

Article

Evaluation of the Inhibitory Effectiveness of Extracts from Capparis Spinosa Leaves and Flowers on the Microbial, Chemical, and Sensory Quality of Local Minced Beef During Cold Storage At 4°C

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Citation: Numan S. B., Mamdoouh O. I. Evaluation of the Inhibitory Effectiveness of Extracts from Capparis Spinosa Leaves and Flowers on the Microbial, Chemical, and Sensory Quality of Local Minced Beef During Cold Storage At 4°C. American Journal of Biology and Natural Sciences 2026, 3(10), 88-95.

Received: 10th Jun 2026

Revised: 11th Aug 2026

Accepted: 20th Sep 2026

Published: 07th Oct 2026



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Abstract: The quality deterioration of ground beef during refrigerated storage is very rapid due to lipid oxidation and microbial contamination. The objective of this research was to assess the inhibitory ability of extracts rich in phenolic compounds and tannins obtained from the leaves and flowers of the caper plant (*Capparis spinosa* L.) at concentrations of (0.25 and 0.50% w/w) on the microbial, chemical and sensory characteristics of local ground beef stored at 4 ± 1 °C for 10 days. Results indicated significant ($P < 0.05$) inhibition of total viable count (TPC) and lipid oxidation (TBARS) in leaf- and flower-extract-treated samples compared to the control. On day 10, the total bacterial count in the control sample was above the allowable limit of microbial acceptability ($7.42 \text{ Log}_{10} \text{ CFU/g}$), while the samples treated with flower extract at 0.50% concentration maintained a low microbial load ($6.14 \text{ Log}_{10} \text{ CFU/g}$). Likewise, on day 10, TBARS values in the control reached 2.25 mg MDA/kg , which was above the rancidity threshold (1.0 mg MDA/kg), while all caper treatments kept the TBARS values within a range of $0.41 - 0.57 \text{ mg MDA/kg}$. Sensory evaluation showed that the flower extract 0.50% was effective in preserving color, odor, texture and overall appearance without producing any off-flavors. These results demonstrate extracts of caper plant as an effective natural preservative for extending the shelf life of refrigerated ground beef.

Keywords: *Capparis spinosa* L., Ground beef, Lipid oxidation, TBARS, Microbial spoilage, Tannins, Natural preservatives.

Introduction

Fresh ground beef is a highly perishable food product that is readily deteriorated rapidly in refrigerated storage at 4 °C [1]. Mincing breaks down cell walls and increases the exposed surface area to atmospheric oxygen, and also spreads endogenous heme iron and spoilage microorganisms throughout the meat matrix [2]. Deterioration occurs mainly through two interacting pathways i.e. microbial growth and autoxidation of lipids and proteins [3]. Lipid oxidation leads to volatile secondary compounds (e.g. malondialdehyde, hexanal, ketones) which are responsible for the rancid

odors and off-flavors and promotes the oxidation of oxymyoglobin to brown metmyoglobin [4]. Microbial growth also results in protein degradation, slime formation and degradation of the structural texture [5]. Synthetic antioxidants, such as BHA and BHT, have been extensively used to prevent oxidation; however, there is increasing consumer health concern over the potential toxicological risks of these synthetic antioxidants, thus driving the shift to natural plant-derived antioxidants in “Clean Label” initiatives [6, 7]. The caper plant (*Capparis spinosa* L.) is a dwarf shrub species that occurs throughout the Mediterranean basin and arid areas, and is a rich source of secondary metabolites such as phenolic acids, tannins (condensed and hydrolyzable), flavonoids (quercetin, rutin, etc.) and glucosinolates [8, 9, 10]. Various parts of the caper plant have shown antimicrobial and antioxidant effects in different food systems in recent studies [11, 12]. Phytochemical analysis revealed that caper leaves and floral parts are rich in phenolic compounds and bioactive constituents confirming its health-promoting and food-preservative properties [13, 14, 15]. Furthermore, the biological activities of caper extracts and essential oils have been demonstrated to be relevant in preserving the quality of beef and fish products and prolonging their shelf life, such as meat and fish burgers [16, 17]. The objective of this study was to assess the inhibitory effectiveness of tannin- and phenol-rich extracts from caper leaves and flowers (0.25 and 0.50% w/w) against total aerobic bacterial growth, lipid oxidation and sensory quality deterioration of local ground beef during refrigerated storage (4 °C) for 10 days.

