with *Aloe vera*, stored in vacuum packaging at 0 °C
- The addition of 2% *Aloe vera* gel not only extends shelf life but also helps maintain the color of beef burgers, thus making it a viable strategy for improving food safety and quality in the meat industry

1 Introduction

Beef burgers are one of the most popular meat products in the world, mainly due to their convenience, low price, expansion of the fast-food market, and the high nutritional quality of some formulations that contribute to consumer health (Hadidi et al., 2022). However, it is a highly perishable food, and its stability is mainly affected by microbial deterioration and the oxidation of structural meat components such as lipids, proteins, and pigments (Olvera-Aguirre et al., 2023). Compared to non-processed raw meat, the higher degree of manipulation of the beef burger makes it more susceptible to spoilage. Higher initial counts of mesophilic and psychrotrophic microorganisms have been found in processed meat products (Mizi et al., 2019).

The process of mincing the meat used in the development of hamburgers changes the muscular structures. It favors the insertion of more oxygen into the product, which can influence oxidation reactions in the meat during storage. Lipid oxidation and myoglobin oxidation are the main oxidative reactions in meat, and one process can contribute to the progression of the other. The primary consequences of lipid oxidation are organoleptic changes, mainly in aroma and texture (Munekata et al., 2023). Therefore, the deteriorative reactions lead to color changes, nutrient loss, and possible toxic compounds, thereby making the product undesirable or improper for consumption (Trujillo-Santiago et al., 2021).

There are many alternatives to increase the shelf life of refrigerated meat products, such as Light-Emitting Diodes (LEDs) and Ultraviolet-LED lights (Finardi et al., 2021), incorporation of active compounds from the agro-industry (D'Ambra et al., 2023) and vacuum packaging (Sauvala et al., 2023). The oxygen concentration in the vacuum packaging is reduced, minimizing its availability for oxidative reactions and microbial growth (Meinert et al., 2023). Vacuum packaging is one of the most employed technologies in meat. Due to packaging

permeability, increasing oxygen concentration inside the packaging during vacuum storage leads to undesirable changes in meat color during the first days of storage (Pergentino dos Santos et al., 2023).

For at least a decade, consumers in Latin America have shown a growing interest in healthy foods, such as products low in sodium and saturated fat (Rios-Mera et al., 2020), and with functional attributes, which can be obtained with the addition of natural antioxidants (Paiva et al., 2021). Using active agents in the food formulation, coating, or packaging is also a promising way to maintain food quality during storage (Hoffmann et al., 2022). Antioxidant agents can act against lipid oxidation, reacting with free compounds and preventing them from forming undesirable products. Moreover, they can reduce changes in the meat pigment. Thus, using active agents combined with vacuum packaging could lead to an extension in meat quality during storage and increase consumer acceptance (Smaoui et al., 2022).

Many active agents are available, and natural ones are gaining attention because they combine high efficacy and ecological nature (Martelli & Giacomini, 2018). *Aloe vera* has attracted attention in many areas of the food sector as a functional food ingredient, natural preservative, and coating for food packaging (Kumar et al., 2022). Research has shown the potential of *Aloe vera* as a food additive, as it contains more than 70 compounds that can act as antimicrobial and antioxidant agents (Kumar et al., 2022). The combination of modified atmosphere packaging (Pergentino dos Santos et al., 2023), refrigeration, and *Aloe vera* can contribute synergistically to product quality during storage. In this context, this study aimed to evaluate the influence of the interaction between packaging atmosphere (1 atm and 0 atm), *Aloe vera* gel concentration (2% and 4%), and temperature (0 °C and 4 °C) on the quality of beef burgers during 7 days storage. Beef burger quality was assessed by pH, lipid oxidation, pigment oxidation, microbiological, and sensory analysis.

2 Material and methods

2.1 Sample preparation

The composition of ground *Aloe vera* gel and ground beef samples used in the present study are shown in Table 1. The fresh *Aloe vera* leaves (*Aloe barbadensis* Miller) leaves (Latitude: 26°55'09" S. Longitude: 49°03'57" O) were immersed in a 0.998 mg L⁻¹ chlorine solution for half an hour + rinsed with distilled water + surface sterilization under UV light with a wavelength (λ) between 230-270 nm for half an hour. Then, the peel was removed with a stainless-steel knife. The pulp was submerged in distilled water (15 minutes/3 times) for removal of the aloin present, then superficially dried and macerated, forming a gel (Ramachandr & Rao, 2008), which was kept frozen ($-18 \pm ^\circ\text{C}$) until use.

Table 1. Nutritional properties data (means $\pm$ standard deviation) of ground beef and *Aloe vera* gel were used in elaborating beef burger samples.

Properties	Ground beef	<i>Aloe vera</i> gel
Moisture (g 100 g ⁻¹)	72.66 $\pm$ 1.48	98.76 $\pm$ 1.35
Water activity	0.98 $\pm$ 0.01	0.96 $\pm$ 0.01
pH	5.23 $\pm$ 0.12	5.87 $\pm$ 0.10
Total ash (g 100 g ⁻¹)	0.83 $\pm$ 0.01	0.46 $\pm$ 0.01
Total protein (g 100 g ⁻¹)	17.41 $\pm$ 2.80	ND
Total lipids (g 100 g ⁻¹)	8.36 $\pm$ 1.45	ND
Ascorbic acid ($\mu\text{g g}^{-1}$)	ND	34.20 $\pm$ 2.00
Total polyphenols ($\mu\text{g of GAE g}^{-1}$)	ND	28.80 $\pm$ 0.30
Antioxidant capacity ($\mu\text{M TEAC g}^{-1}$)	ND	12.70 $\pm$ 0.20

ND: Not determined. GAE: gallic acid. TEAC: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. Each sample was analyzed in triplicate.

The beef burgers developed exclusively with ground beef (*Nelore sirloin* cut/24 hours *postmortem*) were prepared in portions of approximately 100 g each and shaped using a cylindrical molding form (1cm x 10 cm). Concentrations of 2% and 4% (g of Aloe vera g⁻¹ of beef burger) were added to the beef burger formulation (Figure 1), which was then stored under packaging conditions at 1 atm and vacuum packaging conditions (0 atm), both conducted under normal atmospheric conditions, using a commercial vacuum sealer (Cetro®, Bauru, São Paulo-SP, Brazil).



