

Influence of aqueous green tea extract on microbiological activity and biogenic amines formation on minced mutton

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Abstract

The increased demand for meat and meat products necessitates safe preservation in order to meet their desired quality. Natural preservatives have become the safer alternative to artificial preservatives. These preservatives come mainly from plant sources without negative side effects as unlike artificial ones. This study aimed to examine the effect of green tea extract (GTE) on extending the shelf life of fresh mutton under various storage conditions. Lamb leg samples were stored for 0, 3, 6, 9 and 12 days at a temperature of 5°C and frozen storage at -20°C for 30, 60 and 90 days of storage. Minced meat samples were treated with various levels of aqueous extract of green tea leaves to increase the shelf life of fresh mutton. The aqueous extracts were sprayed on minced meat samples at concentrations of 0, 0.5, 1, 1.5 and 2% (GTE1, GTE2, GTE3, GTE4 and GTE5, respectively). The results revealed that the extraction of green tea was instrumental in significantly inhibiting *Escherichia coli* growth in GTE2, GTE4, and GTE5 for up to 4 days in chilled samples. There was no significant effect on frozen samples stored for 30 days. The biogenic amines differed among samples treated with different extracts, with GTE1 having the highest as compared to GTE2, GTE3, GTE4, and GTE5 for samples stored at 5°C for all storage periods. On day 30, cadaverine was not noticed in the mutton but subsequently appeared at an increasing rate in GTE1 as compared to other treated groups after 60 and 90 days of frozen storage. Green tea extract has the potential to act as a natural antibacterial and antioxidant in the preservation of mutton.

1. Introduction

The demand for meat and meat products is increasing globally due to the increase in the population and living standards of a sizable number of the world population (Sohaib and Jamil, 2017; Alnori *et al.*, 2022). Meat supplies high-quality proteins, vital and trace minerals, and a variety of B vitamins in a form that is bioavailable to humans when consumed. Mutton was a special delicacy due to its quality of protein, fat, and trace elements (Saeed *et al.*, 2019; Alnori, 2021). It has been characterized by high water activity as well as a high quantity of readily available nutrients, which enhance rapid microbial growth with resultant slime

texture alterations (Sun and Holley, 2012).

Surface contamination by microbes is commonly thought to produce off-flavors and meat decomposition, although chemical changes that affect meat appearance before microbial degradation might be significant. In general, spoilage affects the sensory acuity of meat and the degree of changes influences consumers' preferences and cooking practices. Due to a lack of sustainable cold chains and other storage facilities, various methods are used in the preservation of meat during transportation. Chilling is one of the short-term storage methods commonly employed (Sharafati-Chaleshtor and Sharafati

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-Chaleshtori, 2017).

Biogenic amines are organic substances with heterocyclic, aliphatic and aromatic structures that are mostly generated by microorganisms in food, particularly meat and meat products, by decarboxylation of amino acids. Amino acid decarboxylases may be found in many microbes, including the Enterobacteriaceae and Micrococcaceae families. Microorganisms also contribute to the production of N-nitrosamines by converting nitrate elements to nitrites and then breaking down proteins into amines (Wang *et al.*, 2015). Some people have severe allergic reactions to foods with high levels of biogenic amines, especially cadaverine, histamine, and putrescine, which can cause breathing problems, hives, vomiting, a high body temperature, high blood pressure, and even more severe toxicological symptoms like headaches, bleeding in the brain, irregular heartbeats, and stomach cramps (Khezrian and Shahbazi, 2018). As a result, biogenic amines in food have gotten a lot of attention (Erol and Ocak, 2020).

