Antioxidant Properties of Aqueous Extraction Green Tea on Some Chemical Properties of Local sheep Meat Stored in Cold Storage

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Abstract. The aim of this study was investigate the effect of adding aqueous extract of green tea on some chemical properties (thiobarbituric acid TBA, total volatile nitrogen TVN, free fatty acid FFA, and peroxide value PV) on sheep meat stored for 0, 3, 6, 9 and 12 days at 1-4 °C. The treatments included: the first treatment was a control treatment without addition (0 %). As for the second, third, fourth and fifth treatments, green tea leaf aqueous extract was added by 0.5, 1, 1.5 and 2% respectively. The results of the study showed a significant decrease for (T.B.A, T.V.N, F.F.A, and P.V) compared with control treatment for all periods. The TBA concentration decreased significantly for the fifth treatment, compared the first treatment which had the highest concentration. As for the addition treatment, the fifth treatment showed a significant decrease for all the periods. As for T.V.N, its value decreased significantly for the fifth treatment (7.01%) at the day 12 of storage period. While the first treatment had the highest (P≤0.01) concentration value of 14.72% for the same storage period. The concentration of P.V decreased significantly for the fifth treatment, (4.22%) for the last storage period (12 days), while the first treatment recorded the highest (P≤0.01) value (6.31%) for the same period of storage. With these results, green tea leaf extract can be used as a natural antioxidant to store meat for long periods in cold storage or to extend the shelf life in term of TBA, TVN, FFA and PV.

1. Introduction
Meat and meat products are a first-class source of protein for the human body, and are rich in iron and zinc, and rich in B-Complex vitamins [1]. Meat in general has the ability to spoil quickly if it is not stored by one of the known keeping methods, Due to the chemical composition and physical characteristics of meat that makes a suitable environment for the growth of organisms such as bacteria [2].
The cold storage is one of the most important methods for saving a fresh meat Lipid oxidation in meat [3]. The most known methods of spoiling meat during the storage in the refrigerator as a consequence of this the nutritional value of meats [4]. Therefore, a number of methods have been introduced to extend the shelf life of meat during the storage period such as using a natural products as a preservatives[5 , 6]. An example of that are Polyphenol Catechins , Epicatechin Gallate (ECG) , EpiGallo Catechin Gallate (EGCG) [7].
Green tea leaf extracts are very important source of active compounds such as flavonoids, phenols, and tannins, which have antioxidant and microbiological effects [8].
The study aimed to investigate the effect of green tea leaf aqueous extract on some chemical properties of minced sheep meat (Thiobarbituric acid TBA, total volatile nitrogen TVN, free fatty acid FFA and P.V peroxide value).

2. Materials and methods

2.1 Extraction of green tea leaves:
10 gm of green tea leaves were weighed and added to 100 ml of distilled water and the mixture was left at room temperature for 12 hours on a magnetic mixture. The extract was filtered by No. 1 filter papers. Then the filtrate was concentrated with a Rotary Vacuum Evaporator at 40 °C [9].

2.2 Sample preparation:
This study sheep meat leg was used after being into medium-sized pieces, minced by an electric machine and then treated with aqueous extract of green tea. The samples were covered with polyethylene bags that emptied from the air and kept at a temperature of 1-4 °C for 0, 3, 6, 9 and 12 days. The treatments were as follows:
The first treatment: the control treatment without any addition.
The second treatment: the treatment with 0.5% of green tea aqueous extract.
The third treatment: the treatment with added 1% of the aqueous extract of green tea.
The fourth treatment: the treatment with 1.5% of the green tea aqueous extract.
The fifth treatment: the treatment with an addition of 2% of the aqueous extract of green tea.

