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Green tea – A potential preservative for extending the shelf life of fresh mutton at ambient temperature (25 ± 2 °C)

K.V. Kumudavally *, H.S. Phanindrakumar, Aisha Tabassum, K. Radhakrishna, A.S. Bawa

Defence Food Research Laboratory, Siddarthanagar, Mysore 570 011, India

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Abstract

This investigation was taken up to evaluate the feasibility of using green tea (GT) to extend the shelf life of fresh mutton, at ambient storage conditions (25 ± 2 °C and 85 ± 5% RH). The ethanolic extract of GT (GTE) was found to significantly inhibit ($P < 0.01$) spoilage microflora, including certain pathogens of acidulant treated mutton (pH 3.8) for up to 4 days. Application of GTE did not cause any deleterious change in sensorial and physical quality and the mutton was acceptable for up to 4 days. While the control samples showed initial signs of spoilage between 20 and 24 h and registered an increase in free fatty acids (FFA) from 1.24 g to 4.1 g/100 g lipid and biogenic amine index (BAI) from 0.27 mg to 4.63 mg/100 g mutton, at the end of two days of storage, the GTE treated sample showed FFA levels of 1.5 g/100 g lipid and BAI of 0.25 mg/100 g mutton at the end of the 4 days. GTE treatment could be effectively used to extend the shelf life of fresh mutton for up to 4 days in Indian climatic conditions, since it significantly ($P < 0.01$) inhibits the formation of these lipolytic and proteolytic degradation products, which are responsible for sensorial spoilage.

Keywords: Green tea; Mutton; Preservation; Microflora; FFA; BAI; Shelf life

1. Introduction

Green tea is being used in more than 160 countries every day, for drinking (Bong-Jeun An et al., 2004). Recently, tea has attracted attention for its health benefits, particularly with respect to its potential for preventing and treating cardiovascular diseases, as tea is a very good source of polyphenols (10–30%, dry leaf weight) including bioactive chemicals, flavonoids and catechins and their derivatives (Jane V. Higdon & Balz Frei, 2003). Several workers have reported the antibacterial property of green tea extracts. The beneficial effect of GT catechin (GTC), on human enteric microflora, in inhibiting the growth of harmful bacteria and promoting the growth of useful bacteria has been reported (Ahn, Kawamura, Kim, Yamamoto, & Mitsuoka, 1991). Catechins, the important phenolic component of GT are known to inhibit bacterial growth (Fukai, Ishigami, & Hara, 1991; Juneja, Okubo, & Hung, 2000; Okubo & Juneja, 1997; Sakanaka & Kim, 1997; Toda, Okubo, Ohnishi, & Shimamura, 1989). Most of the published literature, while noting the antimicrobial activity of green tea catechins against specific cultures, have not exploited this property to preserve fresh mutton which contains heterogeneous micro-organisms (normal flora). There have been too few studies in real foods employing GT as a preservative. The efficacy of antibacterial component *in situ*, i.e. in foods, is expected to be often much lower than *in vivo*, since the active components could bind with food ingredients like proteins and fats and thus exhibit decreased efficiency (Davidson, 1997).

Mutton being a highly perishable commodity requires special attention during handling/storage/transportation. In India, the practice of slaughter, holding and sale of meat under commercial conditions is very much different from that in western countries. In many parts of India, the sheep carcasses are brought to the retail shops from the local

* Corresponding author. Tel.: +91 0821 2473290; fax: +91 821 2473468.
E-mail address: dfrlmysore@sancharnet.in (K.V. Kumudavally).