Figure 1. Preparation of beef burger samples with *Aloe vera* gel.

All the burger samples were frozen for 1 hour at -20 ± 1 °C to prevent water loss during the vacuum-packing process. Then, they were packaged in Nylon Poli packaging (3 coextruded layers of polyamide and low-density polyethylene) (Artvac®, Três Pontas-MG, Brazil) and stored at 0 °C or 4 °C for up to 7 days. Therefore, the raw meat burger samples were prepared according to the 12 treatments shown in Table 2 and analyzed on days 1, 3, 5, and 7. Four of the samples were used as controls (without the addition of *Aloe vera* gel). Storage was conducted in a BOD chamber (Model 371, Tecnal®, Piracicaba-SP, Brazil) with temperature control, and all treatments were performed in triplicate.

Table 2. Treatments used to prepare the beef burger samples.

Treatments	Storage temperature (°C)	Pressure (atm)	<i>Aloe vera</i> gel concentration (g 100 g ⁻¹)
1	0	0	2
2	0	0	4
3	4	0	2
4	4	0	4
5	0	1	2
6	0	1	4
7	4	1	2
8	4	1	4
9 (control 1)	0	0	0
10 (control 2)	4	0	0
11 (control 3)	0	1	0
12 (control 4)	4	1	0

Controls are samples without *Aloe vera* gel addition. Each treatment was analyzed in triplicate.

2.2 Beef burgers analysis

Beef burgers were submitted to the following analyses: pH, lipid oxidation, pigment oxidation, microbiological, and sensory.

2.2.1 pH determination

The pH measurement (pH meter, Thermo Electron Orion® 710A+, Darmstadt, Germany) was determined for ground beef, *Aloe vera* gel, and twelve samples of beef burgers prepared according to the treatments shown in Table 2.

2.2.2 Lipid oxidation

Lipid oxidation was determined using the thiobarbituric acid reactive substances (TBARS) method, according to Lemon (1985). This method detects malondialdehyde (MDA) as an indicator of oxidized compounds. Absorbance was measured with a UV-Vis spectrophotometer (model U1800, Hitachi®, Japan) at 530 nm. A blank standard was prepared without beef burgers. The results were expressed as milligrams of MDA equivalent per kilogram of beef burger.

2.2.3 Pigment oxidation

The pigment oxidation (myoglobin) content was assessed through the metmyoglobin (MMb) content using a sphere spectrophotometer (X-rite®, model SP60, Carlstadt - NJ, USA). Thus, the pigment oxidation was measured by the beef burger surface's reflectance (R), which was calculated according to Equations 1 and 2.

$$\text{MMb (\%)} = \left\{ 1.395 - \left[\frac{(Ar_{572} - Ar_{700})}{Ar_{525} - Ar_{700}} \right] \right\} \times 100 \quad (1)$$

where:

$$Ar = \log \left(\frac{1}{R} \right) \quad (2)$$

2.2.4 Microbiological analysis

For the microbiological analyses, 10 g of each beef burger sample was homogenized with 90 mL of sterile 0.1% peptone water. Serial dilutions were prepared with the same diluent and carried out by deep plating with 1 mL of the sample. The total aerobic mesophilic bacteria count and aerobic psychrotrophic bacteria were carried out using Plate Count Agar (PCA) (Merck®, São Paulo - SP, Brazil), incubated at 35 ± 1 °C for 48 hours and 7 ± 1 °C for 10 days, respectively. The results were presented in log colony forming unit per gram (CFU g⁻¹) (Baird & Bridgewater, 2017).

2.2.5 Sensory analysis

The sensory analysis (color) was carried out by trained judges (n=12), who evaluated the red color of the raw beef burgers. Approximately 100 g of each sample was served to each judge, who evaluated three replicates of each treatment studied.

The sensory panel consisted of individuals with normal vision and color perception trained on raw meat's key color attributes using reference samples. The training took place at the Sensory Analysis Laboratory at the University of Blumenau (Campus 2) under standardized lighting, followed by three practical sessions to assess result consistency.

The sample presentation order was randomized for each sensory judge. The red color was evaluated using the 8-point structural hedonic scale method (1. Very bright red, 2. Bright red, 3. Matte red, 4. Slightly dark

red, 5. Moderately dark red, 6. Dark red to brownish red, 7. Reddish brown, 8. Brown) (Figure 2), according to the meat color scales and their scores proposed by American Meat Science Association (2012). This project was evaluated and approved by the Human Research Ethics Committee of the University of Blumenau (nº 08993219.0.0000.5370).

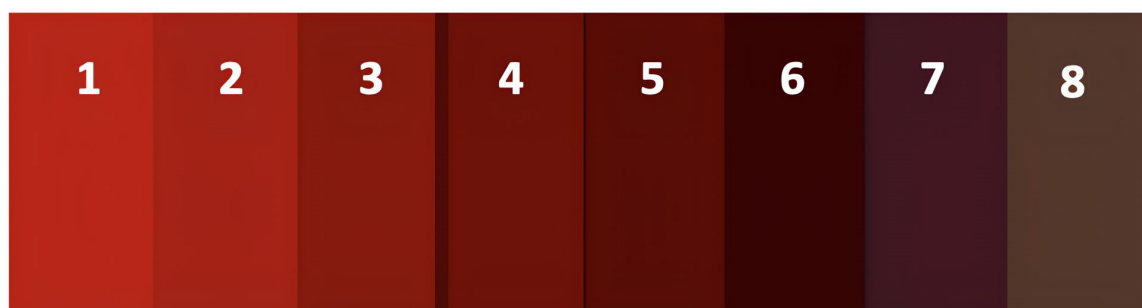


Figure 2. Meat color scales and their respective scores, using the 8-point structural hedonic scale method, where 1 is very bright red, 2 is bright red, 3 is matte red, 4 is slightly dark red, 5 is moderately dark red, 6 is dark red to brownish red, 7 is reddish brown, and 8 is brown, as recommended by American Meat Science Association (2012).