The use of herbs in food preservation dates back to 1550 BC by the ancient Egyptians. Many food-borne diseases are suppressed by the use of herbs such as green tea, which are commonly used in Iraq (Kumudavally *et al.*, 2008; Bancirova, 2010; Durak-Dados *et al.*, 2020). Green tea is consumed every day in more than 160 countries. Tea has recently gained attention for its health advantages. Notably concerning tea is a rich source of polyphenols (10-30% of leaf weight), which include bioactive chemicals, catechins, flavonoids, and their derivatives, and has the ability to prevent and cure cardiovascular diseases (Kumudavally *et al.*, 2008). Because the majority of plant extractions contain phenolic chemicals that are considered natural antimicrobials and antioxidants, it's crucial to measure phenolic content and analyze its contribution to antioxidant and antibacterial activity. Phenolic-rich extractions, such as those obtained from green tea extract, are among the best natural antioxidants. Mutton's shelf life has been extended with natural preservatives like green tea extract because consumers are wary of buying foods with artificial preservatives. Green tea extract may prevent the development of pathogens in mutton, as reported by Zhang *et al.* (2017) and Kumudavally *et al.* (2008). Therefore, this study was aimed at examining the effect of green tea extract on the life span of fresh mutton at different storage conditions. It was also aimed at investigating the role of green tea extract in preventing microbiological deterioration as well as biogenic amines during storage.

2. Materials and methods

2.1 Sample preparation

Green tea leaves were soaked in distilled water to produce aqueous extracts (0.5, 1, 1.5 and 2 g of green tea leaves in a volume of 100 mL of distilled water). The four different concentrations of green tea extract (0.5, 1, 1.5 and 2%) were prepared by soaking green tea leaves (0.5, 1, 1.5 and 2 g) in 100 mL distilled water. The extraction flasks were left on a magnetic stirrer at room temperature for 12 h. A rotary vacuum evaporator (Buchi R-114, Switzerland) was used to concentrate the extractions at 40°C after the extract solution had been filtered through Whatman No.1 filter paper. All the meat samples tested were from a male lamb leg aged 1-2 years, obtained from a butcher. Prior to undergoing aqueous green tea extraction, the meat was mechanically minced into medium-sized pieces (weighing 75±10 g/sample) using an electric machine.

2.2 Preservative treatment

The treatments (cold and frozen storage) consist of one control (GTE1, no sprayed) and spraying the following extracts onto samples: 0.5% green tea aqueous extract (GTE2), 1% green tea aqueous extract (GTE3), 1.5% green tea aqueous extract (GTE4), and 2% green tea aqueous extract (GTE5). The samples were sprayed once with green tea extract (7 mL/sample) using an electric spray gun on day 0. Polyethylene-polyamide bags with an ethyl vinyl acetate sealant layer (30×25 cm and 90 µm thickness) were vacuum packaged with duplicates of each treatment and stored at 5°C for chilled samples after being sprayed with the aqueous green tea extraction. The frozen samples were stored at -20°C until analysis was performed. There was a specific analysis done on cold storage samples at 0, 3, 6, 9, and 12 days following packing (about 24 h post-mortem). Each sampling day consisted of five packages per treatment. Samples were taken from frozen storage at 30, 60, and 90 days. The microbiological and biogenic amines assessment was conducted on three packages from each treatment.

2.3 Identification of biogenic amines

High-performance liquid chromatography (HPLC) was used to analyze the biogenic amines, as described by Latorre-Moratalla *et al.* (2009). Each sample was five grams in weight, and it was centrifuged at 2000 g for 10 min at 5°C after being mixed with 20 mL of 0.4 M perchloric acid. The supernatant was removed twice into a volumetric flask (50 mL) after filtering. One mL of either solution of filtrate or the standard was combined with 200 mL of 2 N NaOH and 300 mL of saturated sodium bicarbonate solutions at room temperature for 30

mins. After that, the reaction mixture was incubated for 45 mins at 40°C with 2 mL of Dansyl chloride solution (10 mg/mL in acetone). The reaction process was stopped by adding 100 mL of a 25% NH₃-N solution and then incubating it for 35 mins at room temperature. The mixture was diluted to a final volume of 5 mL with acetonitrile and then filtered through a nylon membrane filter (pore size of 0.45 μm). Twenty mL of the filtered sample was infused for analysis into the HPLC equipment. The biogenic amines of each treatment were separated on a column (250 mm, 4.6 mm ID; 5 μm) and measured using an HPLC system. The gradient procedure began with 35% H₂O and 65% acetonitrile, followed by a 24-minute increase in acetonitrile. The acetonitrile concentration was reduced to 60% from 20 to 30 mins. One mL/min flow rate of biogenic amines was identified at a wavelength of 254 nm. An internal standard was created using putrescine, cadaverine, and histamine. An aqueous dansyl chloride derivative amine calibration curve was used to determine the quantity of each amine.