Materials and Methods

Plant Material and Extract Preparation

The fresh leaves and flowers of *Capparis spinosa* L. were collected during full flowering period. The samples were shade dried at room temperature (25 ± 2 °C) to constant weight and then ground to fine powder [18]. The active compounds and tannins were extracted with aqueous methanol (80:20 v/v) in a Soxhlet apparatus using the standard procedures for phenolic compound extraction [19, 20]. The obtained extract was filtered and concentrated under reduced pressure with a rotary evaporator at 40 °C to avoid thermal degradation of active compounds [21]. The dried crude extract was stored in airtight dark containers at -20 °C until further use [22].

Preparation of Ground Beef and Treatments

Fresh local beef (*Longissimus dorsi*) was obtained after rigor mortis, from an automated abattoir under hygienic conditions [3]. Surface fat and connective tissues were removed and the meat was minced using a sterile electric mincer equipped with a 4 mm plate [2]. The ground beef was randomly split into five equal treatment groups

- Control: Ground beef with no plant extract added.
- LE 0.25%: Ground beef + 0.25% (w/w) caper leaf extract.
- LE 0.50%: ground beef. + 0.50% (w/w) Caper leaf extract
- FE 0.25%: Ground beef + 0.25% (w/w) caper flower extract.
- FE 0.50%: Ground beef + 0.50% (w/w). caper flower extract .

Each treatment was hand mixed thoroughly for 5 min under refrigerated conditions to homogenize it [3]. Samples were put into sterile polyethylene bags and kept in refrigerator at 4 ± 1 °C. Microbiological, chemical and sensory evaluation was carried out on days 0, 3, 7 and 10.

Microbiological Analysis (Total Plate Count - TPC)

For enumeration of bacteria, 10 g of ground beef was aseptically placed into a sterile stomacher bag containing 90 mL of sterile 0.1% peptone water and homogenized for 2 min [5]. Serial ten fold dilutions were made. Total Plate Count (TPC) was calculated by pour plate method on Nutrient Agar [12]. Plates were incubated at 37 °C for 24–48 h. Colonies were counted and expressed as Log₁₀ CFU/g.

Measurement of Lipid Oxidation (TBARS)

Lipid oxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS), according to the method described by Kakaei et al. [2]. A 10 g meat sample was homogenized with 20 mL of 7.5% trichloroacetic acid (TCA) solution and filtered through Whatman No. 1 filter paper. Then, 5 mL of the filtrate was added to 5 mL of aqueous thiobarbituric acid solution (0.02 M TBA). The solution was incubated in a boiling water bath at 95°C for 30 min, then cooled under

running water and absorbance was taken at 532 nm wavelength using UV-Vis spectrophotometer. TBARS values were calculated from a TMP standard curve and expressed as mg MDA/kg.

Sensory Evaluation

The sensory characteristics (color, odor, texture, and overall appearance) were assessed by a trained panel of 10 members using a 9-point hedonic scale (9 = extremely desirable, 1 = undesirable, with 5 being the threshold of acceptability) according to standard methods for meat product evaluation [18]. Samples were evaluated in normal lighting conditions at an ambient temperature of 22 ± 1 °C.

Statistical Analysis

The experiment was designed in a factorial arrangement (5 treatments $\times$ 4 storage periods) with three replications. Statistical analysis of data was done by Analysis of Variance (ANOVA) using SAS software. Means were compared by Duncan's Multiple Range Test or Least Significant Difference (LSD) at $P < 0.05$.

Results and Discussion

Inhibitory Effect on Total Plate Count (TPC)

The results of Total Plate Count (TPC) measurement in refrigerated ground beef stored at 4 ± 1 °C are shown in Table 1. The differences between storage time, type of treatment and their two-way interaction (Treatment $\times$ Time) were highly statistically significant ($P < 0.05$).