2.3 Statistical analysis

The data analysis was conducted using STATISTICA 13.3 *software* (TIBCO Software Inc., Palo Alto, USA). Each sample was analyzed in triplicate to ensure the accuracy and reliability of the results. All measurements were performed in triplicate, and the outcomes are presented as the mean accompanied by the standard deviation (SD). The significance between mean values was evaluated by the one-way analysis of variance (ANOVA), and it was performed at a significance level of 5% ($p < 0.05$). Tukey's post-hoc test was applied to investigate further and confirm the observed differences.

3 Results and discussion

3.1 Changes in pH during storage

Table 3 shows the pH values of the twelve beef burger samples stored for 7 days. During storage, the beef burger showed stability of pH value ($p > 0.05$). Likewise, the samples showed no differences in the pH values ($p > 0.05$) on the first day of storage, and the values are within the ideal range of 5.20 to 6.00 for fresh meat (Jankowiak et al., 2021).

In fact, pH is an important indicator of meat quality, as it affects the meat's water retention capacity, color, tenderness, and shelf life. The correlation between pH and meat color is evident in the darkening of the meat as pH values increase (Jankowiak et al., 2021).

Differences in pH values were observed from the 3rd to the 7th days, with increases being more significant from the fifth day of storage. During the storage, no pH value was $\geq 6.37 \pm 0.27$ (*Aloe vera* 0%, 1 atm, 4 °C). This behavior was expected because, according to Hoa et al. (2021), the meat pH shows greater variations only at higher temperatures.

Comparing the packages, the pH values at the end of storage are lower for the vacuum-packed meats. The predominance of lactic acid bacteria (LAB) in these packages favors the formation of lactic acid and the consequent drop in pH (Wang et al., 2022).

The increase in pH is associated with the production of nitrogenous compounds, mainly amines, which also originate from microbial growth, especially aerobic microorganisms (Zhu et al., 2022).

Table 3. Results of the pH (mean $\pm$ standard deviation) of the beef burger samples on different days of storage.

Treatments	Storage (day)			
	1	3	5	7
1	5.50 $\pm$ 0.42 ^{Aa}	5.49 $\pm$ 0.28 ^{aB}	5.30 $\pm$ 0.70 ^{aAB}	5.25 $\pm$ 0.93 ^{aABC}
2	5.54 $\pm$ 0.41 ^{Aa}	5.40 $\pm$ 0.28 ^{aB}	5.20 $\pm$ 0.14 ^{aB}	5.40 $\pm$ 0.70 ^{aABC}
3	5.55 $\pm$ 0.28 ^{Aa}	5.60 $\pm$ 0.83 ^{aAB}	5.60 $\pm$ 0.14 ^{aA}	5.65 $\pm$ 0.85 ^{aABC}
4	5.65 $\pm$ 0.28 ^{Aa}	5.50 $\pm$ 0.14 ^{aB}	5.30 $\pm$ 0.14 ^{aAB}	5.20 $\pm$ 0.57 ^{aC}
5	5.50 $\pm$ 0.42 ^{Aa}	5.50 $\pm$ 0.12 ^{aAB}	5.53 $\pm$ 0.26 ^{aAB}	5.38 $\pm$ 0.70 ^{aBC}
6	5.54 $\pm$ 0.41 ^{Aa}	5.50 $\pm$ 1.12 ^{aAB}	5.50 $\pm$ 0.26 ^{aAB}	5.49 $\pm$ 0.42 ^{aBC}
7	5.55 $\pm$ 0.28 ^{Aa}	5.60 $\pm$ 0.27 ^{aB}	6.20 $\pm$ 0.56 ^{aA}	6.00 $\pm$ 0.28 ^{aAB}
8	5.65 $\pm$ 0.28 ^{Aa}	5.60 $\pm$ 0.28 ^{aB}	5.75 $\pm$ 0.14 ^{aA}	6.00 $\pm$ 0.26 ^{aAB}
9 (control 1)	5.23 $\pm$ 0.12 ^{aA}	5.53 $\pm$ 0.01 ^{aB}	5.47 $\pm$ 0.26 ^{aAB}	5.40 $\pm$ 0.14 ^{aC}
10 (control 2)	5.26 $\pm$ 0.10 ^{aA}	6.25 $\pm$ 0.96 ^{aAB}	5.90 $\pm$ 0.40 ^{aA}	5.75 $\pm$ 0.01 ^{aB}
11 (control 3)	5.23 $\pm$ 0.12 ^{aA}	5.60 $\pm$ 0.28 ^{aB}	5.50 $\pm$ 0.93 ^{aAB}	5.40 $\pm$ 0.56 ^{aBC}
12 (control 4)	5.26 $\pm$ 0.10 ^{bA}	6.35 $\pm$ 0.28 ^{aA}	6.10 $\pm$ 0.43 ^{abA}	6.37 $\pm$ 0.27 ^{aA}

^{a-b}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-C}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C.

3.2 Lipid and pigment oxidation

Lipid oxidation is the main non-microbial process responsible for restricting the quality of meat and meat products (D'Ambra et al., 2023).

Lipid oxidation is a reaction in which reactive oxygen species attack the polyunsaturated fatty acids of phospholipids in cell membranes, disintegrating them and allowing these species to enter intracellular structures (Domínguez et al., 2019). The rate and extent of lipid oxidation is influenced by several factors, including the pH of the food, the iron content, the distribution of unsaturated fatty acids, antioxidant levels, temperatures, and storage time. Therefore, all these factors can influence the lipid oxidation of beef burger samples (Geng et al., 2023).

The results of lipid oxidation are presented in Table 4.

In general, it was found that lipid oxidation increased during storage, showing greater stability in storage conditions at 0 °C, in vacuum packaging, and with the addition of 4% *Aloe vera* gel to the hamburger samples (0.23 $\pm$ 0.03 MDA/kg of sample).

Normally, TBA values should be below 1.0 mg MDA/kg of meat, as higher values can indicate significant lipid oxidation and may present a rancid flavor (Carvalho et al., 2019). This limit was only reached in the control samples stored at 4 °C during the 7 days of storage (vacuum packaging: 1.13 $\pm$ 0.05 MDA/kg of sample and 1 atm packaging: 1.42 $\pm$ 0.13 MDA/kg of sample).