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slaughter houses immediately after slaughter and consumption of hot meat (unchilled meat) is a common practice throughout the country. The hot meat (35–37 °C) is held at ambient temperature (25 ± 2 °C) and offered for sale for 18–20 h in the retail shops, unlike in western countries where the carcasses are held at chilled temperature (5 °C) for ageing. Ageing helps in tenderisation which is desirable, whereas when carcasses are held at ambient temperature for long hours, it results in microbial spoilage resulting in undesirable changes like textural changes (which is different from tenderisation), changes in WHC and in other physical, chemical and microbiological parameters. Thus the carcasses had a shelf life of 18–20 h when held at ambient temperature (Narasimha Rao & Sreenivasa Murthy, 1985). Hence an attempt has been made to extend the shelf life of mutton at ambient temperature using natural preservatives like green tea extract, since consumers are reluctant to eat foods containing preservatives of chemical origin. Kumudavally, Srihari, Bhagirathi, Radhakrishna, and Bawa (2005) have observed the inhibitory effect of GTE on mutton flora (heterogenous flora) including certain pathogens. Report on the use of GTE in preserving foods, particularly in extending the shelf life of fresh mutton is not much elucidated in the literature. Hence the present study was taken up to investigate the effect of GTE in preserving raw mutton at ambient storage conditions.

2. Materials and methods

2.1. Mutton

Mutton (leg portions)-weighing 1.5–2 kg from 18 to 20 months old 'Bannur' Sheep (a local breed), after 6–7 h post-mortem, was purchased from a known source in six different batches on different dates. The mutton was washed in potable water and was hung vertically for 1 h (to allow the water to drain).

2.2. Green tea extract (GTE)

Commercially available dry green tea leaves (*Camellia sinensis*) were procured and soaked overweight in 95% ethyl alcohol (1:4 w/v) and filtered after thorough agitation for 10–15 min in a rotary flash shaker.

2.3. Preservative treatment

The washed mutton sample was dipped (1:3 w/v) in hot water (66 °C) containing acidulants (1% each of lactic acid and glacial acetic acid), in a vertical tank (30 cm × 90 cm) and removed immediately. The combination of these acids, coupled with higher temperature, helps to reduce the surface microbial load of meat (Anderson & Marshall, 1990; Anderson, Marshall, & Dickson, 1992). The above treatment also aided in reducing the surface pH of mutton from 5.6 to 3.8, thereby increasing the antibacterial activity of phenolic components, which are known to be active at low pH (Sara Burt, 2004). After the acidulant treatment, the sample was hung vertically for 30 min to drain surface water and sprayed twice with GTE using an electric spray gun (Pilot Power-E88, India) at an interval of 50–60 min (for 1 kg mutton, 100 ml of extract). The above treated mutton (T_4) along with four types of control samples {T_0-without any treatment, T_1-with only ethyl alcohol, T_2-with only acidulants and T_3-with only GTE} were stored in a room where the desired temperature and humidity were maintained (25 ± 2 °C and 85 ± 5% RH) and observed once every 3 h for off odour development till the samples become unacceptable.

2.4. Analytical methods

The samples were periodically analysed for sensory, microbiological, physical and chemical parameters as follows.

2.4.1. Sensory analysis

Sensory attributes viz, colour, odour and texture were evaluated using 8-point hedonic scale by a semi-trained panel consisting of 15 members.

2.4.2. Microbiological analysis

Microbiological media and media components were obtained from Hi-Media laboratories, Mumbai, India. A 10 g sample was homogenised with 90 ml sterile peptone water. Multiple decimal dilutions were made with the sample diluents. The standard plate count (SPC) was enumerated on plate count agar (PCA), yeast and moulds (Y&M) on potato dextrose agar (acidified to pH 3.5 using 10% tartaric acid), Lipolytics on Tributyrin Agar (TBA), *Enterobacteriaceae* count on Violet Red Bile Dextrose agar (VRBDA) and phospholipids degrading organisms on PCA agar containing 5% egg yolk emulsion. The pathogens *S. aureus* and *E. coli* were enumerated using Baird Parker Agar (Harrigan & Mc Cance, 1976) and 4-methyl umbelliferyl β-D-glucoronide (MUG) agar (Srihari & Vijaya Rao, 1998), respectively.

2.5. Physical parameters

Hunter Lab value: The *L* (lightness), *a* (redness) and *b* (yellowness) values of the samples were evaluated using a Tristimulus Colorimeter (Model 1456 MF, Data Lab System, Silvasa, India). A D 65 lamp was used as illuminant at 10^0 observer with an aperture diameter of 1.5 cm. Infinite solid sample was used in a spectrally pure quartz cuvette without a white background. The data was interpreted using 'Chromaflash colour matching system' calibrated with white tiles.