Table 1. Effect of Capparis spinosa extracts on Total Plate Count (Log10 CFU/g) of ground beef at 4 °C

Treatment	Concentration (%)	Day 0	Day 3	Day 7	Day 10	Main Effect (Treatment)
Control	—	5.50 $\pm$ 0.03 f	6.24 $\pm$ 0.09 c	6.32 $\pm$ 0.03 b	7.42 $\pm$ 0.03 a	6.37 $\pm$ 0.79 A
Leaf Extract	0.25%	5.59 $\pm$ 0.01 e	5.88 $\pm$ 0.05 d	6.05 $\pm$ 0.01 d	6.28 $\pm$ 0.03 bc	5.95 $\pm$ 0.29 B
Leaf Extract	0.50%	5.52 $\pm$ 0.01 ef	5.92 $\pm$ 0.04 d	6.00 $\pm$ 0.01 d	6.24 $\pm$ 0.01 c	5.92 $\pm$ 0.30 B
Flower Extract	0.25%	5.54 $\pm$ 0.03 ef	5.86 $\pm$ 0.05 d	5.97 $\pm$ 0.04 d	6.20 $\pm$ 0.03 cd	5.89 $\pm$ 0.27 BC
Flower Extract	0.50%	5.50 $\pm$ 0.03 f	5.82 $\pm$ 0.06 d	5.92 $\pm$ 0.03 d	6.14 $\pm$ 0.02 cd	5.85 $\pm$ 0.27 C
Main Effect (Time)	—	5.53 $\pm$ 0.04 D	5.94 $\pm$ 0.17 C	6.05 $\pm$ 0.16 B	6.46 $\pm$ 0.54 A	Overall Mean: 6.00 $\pm$ 0.43

* Different lowercase letters indicate significant differences for the interaction (Treatment $\times$ Time), and uppercase letters indicate main effects ($P < 0.05$).

The narrative reading of the tabular data indicates that the main effect of time was a progressive, significant ($P < 0.05$) increase in mean microbial loads from day 0 (5.53 ± 0.04 Log10 CFU/g, letter D) to maximum levels on day 10 (6.46 ± 0.54 Log10 CFU/g, letter A). This is typical for the growth of psychrotrophic spoilage bacteria in refrigerated meat because of the high moisture content and the presence of many nutrients [1]. The lowest overall mean bacterial count (5.85 ± 0.27 Log10 CFU/g, letter C) was observed in the 0.50% flower extract at the main effect level of treatments, which significantly outperformed the control sample (6.37 ± 0.79 Log10 CFU/g, letter A) that had the highest microbial spoilage rate. The other treatments showed similar inhibition efficiency (5.89 - 5.95 Log10 CFU/g, letters B and BC). The interaction effect (Treatment $\times$ Time) showed no significant differences among all treatments on

day 0 ($P > 0.05$) as samples share the same significance letters (e, ef, f) confirming the homogeneity of sample preparation. However, with the storage time, the control sample showed a sharp increase, surpassing the maximum acceptable microbiological limit of 7.0 Log₁₀ CFU/g, according to ICMSF, on day 10 with a value of 7.42 ± 0.03 Log₁₀ CFU/g (letter a). In contrast, all caper extract treatments showed significant inhibition of bacterial growth, keeping lower values than the critical limit up to 10 days ($6.14 - 6.28$ Log₁₀ CFU/g, letters bc, c, cd), with the lowest bacterial load being in the 0.50% flower extract (6.14 ± 0.02 Log₁₀ CFU/g, letter cd). The inhibition of these microbes is attributed to the presence of condensed and hydrolyzable phenolic tannins in capers, such as quercetin and rutin, which affect the integrity and permeability of the bacterial membrane, causing leakage of intracellular ions such as K⁺ and ATP [5, 9, 10], and also inhibiting quorum sensing mechanisms and essential metabolic enzymes [1]. The results of the present study are in accordance with the findings of Merlino et al. [12], Al-Rubaie et al. [16], Kakaie et al. [2], and Mahboubi & Mahboubi [11] in terms of inhibition of meat spoilage bacteria using phenol-rich plant extracts.

Inhibitory Effect on Lipid Oxidation (TBARS)

The results of secondary lipid oxidation measurement (TBARS) presented below demonstrate the superiority of caper extracts in reducing the accumulation of harmful oxidative compounds such as malondialdehyde (MDA).