All the treatments with active agents and storage at 0 °C showed greater stability and remained below the limit until the last day of storage. Comparing storage at 0 °C and 4 °C, this storage reveals that lower temperatures enhance lipid oxidation stability during storage time, mainly on the 7th day (Nethra et al., 2023).

However, the incorporation of *Aloe vera* (2 and 4%) contributed to a reduction in lipid oxidation ($p < 0.05$), according to (Kouser et al., 2023).

Table 4. Results of the lipid oxidation (mean $\pm$ standard deviation) of the beef burger samples on different days of storage.

Treatments	Storage (days)			
	TBARS (malondialdehyde mg / kg burger)			
	1	3	5	7
1	0.23 $\pm$ 0.01 ^{aA}	0.26 $\pm$ 0.09 ^{aC}	0.33 $\pm$ 0.03 ^{aC}	0.30 $\pm$ 0.03 ^{aD}
2	0.23 $\pm$ 0.01 ^{aA}	0.25 $\pm$ 0.01 ^{aC}	0.24 $\pm$ 0.03 ^{aD}	0.23 $\pm$ 0.03 ^{aE}
3	0.28 $\pm$ 0.01 ^{cA}	0.39 $\pm$ 0.10 ^{bB}	0.52 $\pm$ 0.15 ^{aAB}	0.60 $\pm$ 0.03 ^{aB}
4	0.28 $\pm$ 0.01 ^{bA}	0.35 $\pm$ 0.03 ^{bB}	0.46 $\pm$ 0.05 ^{aB}	0.48 $\pm$ 0.01 ^{aC}
5	0.23 $\pm$ 0.01 ^{bA}	0.30 $\pm$ 0.06 ^{aBC}	0.32 $\pm$ 0.06 ^{aC}	0.30 $\pm$ 0.01 ^{aD}
6	0.23 $\pm$ 0.01 ^{bA}	0.23 $\pm$ 0.06 ^b	0.23 $\pm$ 0.01 ^{bD}	0.41 $\pm$ 0.01 ^{aC}
7	0.28 $\pm$ 0.01 ^{cA}	0.44 $\pm$ 0.03 ^{bA}	0.59 $\pm$ 0.08 ^{bA}	0.71 $\pm$ 0.13 ^{aB}
8	0.28 $\pm$ 0.01 ^{cA}	0.39 $\pm$ 0.05 ^{bcAB}	0.48 $\pm$ 0.05 ^{bB}	0.63 $\pm$ 0.03 ^{aB}
9 (control 1)	0.23 $\pm$ 0.01 ^{bA}	0.41 $\pm$ 0.03 ^{abA}	0.46 $\pm$ 0.01 ^{aB}	0.51 $\pm$ 0.01 ^{aBC}
10 (control 2)	0.28 $\pm$ 0.01 ^{cA}	0.43 $\pm$ 0.03 ^{bcA}	0.55 $\pm$ 0.05 ^{bA}	1.13 $\pm$ 0.05 ^{aA}
11 (control 3)	0.23 $\pm$ 0.01 ^{cA}	0.41 $\pm$ 0.24 ^{bA}	0.60 $\pm$ 0.06 ^{aA}	0.65 $\pm$ 0.03 ^{aB}
12 (control 4)	0.28 $\pm$ 0.01 ^{dA}	0.46 $\pm$ 0.01 ^{cA}	0.62 $\pm$ 0.03 ^{bA}	1.42 $\pm$ 0.13 ^{aA}

^{a-c}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-E}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C.

Aloe vera gels contain bioactive compounds, including polyphenols, known for their potent free radical scavenging ability. Consequently, the bioactive compounds in *Aloe vera* can operate during the second phase (propagation) of lipid oxidation (Elferjane et al., 2023).

According to Kumar et al. (2022), these bioactive compounds react with radicals present in the burger formulation, forming stable products and preventing the formation of undesirable ones. Trujillo-Santiago et al. (2021) observed a reduction of lipid oxidation when Hierba Santa (*Piper auritum* Kunth) was added to beef burgers, and they credited this behavior to the bioactive compound's presence. Al-Hijazeen (2022) also noted the same behavior using rosemary extract and oregano essential oil, on lipid oxidation of chilled ground chicken meat.

Felicia et al. (2024) reported that incorporating *Aloe vera* to form a biodegradable coating can improve the functionalities of the packaging system, protecting food against the lipid oxidation process. Saha et al. (2023) stated that *Aloe vera* gel provides a barrier to O₂ and CO₂ and acts as a moisture barrier, thus reducing the oxidation process. In addition, these authors reported that consumers prefer to use natural compounds rather than synthetic agents.

Aloe vera not only helps preserve food due to its antioxidant and antimicrobial properties but also contributes to its nutritional value, as it contains vitamins, fatty acids, amino acids, sugars, minerals, and enzymes (Martínez-Burgos et al., 2022).

Vacuum packaging is a barrier between the external environment and the interior of the material, minimizing the transfer of oxygen to the burger. Although vacuum packaging limits the proliferation of aerobic microorganisms, facultative and obligate anaerobes can survive in this environment, leading to meat spoilage (Pellissery et al., 2020).

Pigment oxidation is another important factor related to meat quality, as it is associated with the parameters of the process used to acquire a meat product (Dursun & Güler, 2023). Wang et al. (2021) pointed out that

changes in meat during storage can be influenced by chemical composition, with a higher concentration of intramuscular fat contributing to lipid oxidation and instability of the color of meat products.

Table 5 shows the results values of the pigment oxidation content of the beef burger samples on different days of storage.

Table 5. Results (mean $\pm$ standard deviation) of the pigment oxidation content assessed through the metmyoglobin (MMb) content of the beef burger samples on different days of storage.