Texture: The textural changes of the samples were estimated in a Texture analyser (Model TA HDi, Stable micro systems, UK) using a software, 'Texture expert', Version 1.22. Longitudinal section of deboned mutton chunks (2.5 cm^3) were subjected to force (kg) to compress to

25%, at a speed of 0.5 mm/s and the result was expressed in Newtons.

Water holding capacity (WHC): The WHC was analysed (Wardlaw, Mc Caskill, & Acton, 1973) using 10 g sample and centrifuging at 10,000 rpm for 10 min in a refrigerated centrifuge (4 °C) and expressed as g/100 g mutton.

2.6. Chemical parameters

All the reagents and chemicals used were of analytical reagent (AR) grade obtained from Qualigens Fine Chemicals, (India). Standard palmitic acid and other amines like putrescine, cadaverine and spermidine were obtained from Sigma Chemical Co., St. Louis, MO, USA.

2.6.1. Metmyoglobin (MMb)

MMb was estimated as per the method described by Trout (1989) by homogenising 10 g minced mutton in 100 ml of cold phosphate buffer pH 6.8 and measuring optical density (OD) at 525, 572 and 700 nm using the formula:

$$\%\,\text{Metmyoglobin} = \{1.395 - [(A_{572} - A_{700})/(A_{525} - A_{700})]\} \times 100$$

and expressed as g/100 g of total pigment.

2.6.2. Analysis of free fatty acids (FFA)

Total lipids from the samples were extracted using a chloroform and methanol mixture according to the method of Folch, Lees, and Stanley (1957). After removal of excess solvent, the fat was dried, weighed and analysed for FFA by colorimetric procedure as described by Lowry and Tinsley (1976). The concentration of FFA present in the sample was calculated from the standard curve constructed with different aliquots of palmitic acid solution, treated in the same manner and expressed as % on lipid basis.

2.6.3. Analysis of phospholipids (PL)

Lipid phosphorus from the lipid extract of samples was analysed colorimetrically using 4-amino-3-naphthol-sulfonic acid (ANSA) reagent (Fiske & Subba Row, 1925). The amount of lipid phosphorous present was determined with the help of a linear graph drawn using various aliquots of standard potassium phosphate solution (80–400μg). Phospholipid (PL) content was determined from lipid phosphorous (P) levels ($\text{PL} = P \times 25$) (Leseigneur-Meynier & Grandemer, 1991) and expressed as % on lipid basis.

2.6.4. Analysis of biogenic amines

Extraction of amines from samples and their derivatisation was done as reported by Rosier and Petegham (1988), with a slight modification. Twenty five grams of the sample was homogenised with 50 ml 5% hot (80–90 °C) trichloroacetic acid (TCA) solution for 2 min in a homogeniser. The homogenate was centrifuged at 5000 rpm for 10 min. The supernatant was filtered and 5 ml filtrate was dansylated using 2 ml dansyl chloride (10 mg/2 ml acetone), 1 ml pH 9 buffer concentrate (E.Merck, India) and five drops of 4 N sodium hydroxide solution. After 1 h at 55 °C for the completion of dansylation, the sample was centrifuged at 20,000 rpm for 3 min and the supernatant used for HPLC analysis. Similarly, a stock standard dansyl amine of putrescine, cadaverine and spermidine was prepared using 17–20 mg of the standard amine in 10 ml 5% TCA. From this, working standard was prepared by diluting the stock tenfolds and 1 ml of working standard was subjected to dansylation as above.

HPLC analysis: The dansylated solution (25 μl) was analysed on HPLC (Waters, Model 1525) using RP C_{18} micro Bondapack column and a gradient elution of methanol–water, (starting with 70 ml methanol and 30 ml water and ending with 100 ml methanol over 15 min) and UV detector (Model 2487) at 254 nm. Peak identity was established with the help of standards and peak area was measured using a microprocessor-based integrator. Various amine levels in the mutton were quantified as mg/100 g mutton (on fresh weight basis).

Determination of biogenic amine index (BAI): An index based on the sum of putrescine, cadaverine and spermidine, the major biogenic amines found in mutton was drawn for the acceptance of mutton as per Hernandez-Jover, Izquierdo-Pulido, Vecina-Nogeus, and Vidal-Carou (1996).