2.4 Microbiological analysis

Approximately 10 g of samples for each treatment were diluted to 90 mL with sterile 0.1% peptone solution and homogenized using a stomacher (Seward Stomacher 400, UK). Serial dilutions were prepared using dilution tubes. Counts of total viable bacteria were performed using the surface spread method on plate count agar (PCA; Merck, Germany), incubated at 37°C for 24 h. Enumeration of psychrotrophic bacteria was performed by the surface culture method on the glutamate starch phenol red agar (GSP; Merck, Germany) and incubated

at 35°C for 48 h. *Escherichia coli* counts were determined using the surface culture method on brilliant green agar (BGA; Merck, Germany) with incubation at 35°C for 24 h. All microbiological analyses were performed according to the American Public Health Association (2001) Compendium of Methods for the Microbiological Examination of Foods. Results were collected by manual colony counts on suitably diluted plates and expressed as log CFU/g. Counts were calculated by multiplying the average colony count from duplicate plates by the reciprocal of the dilution factor.

2.5 Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data, and Duncan's multiple range test was used to determine the significant differences between groups. $p < 0.01$ was used to determine significance.

3. Results

3.1 Microorganism content

Tables 1 and 2 show the effects of green tea aqueous extract on spoilage microorganisms in minced mutton kept at temperatures of 5°C and -20°C. The viable cell counts of the control and experimental groups were initially (0 days of storage) in a range of 15-17 log CFU/g. The viable cell counts of GTE1, GTE2, GTE3, GTE4, and GTE5 after 3 days of cold storage at 5°C were 22, 65, 43, 40, and 35 log CFU/g, respectively (Table 1). With a 6, 9 and 12-day storage period, the availability of viable cell counts of bacterial cells in GTE2, GTE3, GTE4, and GTE5 was significantly increased ($p < 0.01$),

Table 1. Changes of microorganism populations (log CFU/g) of minced mutton during cold storage.

Microorganism	Treatments	Time of cold storage (day)				
		0	3	6	9	12
Total viable count	GTE1	17±0.008	22±0.008	7±0.003 ^a	ND	ND
	GTE2	15±0.005	65±0.002 ^b	23±0.007 ^b	9±0.006 ^a	24±0.002 ^a
	GTE3	16±0.008	34±0.004 ^c	19±0.008 ^c	6±0.003 ^b	15±0.004 ^b
	GTE4	17±0.002	40±0.006 ^c	10±0.006 ^d	1±0.008 ^c	4±0.007 ^c
	GTE5	16±0.005	35±0.01 ^d	5±0.004 ^c	66±0.007 ^d	33±0.003 ^d
Psychrotrophic bacteria	GTE1	5±0.008	7±0.004 ^a	21±0.03 ^a	21±0.003 ^a	ND
	GTE2	4±0.006	46±0.001 ^b	44±0.006 ^b	54±0.006 ^b	68±0.002 ^a
	GTE3	5±0.004	39±0.005 ^c	31±0.005 ^c	40±0.004 ^c	78±0.007 ^b
	GTE4	5±0.003	25±0.007 ^c	15±0.002 ^d	35±0.004 ^d	56±0.006 ^c
	GTE5	6±0.005	15±0.03 ^d	9±0.002 ^c	22±0.003 ^c	44±0.003 ^d
<i>E.coli</i>	GTE1	12±0.003	22±0.003 ^a	53±0.004 ^a	18±0.006 ^a	21±0.003 ^a
	GTE2	10±0.002	46±0.008 ^b	9±0.005 ^b	89±0.002 ^b	21±0.004 ^b
	GTE3	11±0.006	41±0.002 ^c	61±0.007 ^c	44±0.004 ^c	32±0.003 ^c
	GTE4	12±0.001	33±0.005 ^c	34±0.002 ^d	26±0.003 ^d	17±0.006 ^d
	GTE5	11±0.005	21±0.04 ^d	11±0.005 ^c	2±0.004 ^c	3±0.002 ^c