Treatments	Storage (day)			
	1	3	5	7
1	13.76 $\pm$ 0.01 ^{cA}	9.53 $\pm$ 0.10 ^{dF}	16.12 $\pm$ 2.00 ^{bEF}	19.43 $\pm$ 0.90 ^{aB}
2	13.76 $\pm$ 0.01 ^{bA}	11.74 $\pm$ 0.15 ^{bE}	16.91 $\pm$ 1.30 ^{aEF}	16.26 $\pm$ 0.30 ^{aC}
3	12.15 $\pm$ 0.01 ^{cA}	24.15 $\pm$ 0.05 ^{aA}	15.65 $\pm$ 0.05 ^{bF}	12.00 $\pm$ 1.90 ^{cD}
4	12.15 $\pm$ 0.01 ^{bA}	17.31 $\pm$ 0.02 ^{aC}	12.72 $\pm$ 0.02 ^{bG}	12.00 $\pm$ 1.50 ^{bD}
5	13.76 $\pm$ 0.01 ^{bA}	14.72 $\pm$ 0.20 ^{bD}	14.42 $\pm$ 1.20 ^{bF}	17.96 $\pm$ 0.50 ^{aC}
6	13.76 $\pm$ 0.01 ^{bcA}	12.55 $\pm$ 0.06 ^{cE}	15.41 $\pm$ 1.00 ^{abF}	16.81 $\pm$ 0.85 ^{aC}
7	12.15 $\pm$ 0.01 ^{cA}	15.73 $\pm$ 0.05 ^{bD}	18.24 $\pm$ 0.01 ^{aDE}	18.77 $\pm$ 0.01 ^{aC}
8	12.15 $\pm$ 0.01 ^{cA}	19.51 $\pm$ 0.09 ^{aB}	19.51 $\pm$ 0.09 ^{aD}	13.27 $\pm$ 0.15 ^{bcD}
9 (control 1)	13.76 $\pm$ 0.01 ^{bA}	11.74 $\pm$ 0.50 ^{aE}	20.52 $\pm$ 1.08 ^{aCD}	20.48 $\pm$ 0.50 ^{aB}
10 (control 2)	12.15 $\pm$ 0.01 ^{cA}	23.19 $\pm$ 1.00 ^{abA}	21.09 $\pm$ 1.05 ^{bC}	27.50 $\pm$ 2.00 ^{aA}
11 (control 3)	13.76 $\pm$ 0.01 ^{dA}	20.06 $\pm$ 0.25 ^{cB}	23.25 $\pm$ 2.10 ^{aB}	21.96 $\pm$ 0.03 ^{bB}
12 (control 4)	12.15 $\pm$ 0.01 ^{dA}	15.60 $\pm$ 3.00 ^{cD}	26.38 $\pm$ 2.00 ^{bA}	28.60 $\pm$ 0.01 ^{aA}

^{a-d}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-G}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C.

In this study, during the seven days of storage, higher metmyoglobin values (MMB) were observed in control beef burger samples, without *Aloe vera* gel (Samples 10 and 12), at 4 °C ($p < 0.05$).

According to Vieira et al. (2022), one of the most common ways of reducing oxidative processes in animal products is to use synthetic antioxidants. However, the use of natural substances, such as *Aloe vera* gel, to improve the oxidative stability of these products has aroused greater interest, given consumer demand for natural products and their willingness to pay for them (Yilmaz et al., 2024).

Zhou et al. (2023) emphasized that *Aloe vera* gel showed an antioxidant effect, reducing the formation of metmyoglobin over time.

Vieira et al. (2022) stated that lamb meat could have a higher shelf life when enriched with components with antioxidant properties, counteracting the effect of oxidation and microbial spoilage.

In the present work, a fluctuation of MMb values was also verified when using vacuum and conventional packaging. This behavior is common to conventional packing (1 atm), but in vacuum packaging, this behavior could be associated with residual oxygen remaining, which facilitated the formation of metmyoglobin. Reduced oxygen (vacuum packaging) is favorable for preserving meat, as it inhibits the growth of deteriorating aerobic bacteria (Reyes et al., 2022). However, the absence of oxygen can also lead to the formation of metmyoglobin, resulting in an undesirable brown color, rejected by consumers (Olvera-Aguirre et al., 2023).

Oxygen in the environment (conventional packaging) promotes the oxidation of myoglobin, resulting in the formation of oxymyoglobin. This process gives the meat a vibrant red color, but it can accelerate the deterioration of meat, as it favors lipid oxidation and the growth of aerobic microorganisms (Kondjoyan et al.,

2022). For these specific storage conditions, the internal oxygen content transforms metmyoglobin in the meat into oxymyoglobin (Henriott et al., 2020b).

Ruedt et al. (2023) emphasized that the increase in the MMb content in meat is the main cause of undesirable changes in its color, influencing the rejection of the product when the concentrations of metamioglobin exceed 20% of the initial value.

Therefore, the concentrations of metmyoglobin during the seven days of storage would indicate that only samples 3, 4 (vacuum, with 2 and 4% *Aloe vera* gel and at 4 °C), and sample 8 (1 atm packaging, with 4% *Aloe vera* gel and 4 °C) would present sensory acceptance (color). The lower level of pigment oxidation in these three samples indicates that the use of a storage temperature of 4 °C, combined with the application of *Aloe vera* gel, favors the color quality of the burgers studied.

Barbieri et al. (2021) observed that the application of low storage temperatures effectively controlled the oxidation of pigments in beef burgers in conventional packaging.

However, Coaguila Gonza et al. (2023) reported that the formation of ice crystals during storage at low temperatures can cause increased oxidation of myoglobin, denaturation, and reduction of the myoglobin redox system due to damage to the protein in the Longissimus dorsi muscle of Zebu Nellore (*Bos indicus*). Henriott et al. (2020a) also related an increase in the concentration of metmyoglobin in frozen beef steaks to changes in its color due to increased oxidation of oxymyoglobin and deoxymyoglobin.

3.3 Microbial growth

Microbial control during processing and storage is crucial to beef burgers' quality and shelf life. Grinding, deficiency of food additives (preservatives and synthetic antioxidants), and absence of thermal treatments lead to microbiological and chemical spoilage and, consequently, the relatively short shelf life of these beef burgers (Mujović et al., 2023).

In this study, total mesophilic and psychrophilic bacteria were quantified in hamburger samples to assess the effect of refrigeration temperature, combined with the addition of *Aloe vera* gel and the internal atmosphere of the packaging, on the growth of these microorganisms.