Table 2. Effect of *Capparis spinosa* extracts on TBARS values (mg MDA/kg) of ground beef stored at 4 °C

Treatment	Concentration (%)	Day 0	Day 3	Day 7	Day 10	Main Effect (Treatment)	
Control	—	0.16 ± 0.01 g	0.44 ± 0.06 c	1.10 ± 0.14 b	2.25 ± 0.35 a	0.99 ± 0.93 A	
Leaf Extract	0.25%	0.15 ± 0.02 g	0.26 ± 0.04 efg	0.40 ± 0.01 cd	0.57 ± 0.01 c	0.34 ± 0.18 B	
Leaf Extract	0.50%	0.15 ± 0.00 g	0.26 ± 0.01 efg	0.36 ± 0.01 de	0.50 ± 0.01 c	0.32 ± 0.15 B	
Flower Extract	0.25%	0.14 ± 0.01 g	0.23 ± 0.03 fg	0.36 ± 0.02 de	0.47 ± 0.02 c	0.30 ± 0.14 B	
Flower Extract	0.50%	0.15 ± 0.01 g	0.21 ± 0.03 fg	0.30 ± 0.01 ef	0.41 ± 0.01 cd	0.27 ± 0.11 B	
Main Effect (Time)	—	0.15 ± 0.01 D	0.28 ± 0.09 C	0.50 ± 0.34 B	0.84 ± 0.79 A	Overall Mean: 0.44 ± 0.48	

* Different lowercase letters indicate significant differences for the interaction (Treatment × Time), and uppercase letters indicate main effects ($P < 0.05$).

TBARS data revealed a constant significant increase ($P < 0.05$) of rancidity values from day 0 (letter D) = 0.15 ± 0.01 mg MDA/kg to day 10 (letter A) = 0.84 ± 0.79 mg MDA/kg resulting from the natural autooxidation of unsaturated fatty acids by oxygen and free iron liberated during mincing [3]. The main effect of time At the treatment level, the control exhibited a very high average oxidation level (0.99 ± 0.93 mg MDA/kg, letter A), while all caper treatments were able to reduce the total average oxidation by 65–72% (0.27 – 0.34 mg MDA/kg, letter B). Regarding the interaction effect, although the initial values were similar for all groups on day 0 ($0.14 - 0.16$ mg MDA/kg, letter g) the control sample deteriorated rapidly, surpassing the sensory threshold for rancidity (1.0 mg MDA/kg) on day 7 (1.10 ± 0.14 mg MDA/kg, letter b) and reaching 2.25 ± 0.35 mg MDA/kg on day 10 (letter a). Conversely, the caper samples showed good oxidative stability, with TBARS values at day 10 within the range of $0.41 - 0.57$ mg MDA/kg (letters c, cd), which was less than half of the critical limit, with the 0.50% flower

extract at the top with 0.41 ± 0.01 mg MDA/kg (letter cd). The oxidative inhibition efficacy is due to the ability of phenolic and flavonoid compounds present in caper parts to donate hydrogen atoms to free radicals, thus breaking the oxidative chain reaction [13, 4, 14], and the high capacity of tannins to chelate ferrous ions (Fe^{2+}) and limit the oxidation-promoting Fenton reactions [23]. This mechanism is consistent with the results confirmed by El-Ghorab et al. [21], Stefanucci et al. [15], Shafaghi Rad & Nouri [17], Nikmaram et al. [7], and Munekata et al. [6].

Preservation of Sensory Attributes

The data presented in the table below show sensory evaluation scores (color, odor, texture, and overall appearance), which determine consumer acceptability of the final product.

Table 3. Sensory evaluation scores of ground beef during refrigerated storage at 4 °C