Values are presented as mean±SE. Values with different superscripts in the same column are statistically significantly different ($p < 0.01$). ND: not detected. GTE1: no sprayed, GTE2: 0.5% green tea aqueous extract, GTE3: 1% green tea aqueous extract, GTE4: 1.5% green tea aqueous extract, GTE5: 2% green tea aqueous extract.

Table 2. Impact of freezing on the microbial load (in log CFU/g) of minced mutton.

Microorganism	Treatments	Time of frozen storage (day)		
		30	60	90
Total viable count	GTE1	22±0.003 ^a	22±0.003 ^a	7±0.003 ^a
	GTE2	65±0.002 ^b	65±0.002 ^b	23±0.007 ^b
	GTE3	34±0.005 ^c	34±0.005 ^c	19±0.008 ^c
	GTE4	40±0.001 ^c	40±0.001 ^c	10±0.006 ^d
	GTE5	35±0.007 ^d	35±0.007 ^d	5±0.004 ^e
Psychrotrophic bacteria	GTE1	18±0.002 ^a	41±0.003 ^b	7±0.003 ^a
	GTE2	16±0.001 ^b	22±0.002 ^b	23±0.007 ^b
	GTE3	17±0.004 ^c	3±0.005 ^a	19±0.008 ^c
	GTE4	16±0.003 ^c	65±0.001 ^c	45±0.006 ^d
	GTE5	15±0.006 ^d	36±0.007 ^d	21±0.004 ^c
<i>E. coli</i>	GTE1	12±0.003	53±0.004 ^a	18±0.006 ^a
	GTE2	10±0.002	9±0.005 ^b	89±0.002 ^b
	GTE3	11±0.006	61±0.007 ^c	44±0.004 ^c
	GTE4	12±0.001	34±0.002 ^d	26±0.003 ^d
	GTE5	11±0.005	11±0.005 ^c	2±0.004 ^c

Values are presented as mean±SE. Values with different superscripts in the same column are statistically significantly different ($p < 0.01$). ND: not detected. GTE1: no sprayed, GTE2: 0.5% green tea aqueous extract, GTE3: 1% green tea aqueous extract, GTE4: 1.5% green tea aqueous extract, GTE5: 2% green tea aqueous extract.

unlike GTE1, which was the lowest or/not detected. Lower viable cell counts of bacterial cells ($p < 0.01$) were observed in GTE5 samples at 90 days as compared to 30 days for GTE5 and GTE1 samples (Table 2). In the samples treated with green tea aqueous extract (GTE2, GTE3, GTE4, and GTE5), there was a statistically significant ($p < 0.01$) drop after 3 days of storage, that an increase in psychrotrophic bacteria and other meat deterioration species as compared to day one of storage.

3.2 Biogenic amines

Histamine, cadaverine, and putrescine are three commonly detected biogenic amines in food that are frequently utilized as indicators of freshness or microbiological deterioration. Tables 3 and 4 show the levels of biogenic amines in minced mutton under two different storage conditions. Except for day one, cadaverine, histamine, and putrescine were the most prevalent biogenic amines for chilling temperature

Table 3. Changes of biogenic amines (mg/kg) of minced mutton during cold storage.