Among the pathogenic mesophilic and psychrophilic aerobic bacteria that may be present in refrigerated hamburgers, the following stand out: *Salmonella* spp. (must be absent), pathogenic *Escherichia coli* (must be absent), *Staphylococcus aureus* (tolerated up to 10^3 CFU g⁻¹), and *Listeria monocytogenes* (must be absent) (Soares et al., 2021).

In addition to pathogenic bacteria, the most common aerobic mesophilic and psychrophilic spoilage microorganisms include *Lactobacillus* spp. (tolerated up to 10^7 CFU g⁻¹), *Enterobacteriaceae* (limit of 10^3 CFU g⁻¹), *Pseudomonas* spp. (limit of 10^7 CFU g⁻¹), and *S. aureus* (limit of 10^3 CFU g⁻¹). These microorganisms can cause changes in the flavor, odor, texture, and appearance of the hamburger, compromising its acceptability to consumers (Karanth et al., 2023).

The mesophilic and psychrotrophic bacterial counts of the beef burger samples are shown in Tables 6 and 7. Aerobic mesophilic and aerobic psychrotrophic bacterial counts were reported up to ≤ 7 log CFU g⁻¹, which is the threshold at which the meat is considered spoiled or unacceptable for consumption. These values were established based on the limits defined for the main spoilage microorganisms found in refrigerated hamburgers (Ercolini et al., 2009).

Evaluating total aerobic mesophilic bacteria count, it can be noted that control samples 10 and 12 reached the limit already on day 3 (Table 6). Both samples were prepared without the presence of *Aloe vera* and stored at 4 °C, indicating that increasing storage temperature promotes microbial growth, mainly when no additive is used in beef burger formulation. At the end of storage, the lowest count for aerobic mesophilic bacteria was found in samples 1 and 2 (Table 6). This is in line with Santiesteban-López et al. (2022), which described the use of natural antimicrobials as a strategy to improve the shelf life and safety of meat products.

Table 6. Results (mean $\pm$ standard deviation) of the total aerobic mesophilic bacteria count expressed as log colony forming unit per gram (CFU g⁻¹) of the beef burger samples on different days of storage.

Treatments	Storage (day)			
	1	3	5	7
1	4.78 $\pm$ 0.09 ^{aA}	5.57 $\pm$ 0.01 ^{bAB}	5.31 $\pm$ 0.12 ^{bA}	5.32 $\pm$ 0.39 ^{bA}
2	4.78 $\pm$ 0.09 ^{aA}	5.20 $\pm$ 0.01 ^{bBE}	5.40 $\pm$ 0.01 ^{cA}	5.39 $\pm$ 0.39 ^{dA}
3	5.12 $\pm$ 0.08 ^{aB}	6.46 $\pm$ 0.59 ^{bAC}	6.38 $\pm$ 0.14 ^{bB}	6.55 $\pm$ 0.01 ^{bB}
4	5.12 $\pm$ 0.08 ^{aB}	6.41 $\pm$ 0.01 ^{bBC}	6.49 $\pm$ 0.02 ^{bC}	> 7.00
5	4.78 $\pm$ 0.09 ^{aA}	5.90 $\pm$ 0.06 ^{bA}	> 7.00	> 7.00
6	4.78 $\pm$ 0.09 ^{aA}	5.52 $\pm$ 0.16 ^{ABE}	6.62 $\pm$ 0.2 ^{cABC}	6.01 $\pm$ 0.06 ^{bAB}
7	5.12 $\pm$ 0.08 ^{aB}	6.09 $\pm$ 0.10 ^{bAD}	> 7.00	> 7.00
8	5.12 $\pm$ 0.08 ^{aB}	5.00 $\pm$ 0.15 ^{aE}	> 7.00	> 7.00
9 (control 1)	4.78 $\pm$ 0.09 ^{aA}	6.59 $\pm$ 0.06 ^{bAB}	> 7.00	> 7.00
10 (control 2)	5.12 $\pm$ 0.08 ^{aB}	> 7.00	> 7.00	> 7.00
11 (control 3)	4.78 $\pm$ 0.09 ^{aA}	5.72 $\pm$ 0.23 ^{bBC}	5.52 $\pm$ 0.38 ^{cA}	5.69 $\pm$ 0.05 ^{cAB}
12 (control 4)	5.12 $\pm$ 0.08 ^{aB}	> 7.00	> 7.00	> 7.00

^{a-c}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-G}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C.

Table 7. Results (mean $\pm$ standard deviation) of the total psychrotrophic bacteria count expressed as log colony forming unit per gram (CFU g⁻¹) of the beef burger samples on different days of storage.

Treatments	Storage (day)			
	1	3	5	7
1	5.07 $\pm$ 0.08 ^{aA}	5.58 $\pm$ 0.09 ^{bA}	6.37 $\pm$ 0.13 ^{cAB}	6.52 $\pm$ 0.01 ^{cA}
2	5.07 $\pm$ 0.08 ^{aA}	5.44 $\pm$ 0.03 ^{abA}	6.02 $\pm$ 0.02 ^{bA}	6.11 $\pm$ 0.38 ^{bA}
3	4.78 $\pm$ 0.08 ^{ab}	6.97 $\pm$ 0.06 ^{bB}	7.00 $\pm$ 0.12 ^{bB}	> 7.00
4	4.78 $\pm$ 0.08 ^{ab}	6.40 $\pm$ 0.02 ^b	6.69 $\pm$ 0.02 ^{bAB}	> 7.00
5	5.07 $\pm$ 0.08 ^{aA}	6.58 $\pm$ 0.21 ^{bBC}	6.96 $\pm$ 0.04 ^{Bb}	> 7.00
6	5.07 $\pm$ 0.08 ^{aA}	6.43 $\pm$ 0.15 ^{bBC}	6.51 $\pm$ 0.19 ^{bAB}	6.62 $\pm$ 0.07 ^{bA}
7	4.78 $\pm$ 0.08 ^{ab}	> 7.00	> 7.00	> 7.00
8	4.78 $\pm$ 0.08 ^{ab}	> 7.00	> 7.00	> 7.00
9 (control 1)	5.07 $\pm$ 0.08 ^{aA}	5.53 $\pm$ 0.25 ^{abA}	6.69 $\pm$ 0.38 ^{bAB}	6.95 $\pm$ 0.54 ^{bA}
10 (control 2)	4.78 $\pm$ 0.08 ^{ab}	6.51 $\pm$ 0.22 ^{bC}	> 7.00	> 7.00
11 (control 3)	5.07 $\pm$ 0.08 ^{aA}	6.92 $\pm$ 0.08 ^{bBC}	> 7.00	> 7.00
12 (control 4)	4.78 $\pm$ 0.08 ^{aB}	> 7.00	> 7.00	> 7.00

^{a-c}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-C}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C.