2.7. Statistical analysis

Data obtained were subjected to one way analysis of variance (ANOVA) and the levels were differentiated using Duncan's multiple range test (Steel & Torrie, 1980). Significance level was established at $P < 0.01$.

3. Results and discussion

Table 1 shows the sensory attributes of the control (T_0) and treated (T_4) samples during storage at ambient conditions. Since control samples (T_0, T_1, T_2 and T_4) were acceptable only up to 18 h, 24 h, 28 h and 72 h, respectively, samples with acidulants and GTE treatment (T_4) which were keeping good for up to 4 days, were considered for detailed analysis and compared with control samples-T_0 {which were analysed only up to 48 h (2 days), since they spoiled completely by that time}. Higher scores for colour, odour and texture were observed for T_4 samples as compared to one day stored control samples (T_0), till the end of the four days of storage. A very slight off odour was noticed on the fifth day and the texture was slightly dried-like in case of T_4 samples because of surface dehydration, though the colour score was still in the acceptable limit.

Table 2 shows the microbial profile of the control and the treated mutton during storage at ambient temperature. The SPC levels were well within the acceptable limit in case of treated samples up to 4 days of storage (Carl,

Table 1
Sensory attributes of preservative treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH

Storage period	Control			Acidulant + GT extract treated		
	Colour	Odour	Texture	Colour	Odour	Texture
0 day (fresh)	7.95 ± 0.09	7.96 ± 0.11	7.30 ± 0.11	7.93 ± 0.09	7.94 ± 0.08	7.92 ± 0.08
1 day	3.26 ± 0.19	2.11 ± 0.12	4.20 ± 0.19	6.78 ± 0.12	7.24 ± 0.06	7.82 ± 0.06
2 day	1.16 ± 0.05	1.07 ± 0.07	1.20 ± 0.08	6.21 ± 0.10	6.89 ± 0.09	6.0 ± 0.04
3 day	ND	ND	ND	6.16 ± 0.09	6.07 ± 0.09	5.42 ± 0.07
4 day	ND	ND	ND	6.09 ± 0.10	5.95 ± 0.07	5.20 ± 0.04
5 day	ND	ND	ND	6.00 ± 0.11	4.14 ± 0.07	4.10 ± 0.04

Mean ± SD ($n = 12$).
ND = Not done.
On 8 point hedonic scale (1 = extremely undesirable, 8 = extremely desirable).

Table 2
Effect of acidulant and GT E (T_4) treatment on the microbial profile (Logcfu/g) of raw mutton during the storage at 25 ± 2 °C and 85 ± 5% RH

Parameter	Control			Acidulant + GT extract treated					
	0 day	1 day	2 day	0 day	1 day	2 day	3 day	4 day	5 day
TPC	4.82 ± 0.21^{a}	7.98 ± 0.21^{b}	9.24 ± 0.31^{c}	4.72 ± 0.10^{a}	5.12 ± 0.09^{ab}	5.19 ± 0.10^{ab}	5.29 ± 0.09^{ab}	5.95 ± 0.05^{ab}	6.85 ± 0.10^{ab}
Entero bacteriaceae	2.95 ± 0.33^{a}	7.04 ± 0.09^{b}	8.79 ± 0.09^{c}	2.88 ± 0.07^{a}	3.85 ± 0.06^{ab}	3.95 ± 0.09^{ab}	4.25 ± 0.07^{ab}	5.15 ± 0.07^{ab}	6.53 ± 0.11^{ab}
Lypolytics	4.19 ± 0.18^{a}	7.82 ± 0.08^{b}	8.98 ± 0.07^{c}	3.95 ± 0.06^{a}	4.85 ± 0.05^{ab}	4.94 ± 0.07^{ab}	5.04 ± 0.08^{ab}	5.15 ± 0.07^{ab}	6.63 ± 0.09^{ab}
PL breaking	4.38 ± 0.16^{a}	7.72 ± 0.07^{b}	9.14 ± 0.05^{c}	4.29 ± 0.09^{a}	4.90 ± 0.10^{ab}	5.10 ± 0.11^{ab}	5.15 ± 0.09^{ab}	5.80 ± 0.11^{ab}	6.41 ± 0.06^{ab}
Y&M	2.11 ± 0.08^{a}	4.61 ± 0.10^{b}	7.91 ± 0.06^{c}	1.97 ± 0.07^{a}	Absent	Absent	Absent	Absent	1.90 ± 0.05^{a}
S. aureus	2.64 ± 0.09^{a}	5.79 ± 0.12^{b}	6.46 ± 0.10^{c}	2.53 ± 0.08^{a}	Absent	Absent	Absent	Absent	Absent
E. coli	2.74 ± 0.08^{a}	4.62 ± 0.09^{b}	6.73 ± 0.07^{c}	2.62 ± 0.06^{a}	Absent	Absent	Absent	Absent	Absent