Biogenic amines	Treatments	Time of cold storage (day)				
		0	3	6	9	12
Cadaverine	GTE1	ND	0.77±0.01 ^a	1.77±0.008 ^a	2.76±0.01 ^a	3.89±0.01 ^a
	GTE2	ND	0.64±0.008 ^b	1.34±0.01 ^b	2.02±0.01 ^b	3.0±0.01 ^b
	GTE3	ND	0.53±0.008 ^c	1.02±0.01 ^c	1.21±0.01 ^c	2.23±0.01 ^c
	GTE4	ND	0.37±0.006 ^d	0.89±0.008 ^d	1.02±0.01 ^d	1.74±0.02 ^d
	GTE5	ND	0.18±0.008 ^e	0.41±0.008 ^c	0.78±0.01 ^c	1.12±0.005 ^c
Histamine	GTE1	ND	0.07±0.006 ^a	0.33±0.005 ^a	0.90±0.008 ^a	1.83±0.008 ^a
	GTE2	ND	0.01±0.005 ^b	0.09±0.005 ^b	0.38±0.02 ^b	0.95±0.01 ^b
	GTE3	ND	ND ^b	0.05±0.003 ^c	0.11±0.005 ^c	0.53±0.01 ^c
	GTE4	ND	ND ^b	0.06±0.003 ^d	0.06±0.001 ^d	0.07±0.005 ^d
	GTE5	ND	ND ^b	ND ^d	ND ^d	0.01±0.01 ^c
Putrescine	GTE1	ND	0.008±0.001 ^a	0.06±0.005 ^a	0.75±0.006 ^a	1.44±0.01 ^a
	GTE2	ND	ND ^b	0.03±0.003 ^b	0.23±0.01 ^b	1.01±0.008 ^b
	GTE3	ND	ND ^b	0.008±0.0005 ^c	0.07±0.003 ^c	0.75±0.01 ^c
	GTE4	ND	ND ^b	0.002±0.0003 ^c	0.02±0.005 ^d	0.42±0.005 ^d
	GTE5	ND	ND ^b	ND ^c	ND ^d	0.08±0.008 ^c

Values are presented as mean±SE. Values with different superscripts in the same column are statistically significantly different ($p < 0.01$). ND: not detected. GTE1: no sprayed, GTE2: 0.5% green tea aqueous extract, GTE3: 1% green tea aqueous extract, GTE4: 1.5% green tea aqueous extract, GTE5: 2% green tea aqueous extract.

Table 4. Changes of biogenic amines (mg/kg) of minced mutton during frozen storage.

Biogenic amines	Treatments	Time of frozen storage (day)		
		30	60	90
Cadaverine	GTE1	ND	0.77±0.004 ^a	1.12±0.008 ^a
	GTE2	ND	0.64±0.008 ^b	0.80±0.01 ^b
	GTE3	ND	0.53±0.008 ^c	0.73±0.004 ^c
	GTE4	ND	ND	0.54±0.008 ^d
	GTE5	ND	ND	0.45±0.008 ^c
Histamine	GTE1	ND	ND	0.73±0.008 ^a
	GTE2	ND	ND	0.42±0.02 ^b
	GTE3	ND	ND	0.05±0.005 ^c
	GTE4	ND	ND	0.6±0.005 ^d
	GTE5	ND	ND	ND
Putrescine	GTE1	ND	0.002±0.0005 ^a	0.01±0.005 ^a
	GTE2	ND	ND ^b	0.003±0.003 ^b
	GTE3	ND	ND ^b	ND ^c
	GTE4	ND	ND ^b	ND ^c
	GTE5	ND	ND ^b	ND ^c

Values are presented as mean±SE. Values with different superscripts in the same column are statistically significantly different ($p < 0.01$). ND: not detected. GTE1: no sprayed, GTE2: 0.5% green tea aqueous extract, GTE3: 1% green tea aqueous extract, GTE4: 1.5% green tea aqueous extract, GTE5: 2% green tea aqueous extract.

storage. They were drastically increased on day 3 with a significant difference ($p < 0.01$) among the five groups from day 9 to 12. The level of cadaverine, histamine, and putrescine was the highest in the control group (GTE1), but it went down as the amount of green tea extract in the treated groups went up. Biogenic amine formation in mutton during frozen storage is shown in Table 4. The concentration of cadaverine was highest at 1.12 mg/kg, followed by histamine (0.73 mg/kg) and putrescine (0.01 mg/kg) at the 90th day of storage in GTE1. The present study found that during chill storage, histamine and putrescine concentrations were significantly lower ($p < 0.01$) in GTE5 when compared to GTE1 and GTE2, even though it was not detected at days 0, 3, 6 and 9.