Regarding psychrotrophic bacteria counts, samples 7, 8, and 12 reached the limit for microbial counts already on the 3rd day. These samples represent beef burgers stored in conventional packaging (pressure = 1 atm) at 4 °C, highlighting the importance of lowering the temperature to reduce psychrotrophic bacteria growth (Jin et al., 2022).

At the end of storage, samples 1 and 2 showed the lowest counts for psychrotrophic bacteria, which is the same condition that reduced mesophilic growth. It indicates that storing at 0 °C, in vacuum packaging, and using *Aloe vera* gel can reduce mesophilic growth, promoting product safety.

The behavior of the results is in accordance with those obtained by Ząbek et al. (2021), which studied the effect of vacuum packaging on microbiological and physicochemical parameters of lamb meat, during refrigerated storage.

Aksu et al. (2023) found that the combination of vacuum packaging and spray-dried raspberry powder (antimicrobial and natural antioxidant) generally resulted in lower bacterial growth in beef during refrigerated storage. Comparing mesophilic and psychrotrophic growth, the growth of psychrotrophic bacteria was more pronounced, reaching higher values at the end of storage. The optimum growth temperature for psychrotrophic is 0 to 7 °C, while mesophilic growth is favored at temperatures close to 30 °C, which justifies the greater growth of psychrotrophic in refrigerated conditions (García-Descalzo et al., 2022).

Temperature significantly influences the growth rate and reproductive strategies of microorganisms. However, the temperature that is optimal for growth may not always align with the ideal conditions for all cellular activities (Gonzalez & Aranda, 2023).

During bacterial growth, various chemicals are produced, many of which are catalyzed by enzymes that are sensitive to temperature changes. Each microorganism species has a particular temperature range in which it thrives, though this range can vary widely between species. Additionally, some microorganisms show greater adaptability to different temperatures, while others have more rigid and specific temperature requirements (Gonzalez & Aranda, 2023).

About the packaging atmosphere, it is evident that aerobic conditions (1 atm) favored microbial growth in the beef burger. Conversely, microbial growth in vacuum packaging occurs due to anaerobic bacteria, primarily LAB, which produce lactic acid as their main metabolite (Abedi & Hashemi, 2020). Thus, the increase in microbial counts under these conditions is related to the drop in the food pH (Table 3), as occurred on the 5th and 7th day of storage for vacuum packaging samples stored at 4 °C.

According to Skwarek & Karwowska (2023), the antioxidant and antimicrobial actions are the primary bioactive properties of many vegetables, attributed to their rich phenolic composition. Moreover, the incorporation of plant-based ingredients into meat products has already proven to be a successful and consumer-accepted strategy, particularly considering health and sustainability aspects. Therefore, plant extracts, such as *Aloe vera* gel, are potential ingredients for improving the nutritional, microbiological, and sensory quality of meat products (Aksu et al., 2023).

3.4 Sensory analysis

The detection of color by the human eye is influenced by light reflection, *i.e.*, when light strikes meat, it can be absorbed, reflected, or scattered. The light must reflect off the viewed object and return to the eye to be detected. The eye perceives the reflected light, which is captured and transmitted to the brain, where color is interpreted. The wavelengths of light absorbed by the beef burger are not perceptible to the eye (American Meat Science Association, 2012).

Ribeiro et al. (2021) stated that the product's visual appearance is of great importance because it strongly influences the consumer's purchase decision. These authors also highlighted that consumers discriminate against meat cuts that have lost their fresh appearance, and meat that becomes discolored is often ground and marketed in a reduced-value form.

The results of the sensory color analysis of the beef burger samples are shown in Table 8.

Table 8. Sensory analysis (color) results (mean $\pm$ standard deviation) of beef burger samples during 7 days of storage and according to the meat color scales by American Meat Science Association (2012).

Treatments	Storage (day)			
	1	3	5	7
1	2.28 $\pm$ 0.03 ^{bb}	6.20 $\pm$ 0.90 ^{aABCD}	4.80 $\pm$ 1.00 ^{abBCDE}	4.20 $\pm$ 0.40 ^{bd}
2	2.28 $\pm$ 0.03 ^{bb}	5.89 $\pm$ 1.00 ^{aABCD}	4.21 $\pm$ 0.50 ^{bd}	6.00 $\pm$ 1.00 ^{aABC}
3	2.60 $\pm$ 0.10 ^{ba}	4.31 $\pm$ 0.80 ^{ad}	5.40 $\pm$ 0.65 ^{aBCD}	4.86 $\pm$ 0.90 ^{aCD}
4	2.60 $\pm$ 0.10 ^{ba}	4.90 $\pm$ 0.30 ^{aBCD}	5.30 $\pm$ 0.80 ^{aBCDE}	4.95 $\pm$ 0.20 ^{aBCD}
5	2.28 $\pm$ 0.03 ^{cb}	3.30 $\pm$ 0.80 ^{be}	4.94 $\pm$ 1.00 ^{aBCD}	5.14 $\pm$ 0.70 ^{abBCD}
6	2.28 $\pm$ 0.03 ^{bb}	6.14 $\pm$ 1.10 ^{aABCD}	5.95 $\pm$ 0.65 ^{aABC}	6.34 $\pm$ 1.00 ^{aAB}
7	2.60 $\pm$ 0.10 ^{ca}	6.07 $\pm$ 0.70 ^{aA}	3.25 $\pm$ 0.30 ^{bf}	3.46 $\pm$ 0.20 ^{be}
8	2.60 $\pm$ 0.10 ^{ca}	5.92 $\pm$ 0.20 ^{aABC}	3.84 $\pm$ 0.90 ^{beF}	3.70 $\pm$ 0.70 ^{be}
9 (control 1)	2.28 $\pm$ 0.03 ^{db}	3.94 $\pm$ 0.20 ^{ce}	4.94 $\pm$ 0.20 ^{bc}	6.20 $\pm$ 0.80 ^{aAB}
10 (control 2)	2.60 $\pm$ 0.10 ^{ca}	4.35 $\pm$ 0.80 ^{bCD}	5.84 $\pm$ 0.60 ^{aABC}	6.42 $\pm$ 0.60 ^{aAB}
11 (control 3)	2.28 $\pm$ 0.03 ^{bb}	4.06 $\pm$ 0.70 ^{bCDE}	6.24 $\pm$ 0.50 ^{aAB}	7.05 $\pm$ 1.10 ^{aA}
12 (control 4)	2.60 $\pm$ 0.10 ^{ba}	7.20 $\pm$ 1.10 ^{aA}	7.70 $\pm$ 0.90 ^{aA}	7.32 $\pm$ 1.10 ^{aA}