Values are means ± SD ($n = 10$). The means having different superscripts in a row are significantly different ($P < 0.01$).

1975). Significant ($P < 0.01$) decrease in other meat spoilage organisms was noticed in treated samples at the end of four days of storage as compared with 1 day stored control samples, which were sensorially unacceptable. While yeast and moulds were absent in treated samples (4 day stored), the pathogens *S. aureus* and *E. coli* were absent throughout the storage period. The broad spectrum of antibacterial activity of GT extract is found to be due to the presence of polyphenolic compounds (catechins) which act on the microbial cell, leading to leakage of cell material and which also affect the RNA and DNA metabolism (Ultee, Kets, & Smid, 1999). Juneja et al. (2000), Sakanaka and Kim (1997) and Ahn et al. (1991) have confirmed the inhibitory activity of green tea catechins against several food borne bacteria. Studies have shown that, GT catechins effectively inhibit the growth of several strains of pathogens like V*ibrio*, S*taphylococcus, Campylobacter*, etc. (Toda et al., 1989). In the present study, the phenolic component of green tea is found to exert a profound inhibitory activity against spoilage organisms including certain pathogens in treated mutton at low pH (GTE + acidulants) and thus help in extending the shelf life for up to 4 days.

Colour is one of the most important sensory attributes, which determines the acceptability rate. The red colour of the meat is mainly due to the myoglobin content. Myoglobin is highly susceptible to oxidation to MMb due to lipid oxidation and microbial spoilage. Fig. 1 shows the effect of GTE treatment on the MMb formation during storage. Control samples, upon storage for two days, showed a significant increase ($P < 0.01$) in MMb levels (from 32.5% to 63.41%), whereas treated samples showed a marginal increase in the MMb level up to 4 days. A positive correlation ($r > 0.95$) was noticed between MMb levels and SPC in both control and treated samples. Verma and Sahoo (2000, 2001) observed a positive correlation between MMb levels and sensory and microbiological quality during storage of minced chevon at 4 °C. Neil and Hastings (1925) and Smith and Alford (1969) reported that, *Pnemococci, Pseudomonas*

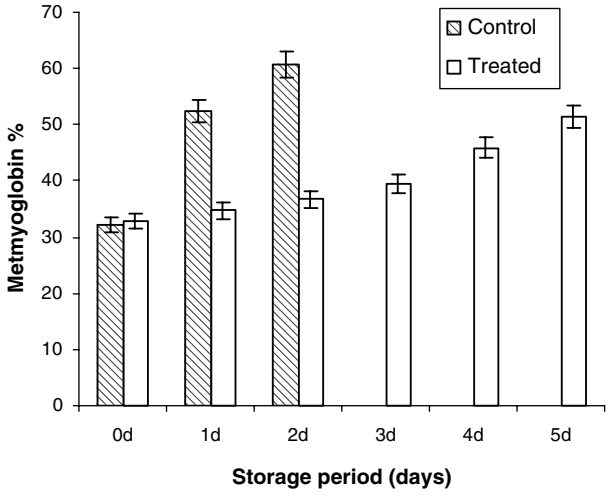

Fig. 1. Changes in metmyoglobin level in control and treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH ($n = 9$).

ovalis, Micrococcus bring about oxidation by the production of peroxides. Signs of brownish discoloration were observed, when atleast 60% of unstable reduced myoglobin present become oxidised to MMb (Armstrong, 1993). Discoloration of meat due to microbial growth as reported by Schweigert (1956) is due to the reduction of oxygen tension.