4. Discussion

4.1 Microorganism content

The green tea aqueous extract used in the present study was discovered to have a strong inhibitory effect against both spoilage and some pathogenic microorganisms in the treated meat as which might be due to the lowered pH. Lowering meat pH may help in increasing the shelf life by up to 4 days (Kumudavally *et al.*, 2008). All of this lines up with the findings of Bouarab-Chibane *et al.* (2017) who reported that during a 12-day storage period at 4°C, ground beef patties added with and without 10 g/kg of each of the five plant extracts (green tea leaves, pomegranate peel, prune flesh, gaillac red wine powder and grape seed extracts) showed no significant reduction in total viable counts, but they all significantly inhibited lipid peroxidation, as it's the

most common form of chemical deterioration of meat quality. The initial *E. coli* of meat samples ranged between 10 and 12 log CFU/g, as indicated in Table 1, representing the meat used in this study being of good quality. Previous studies have revealed that a combination of antimicrobials with essential oils and/or extracts may effectively limit *E. coli* growth in food models (Khezrian and Shahbazi, 2018). This is consistent with our findings. For fermented foods, tea polyphenols might be a good alternative because of their synergistic effect on meat quality and shelf life (Li, 2015). However, Bancirova (2010) has examined the antibacterial properties of green and black teas against gram-negative pathogens (*E. coli*). However, no changes in antimicrobial impact or antioxidant activity were found. These discrepancies may be attributable to variations in the teas' geographical origins, leaf ages, or leaf quality.

Green tea extract has a wide range of antibacterial action, which is attributed to the existence of polyphenolic chemicals like catechins, which act on microbial cells, causing cell material leakage and affecting RNA and DNA metabolism (Kumudavally *et al.*, 2008). Similarly, lean and minced meats have been observed to have a low total viable count (Khezrian and Shahbazi, 2018). Samples GTE4 and GTE5 had the largest number of psychrotrophic bacteria, as indicated in Table 2. However, it appears that the formation of biogenic amines in red meat is significantly influenced by the total viable count and psychrotrophic bacteria. There was no direct correlation between biogenic amines

content and microbial loads in fermented products, which is difficult because of the formation of biogenic amines caused by microbial activity, which was strongly influenced by the strain. These results are consistent with Ikončić *et al.* (2013), who stated that cadaverine and putrescine concentrations might be utilized as chemical markers of raw meat and hygienic practices since their buildup is related to the activities of contaminating bacteria. The putrescine and cadaverine concentrations have been reduced in meat treated with bamboo leaves and tea polyphenols.

This study supports evidence from previous studies (Fan *et al.*, 2015; Zhang *et al.*, 2017). On day 30 of icing storage, the *E. coli* of minced meat samples did not significantly differ according to the treatments and ranged between 10 and 12 log CFU/g. Previous findings reported that the Enterobacteriaceae family is one of the most prevalent microbes linked to red meat deterioration when kept for lengthy periods (Khezrian and Shahbazi, 2018). As a result, in red meat, *E. coli* was the most common. In the current research, the GTE1, GTE2, GTE3, and GTE4 had a linear increase in *E. coli* count, but GTE5 had a downward trend. The findings in this study are consistent with the results of Rubio *et al.* (2016), who clarified that the *E. coli* counts in lamb meat increased significantly during storage. However, there are numerous probable modes of action, including cell wall destruction leading to internal component leaking, morphological changes, and ROS production in bacterial cells, which leads to oxidative stress. Polyphenols may affect protein synthesis and metabolism in bacterial cells as well as restrict the synthesis of DNA by reducing ATP production, gyrase activity, and biofilm development (Efenberger-Szmechtyk *et al.*, 2021).