^{a-d}Different and superscript lowercase letters expressed in the same line indicate significant differences between samples ($p < 0.05$). ^{A-F}Different capital letters and superscripts in the same column indicate significant differences between samples ($p < 0.05$). Sample 1 = 0 atm, 2% *Aloe vera* gel, and 0 °C. Sample 2 = 0 atm, 4% *Aloe vera* gel, and 0 °C. Sample 3 = 0 atm, 2% *Aloe vera* gel, and 4 °C. Sample 4 = 0 atm, 4% *Aloe vera* gel, and 4 °C. Sample 5 = 1 atm, 2% *Aloe vera* gel, and 0 °C. Sample 6 = 1 atm, 4% *Aloe vera* gel, and 0 °C. Sample 7 = 1 atm, 2% *Aloe vera* gel, and 4 °C. Sample 8 = 1 atm, 4% *Aloe vera* gel, and 4 °C. Sample 9 (control 1) = 0 atm, 0% *Aloe vera* gel, and 0 °C. Sample 10 (control 2) = 0 atm, 0% *Aloe vera* gel, and 4 °C. Sample 11 (control 3) = 1 atm, 0% *Aloe vera* gel, and 0 °C. Sample 12 (control 4) = 1 atm, 0% *Aloe vera* gel, and 4 °C. The red color was evaluated using the 8-point structural hedonic scale method (1. Very bright red, 2. Bright red, 3. Matte red, 4. Slightly dark red, 5. Moderately dark red, 6. Dark red to brownish red, 7. Reddish brown, 8. Brown), according to the meat color scales and their scores proposed by American Meat Science Association (2012).

The stability between the results was verified on the first day of the beef burger color analysis, which was variable between the bright red and matte red. However, miscellaneous results regarding the color of the beef burger samples were observed during storage, *i.e.*, the sensory judges could not identify the interference of the form of packaging, temperature, and concentration of *Aloe vera* gel. Overall, the sensory judges observed an increase in the darkening of the red color on the 3rd, 5th, and 7th days of beef burger sample storage.

Ribeiro et al. (2021) observed that the rate of meat browning is related to the effectiveness of oxidation processes and enzymatic reducing systems in controlling the levels of metmyoglobin in meat. Therefore, the products of lipid oxidation catalyze the oxidation of oxymyoglobin to metmyoglobin, and the direct antioxidant action of *Aloe vera* gel on membrane lipids can indirectly delay the oxidation of oxymyoglobin and result in alterations to the meat.

In the present study, sensory judges more evidently observed this behavior on the 7th day of storing beef burgers.

The forms of myoglobin are primarily responsible for the color changes in meat. According to Han et al. (2024), it is believed that the fat content in meat and oxidative reactions damage the protein, which leads to rehydration and lipid oxidation, converting myoglobin into oxymyoglobin. Oxymyoglobin is responsible for the bright red color, as fat reflects light, resulting in the meat's bright appearance. The redness of meat is directly related to oxymyoglobin, and oxidative reactions cause a loss of this redness (Ruedt et al., 2023). The oxidation of myoglobin and oxymyoglobin produces metmyoglobin, which is responsible for the brown color in meat (Dursun & Güler, 2023).

On the 7th day of beef burger sample storage, it was seen that the absence of *Aloe vera* gel and the use of a concentration equal to 4% of this gel accelerated using a temperature of 0 °C, the oxidation of Fe⁺² in myoglobin (Rojas & Brewer, 2008). Consequently, the discoloration occurred with increasing metmyoglobin, responsible for the brown color.

Therefore, the gel prepared from *Aloe vera* represents a source of antioxidant compounds with potential application in food technology.

However, Uşan et al. (2022) also observed that impurities in an ingredient, such as *Aloe vera* gel, can contribute to a strong pro-oxidant effect in meat.

These authors cited that metals present when using a higher gel concentration may be responsible for the opposite effect. This observation may be related to the brown color recorded in the beef burger samples with the addition of 4% *Aloe vera* gel.

4 Conclusion

The influence of *Aloe vera* gel, atmospheric pressure inside the packaging, and temperature on the quality of beef burgers during refrigerated storage was evaluated. All treatments showed an increase in pH values from the fifth day of storage. The concentration of *Aloe vera* gel (2% or 4%) helped reduce lipid oxidation and pigment oxidation, resulting in improved oxidative stability, particularly at 0 °C.

Regarding metmyoglobin (MMb) content, during the seven days of storage, the best results were observed in vacuum-packed samples with 2% or 4% *Aloe vera* gel stored at 4 °C (treatments 3 and 4), as well as in conventionally packaged samples (1 atm) with 4% *Aloe vera* gel at 4 °C (treatment 8).

The lowest counts of mesophilic aerobic bacteria were found in samples treated with *Aloe vera*, stored in vacuum packaging at 0 °C (treatments 1 and 2). However, no significant effect of temperature or atmospheric pressure was observed in reducing the count of psychrotrophic bacteria.

The use of 2% *Aloe vera* gel, regardless of the storage method (with or without vacuum, at 0 °C or 4 °C), helped maintain the desirable color of fresh beef burgers.

Overall, the combination of 2% *Aloe vera* gel, especially with vacuum packaging and storage at 0 °C, proved to be a promising strategy for preserving the quality and safety of raw beef burgers.

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