Aerobic bacteria such as *Pseudomonas* species have largely been associated with spoilage of meat stored aerobically at 25 °C and hence discoloration due to metmyoglobin formation by reducing oxygen tension (Robach & Costilow, 1962; Stanbridge & Davies, 1998). The lower degree of discoloration noticed in treated samples during storage is due to the inhibitory effect of GTE against the spoilage organisms (Table 2).

Fig. 2 shows the Hunter '*a*' values (redness) of treated and untreated samples during storage. Since there was not much change in '*L*' and '*b*' values during storage of control samples, only '*a*' values (redness) were considered. Treated samples exhibited a slight decrease in '*a*' values for up to five days of storage. The control samples showed more than 50% reduction in '*a*' values during the end of 2 days of storage and the values were correlating with the sensory colour score ($r > 0.98$). O'Sullivan et al. (2004) studied the influence of concentrate composition on retail packaged beef quality and observed a negative correlation between 'a' values and metmyoglobin content during storage. Similar negative correlation ($r > 0.97$) were observed in the present study between '*a*' values and metmyoglobin levels in control samples during storage.

Fig. 3 shows the force required (Newtons) to compress 1 in. cube of control and treated samples to its 25% thickness. The treated samples exhibited least changes in texture at the end of 4 days storage as compared to control samples and there was more than 50% textural loss in 2 days in the control. *Pseudomonas* species responsible for spoilage of mutton stored aerobically at 25 °C (Stanbridge & Davies, 1998), are known to be proteolytic and excessive proteolysis could produce inferior texture, abnormal softness and an unacceptable mushiness (Tabilo, Flores, Fiszman, & Toldra, 1999) as observed in the case of the control samples. The firm texture noticed in the treated samples during storage of up to 4 days, correlated ($r > 0.96$) with the sensory and microbiological (SPC) characteristics, indicating that the GTE is able to inhibit the growth and proliferation of this proteolytic organism to a great extent.

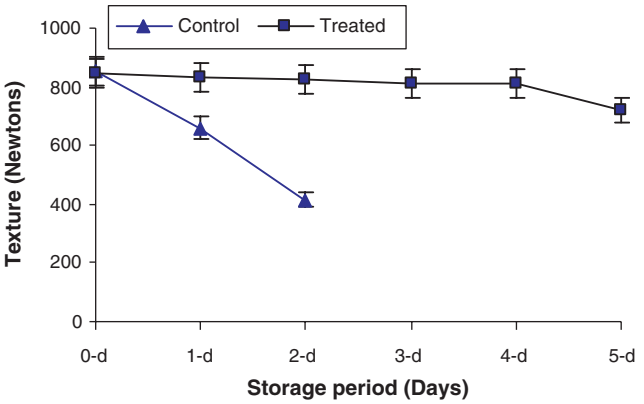

Fig. 3. Textural alterations in control and treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH ($n = 12$).

WHC is an important parameter which affects the eating quality with respect to juiciness of meat and meat products (Hamm, 1986). Fig. 4 shows the effect of treatment on the WHC of fresh mutton during storage at ambient temperature. Significantly lower ($P < 0.01$) losses in the WHC were noticed in treated samples, till 4 days of storage as compared to the control (2 days). In the control samples, there were around a 17% and 26% decrease in WHC during 1st and 2nd day storage, respectively. An increase in WHC during aging, due to the degradation of the cytoskeleton muscle of pork, has been reported (Kristensen & Purslaw, 2001). But in the present study, decrease in WHC was noticed during microbial spoilage in control samples which were correlating well ($r > 0.96$) with microbial counts (SPC). The minimum loss in WHC observed in treated