4.2 Biogenic amines

The current finding is consistent with Sun *et al.* (2018), who reported that increasing the levels of green tea extract has a role in enzymatic inhibition that may delay the synthesis of cadaverine, histamine and putrescine. A cut-off point for raw mutton acceptability has been established based on the presence of cadaverine and other amino acids, showing that the treated samples are of acceptable quality for up to four days of storage (Yu *et al.*, 2020). Cadaverine and other biogenic amines were missing in control and treated samples on zero day of storage until appearing at the end of the first and fifth days of storage. The present study's results corroborate those of Kumudavally *et al.* (2008). A possible explanation for this might be that green tea extract inhibits the formation of these amines because of its content of polyphenols that have a synergistic effect in combination with antibiotics (Parvez *et al.*, 2019).

Similarly, Fan *et al.* (2015) also reported that histamine is the most toxicologically significant biogenic amine that leads to headaches, urticaria, flushing, hypotension, stomach cramps, chemical intoxication, and other health issues. Histamine's potential toxicity can be amplified by other biogenic amines like putrescine and cadaverine (Durak-Dados *et al.*, 2020). A possible explanation for the variations between the five present treatment groups (GTE1, GTE2, GTE3, GTE4 and GTE5) were that the anaerobic environment prevents the proliferation of bacteria able to produce biogenic amines. This would explain why green tea extract suppresses the synthesis of cadaverine and putrescine.

The initial concentrations of other amines were not detected for all treatment groups. A possible explanation for focusing on biogenic amine formation in either cold or frozen storage is that the amount of biogenic amines in foods is relevant in terms of food quality, shelf life, and public health (Gardini *et al.*, 2016). Cadaverine, histamine, and putrescine were not detected after 30 and 60 days of frozen storage. Those are consistent with Efenberger-Szmechtyk *et al.* (2021), who stated that all the herbal extracts prevented both biogenic and nitrosamine production. However, cadaverine was the most abundant source of amines in all treatments and storage conditions of this study. This finding is contrary to a previous study that reported the absence of cadaverine in raw beef or raw ground beef, although there were modest levels of histamine and putrescine (Durak-Dados *et al.*, 2020). Green tea flavonoids may have antibacterial effects on food spoilage microbes, as previously observed in beef patties (Gai *et al.*, 2015), because the cadaverine concentration has decreased proportionately to the amount of green tea extracts utilized in our investigation. The most intriguing finding was that the levels of biogenic amines increased over time without any influence from green tea extract. Differences in biogenic amine concentrations were discovered as a result of increased storage time, as well as decreased amounts of added green tea extract treatments. The levels of biogenic amines are positively related to storage duration and are used as a measure of proteolytic activity. Cadaverine has been identified as a useful biomarker for determining spoilage levels at threshold (Kumudavally *et al.*, 2011). In the current study, cadaverine was missing in mutton at day 30, but it rose after 60 and 90 days of frozen storage. According to this study, the treated samples did not deteriorate after 60 days of storage. The same finding was also reported by Ruiz-Capillas *et al.* (2004), who reported an increase in histamine, putrescine, cadaverine, and agmatine. This elevation was only a little and occurred at the end of the study. The biogenic amines found in this study showed that the green tea extract treatment protects proteins

against degradation. The biogenic amine concentration was proportional to the level of green tea extract utilized. Because biogenic amines are generated by bacterial decarboxylase activity, this impact would be antimicrobial (Gai *et al.*, 2015).

4. Conclusion

In this investigation, green tea aqueous extract exhibited a strong bacteriostatic effect on meat, thereby inhibiting the formation of biogenic amines due to its antibacterial and antioxidant components. This, therefore, warrants its practical application in increasing the shelf life of fresh mutton during storage. As a consequence, the spraying treatments using green tea aqueous extract reduced the count of microorganisms and biogenic amines. Green tea extract levels in all treated samples were below toxic levels. Therefore, the aqueous extract of green tea shows the potential to be used as a preservative in mutton to prolong shelf life.

Conflict of interest

The authors declare no conflict of interest.

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