Sensory Attribute	Treatment	Concentration (%)	Day 0	Day 7	Main Effect (Treatment)
Color	Control	—	8.47 ± 0.50 a	3.50 ± 0.50 c	5.98 ± 2.76 B
Color	Flower Extract	0.25%	8.27 ± 0.25 a	7.27 ± 0.25 b	7.77 ± 0.59 A
Color	Flower Extract	0.50%	8.37 ± 0.40 a	7.53 ± 0.47 b	7.95 ± 0.60 A
Color	Leaf Extract	0.25%	8.50 ± 0.10 a	7.10 ± 0.17 b	7.80 ± 0.78 A
Color	Leaf Extract	0.50%	8.37 ± 0.15 a	7.23 ± 0.40 b	7.80 ± 0.68 A
Odor	Control	—	8.30 ± 0.30 a	3.67 ± 1.15 e	5.98 ± 2.65 B
Odor	Flower Extract	0.25%	8.33 ± 0.50 a	7.10 ± 0.17 cd	7.72 ± 0.75 A
Odor	Flower Extract	0.50%	8.10 ± 0.46 abc	7.33 ± 0.29 abcd	7.72 ± 0.54 A
Odor	Leaf Extract	0.25%	8.20 ± 0.62 ab	6.90 ± 0.17 d	7.55 ± 0.82 A
Odor	Leaf Extract	0.50%	8.13 ± 0.74 abc	7.17 ± 0.29 bcd	7.65 ± 0.73 A
Texture	Control	—	8.33 ± 0.58 ab	3.33 ± 0.58 d	5.83 ± 2.79 B
Texture	Flower Extract	0.25%	8.30 ± 0.26 ab	7.27 ± 0.25 c	7.78 ± 0.61 A
Texture	Flower Extract	0.50%	8.37 ± 0.32 a	7.67 ± 0.29 bc	8.02 ± 0.47 A
Texture	Leaf Extract	0.25%	8.50 ± 0.50 a	7.10 ± 0.17 c	7.80 ± 0.84 A
Texture	Leaf Extract	0.50%	8.17 ± 0.15 ab	7.33 ± 0.29 c	7.75 ± 0.50 A

Sensory Attribute	Treatment	Concentration (%)	Day 0	Day 7	Main Effect (Treatment)
Appearance	Control	—	8.30 ± 0.26 a	3.00 ± 0.00 d	5.65 ± 2.91 B
Appearance	Flower Extract	0.25%	8.17 ± 0.25 a	7.17 ± 0.29 c	7.67 ± 0.60 A
Appearance	Flower Extract	0.50%	8.10 ± 0.46 a	7.87 ± 0.23 ab	7.98 ± 0.35 A
Appearance	Leaf Extract	0.25%	8.17 ± 0.29 a	7.17 ± 0.29 c	7.67 ± 0.61 A
Appearance	Leaf Extract	0.50%	8.20 ± 0.20 a	7.53 ± 0.50 bc	7.87 ± 0.50 A

* Different lowercase letters indicate significant differences for the interaction (Treatment × Time), and uppercase letters indicate main effects ($P < 0.05$). Sensory evaluation for the control sample was discontinued on day 10 as it was unviable for sensory evaluation due to severe contamination and rancidity.

The sensory evaluation discussion fits perfectly with the microbiological and chemical analyzes. On day 0, all samples received high and statistically similar sensory scores (8.10 - 8.50) for the four attributes, indicating that the addition of extracts at 0.25% and 0.50% did not cause any initial adverse effects on palatability [17]. The control sample had all the sensory scores below the limit of acceptability (<5.0) in the 7th day with 3.50, 3.67, 3.33, and 3.00 for color, odor, texture and overall appearance respectively. The control showed color deterioration due to oxidative attack [3] which oxidized the red oxymyoglobin to brown metmyoglobin ($\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$). The odor scores dropped due to accumulation of rancid aldehydes and products of bacterial protein degradation [2]. Loss of desirable texture and appearance due to enzymatic breakdown and formation of surface slime [1]. The control sample spoiled and therefore testing could not be done on day 10 and the sensory testing was only done on day 7. In contrast, all meat samples treated with caper extracts had excellent sensory attributes through day 7 (6.90 - 7.87) and the 0.50% flower extract had the highest scores (color: 7.53, odor: 7.33, texture: 7.67, overall appearance: 7.87). This effective preservation of sensory properties is due to phenolic antioxidants that reduce ferric ions back to a color-preserving ferrous state [16] and tannins that inhibit bacterial enzymes that break down muscle protein tissue [4]. This demonstrates that caper can extend the shelf life of refrigerated ground beef by two-fold with no detrimental effects on its taste and odor as suggested by Al-Rubaie et al. [16] and Nikmaram et al. [7].

Conclusion

The addition of leaf and flower extracts of *Capparis spinosa* L. was effective in inhibiting microbial growth and lipid oxidation of local ground beef during refrigerated storage at 4 °C. Microbial loads were maintained below the acceptable ICMSF limits (7.0 Log₁₀ CFU/g) and TBARS chemical values were maintained below the rancidity threshold (1.0 mg MDA/kg) for up to 10 days at concentrations of 0.25% and 0.50% while maintaining sensory quality. The flower extract 0.50% was the most effective treatment among all the treatments. Caper extracts can be a promising plant-based substitute to synthetic additives for meat preservation under clean label requirements.

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