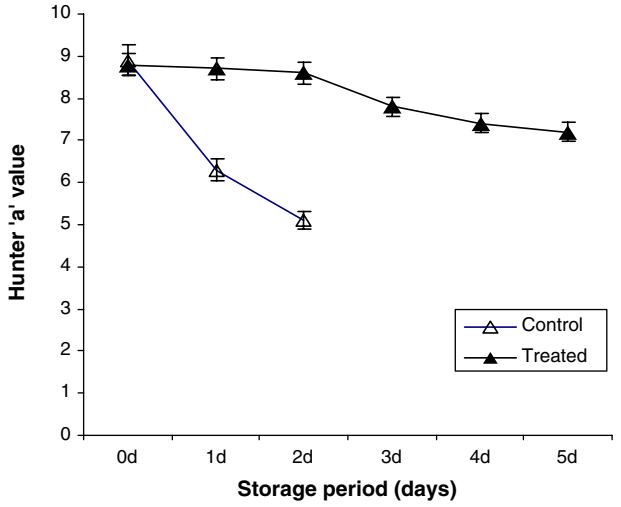

Fig. 2. Changes in Hunter '*a*' values (redness) in control and treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH ($n = 12$).

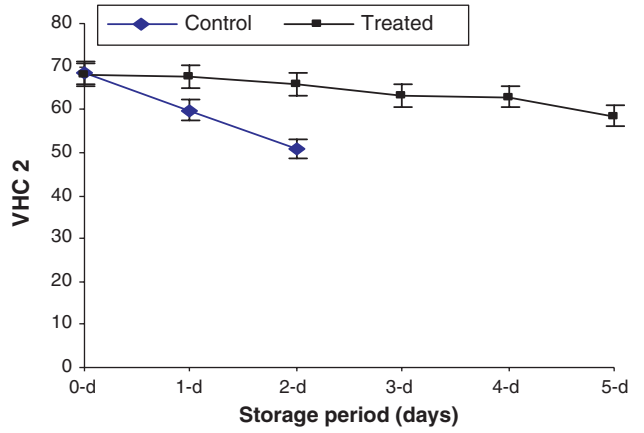

Fig. 4. Changes in WHC in control and treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH ($n = 14$).

samples may be due to lower pH and less degree of microbial spoilage as reported by Verma and Sahoo (2000, 2001).

Biogenic amines are reliable chemical markers for the quality assessment of meat and meat products. Table 3 shows the cadaverine and BAI of samples during storage. Cadaverine was absent in control fresh (0 day) as well as in treated samples for up to 4 days of storage and appeared at the end of the 1st day and the 5th day of storage in the case of control and treated samples, respectively. Kumudavally, Shobha, Vasundhara, and Radhakrishna (2001) have reported that the appearance of cadaverine can be used as a cut off limit for the acceptance of raw mutton, indicating that the treated samples are of acceptable quality for up to 4 days of storage. Biodegradative decarboxylase is found to be responsible for biogenic amine formation during meat putrefaction (Chitra & Sakaguchi, 1995). In the presence of GTE, this enzyme inhibition could be considered as an explanation for the delay in cadaverine and other amine formation as indicated by the lower levels of BAI. Edwards, Dainty, and Hibbard (1983) and Durlu-Ozkaya, Ayhan, and Vural (2001) have studied the relationship between bacterial load and diamine concentrations in meat samples as well in model systems and reported a positive correlation between *Enterobacteriace* and *Pseudamonas* levels with diamine concentration. GTE treatment is found to be helpful in inhibiting these organisms as seen by the lower levels of biogenic amines. A significant ($P < 0.01$) difference in the levels of BAI was noticed only after 4 days of storage in the case of the treated samples as compared to the '0' day samples. Amino acid decarboxylase capability of micro-organism isolated in fermented meat products was studied (Silla Santos, 1998) and it was reported that Enterobacteria were strong producers of biogenic amines. In the present study, the levels of BAI/cadaverine correlated positively ($r > 0.97$) with *Enterobacteriaceae*. Hernandez-Jover et al. (1996) have reported a BAI value of <5 mg/kg to correspond with the freshness of pork and in the present investigation, BAI values were within the acceptable limit of freshness in treated samples for up to 4 days of storage.

Table 3
Effect of acidulant and GT E (T_4) treatment on the biogenic amine profile (mg/100 g) of raw mutton during storage at 25 ± 2 °C and 85 ± 5% RH

Storage period	Control		Acidulant + GT Extract Treated	
	Cadaverine	BAI	Cadaverine	BAI
0 day	Absent	0.25 ± 0.08^{a}	Absent	0.23 ± 0.06^{a}
1 day	0.41 ± 0.09^{a}	2.06 ± 0.41^{b}	Absent	0.25 ± 0.05^{a}
2 day	0.76 ± 0.16^{b}	4.63 ± 0.37^{c}	Absent	0.28 ± 0.07^{a}
3 day	Not done	Not done	Absent	0.31 ± 0.07^{a}
4 day	Not done	Not done	Absent	0.36 ± 0.06^{a}
5 day	Not done	Not done	0.24 ± 0.06	0.92 ± 0.10^{b}

Values are mean ± SD, ($n = 12$) the means having different superscripts in a column are significantly different ($P < 0.01$).

Fig. 5 shows the changes in FFA and PL during storage of untreated and treated samples. Treated samples registered nearly a 25% increase in FFA and 13% decrease in PL levels at the end of 4 days of storage, whereas the control samples registered a 83% increase in FFA at the end of 1 day of storage itself and exhibited symptoms of slight spoilage corresponding to a decrease of 40% in PL levels. FFA and PL contents have been reported as reliable indicators of bacterial quality of raw mutton (Vasundhara & Kumudavally, 1989; Vasundhara, Kumudavally, & Sharma, 1983). Increase in FFA noticed in the control samples in the present study, may be due to the decrease in PL and triglyceride content. Alasnier, David-Briand, and Gandemer (2000), studied lipolysis in muscle during refrigerated storage and reported that FFA formation is due to the breakdown of triglyceride and PL. A positive correlation ($r > 0.97$) between lipolytic bacteria and FFA content

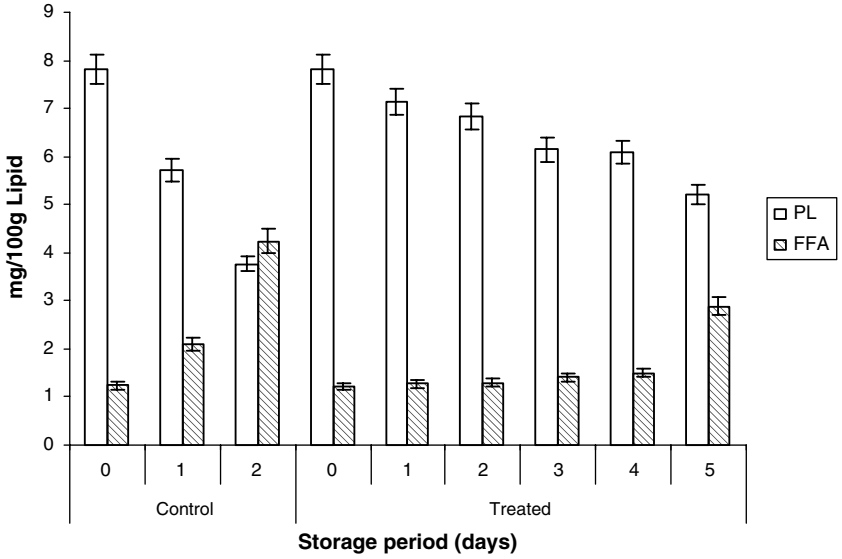

Fig. 5. Changes in FFA and PL levels in control and treated (T_4) samples during storage at 25 ± 2 °C and 85 ± 5% RH ($n = 7$).

and a negative correlation ($r > 0.94$) between phospholipid breaking organisms and PL levels were noticed both in the case of the control and treated samples. Psychrotrophic bacteria, mainly *Pseudomonas* species produce lipase and phospholipase causing an increase in FFA levels (Chung-Wang, Bailey, & Marshall, 1997; Koka & Weimer, 2001). In the present study, a significant increase ($P < 0.01$) in FFA levels of treated samples only on the fifth day of storage, indicates the inhibitory effect of GTE on psychrotrophic bacteria.

4. Conclusion

Studies show that GTE had a profound bacteriostatic effect on meat spoilage organisms including certain pathogens, indicating that the antibacterial component of GTE supports its practical use for extending the shelf life of fresh mutton for up to four days, at room temperature, without adversely affecting its physical, chemical and organoleptic parameters. Since green tea is consumed by people as a daily beverage all over the world, extracts of green tea may be safe to use in food systems to extend the shelf life.

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