2.3 Thiobarbituric acid (TBA) value
Fat oxidation was measured as TBA estimation according to [10] by using the following equation:

\[ \text{TBA value (MDA) Malonaldehyde } = A_{530} \times 5.2 \text{ mg/kg of meat}. \]

2.4 Free fatty acids (FFA)
The concentration of free fatty acids (F.F.A) was determined according to the method of [11] by using the following equation:

\[ \text{F.F.A} = \frac{\text{number of milliliter of NaOH} \times \text{molarity} \times 0.0282}{\text{sample weight in gram}} \times 100 \]

2.5 Total volatile nitrogen (TVN)
The total volatile nitrogen in the meat samples was determined according to the method described by [12] according to the following equation:

\[ \text{TVN} \times \frac{V \times (300 + \text{MO}) \times 14}{500} \]

When :
V= volume of hydrochloric acid ml.
MO= moisture percentage.

2.6 Peroxide value (P.V):
The peroxide value was determined according to the method of [11] according to the following equation:

\[ \text{P.V} = \frac{\text{number of millimeters of sodium Thio Sulphate } \times 0.01 \times 1000}{\text{sample weight in gram}} \times 100 \]
2.7 Statistical Analysis:
ANOVA was carried out by SPSS version 16 on chemical Data are expressed as mean ± SD [13].

3. Results and Discussion
3.1 Thiobarbituric acid (TBA)
Table (1) shows the effect of adding aqueous extract of green tea leaves to minced meat stored by refrigerating to the significant differences (P≤0.01) between the different treatments in the value of TBA. There was no significant differences between the treatments during the 0-day period of the experiment, values which were 0.57, 0.58, 0.57, 0.56 and 0.57 mg malonealdehyde/ Kg meat for T1, T2, T3, T4 and T5 respectively.

The results in Table (1) showed significant differences (P≤0.01) among treatments at the same periods in TBA value 3, 6, 9, and 12 days, showed addition treatments the lowest values of the TBA compared to the control treatment that recorded the highest value and for all periods.

The results in Table (1) showed TBA value of the control treatment was the highest among the treatments (0.94, 1.83, 3.14 and 4.43), while the treatment T5 (2%) recorded the lowest value (0.59, 0.70, 1.16 and 1.77) for the periods 3, 6, 9 and 12 days.

The results agree with [14] when using rosemary and green tea extracts to extend shelf life and improve of meat quality during cold storage found decrease in the TBA value. It also agrees with what he found [15]. Cause of decrease in the value of TBA When adding the aqueous extract of green tea the contains of compounds work the antioxidants , such as Catechins and ECG and EGCG [16]. That and found in green tea leaves, It work to reduce fat oxidation.

increase of the TBA value during the storage period, due to the increase in the concentration of malondialdehyde, produced during fat oxidation [17].

Table 1. Effect of different concentration of green tea leaves extract on TBA value (mg malondialdehyde MDA/kg) of sheep meat minced stored at cold temperature (Mean±S.E).

| Treatments | Storage period (days) |
|------------|-----------------------|
|            | 0    | 3  | 6       | 9       | 12       |
| T1(%)      | 0.57±0.01 | 0.94 ± 0.01 | 1.83±0.008 | 3.14± 0.008 | 4.43±0.01 |
|            | A*   | A  | A       | A       | A        |
| T2(0.5)    | 0.58±0.01 | 0.76±0.005 | 0.95±0.008 | 2.12±0.02  | 2.35±0.008 |
|            | A    | B  | B       | B       | B        |
| T3(1%)     | 0.57±0.005 | 0.67±0.005 | 0.86±0.008 | 1.95±0.005 | 2.02±0.01 |
|            | A    | C  | C       | C       | C        |
| T4(1.5)    | 0.56±0.01 | 0.65±0.005 | 0.75±0.006 | 1.64±0.02  | 1.95±0.003 |
|            | A    | D  | D       | D       | D        |
| T5(2%)     | 0.57±0.005 | 0.59±0.01 | 0.70±0.005 | 1.16±0.01  | 1.77±0.01 |
|            | A    | E  | E       | E       | E        |

P. value N.S** | 0.01 | 0.01 | 0.01 | 0.01 |

* Means large letters among treatments for each period are significantly different (P≤0.01)
** Non significant

3.2 Total Volatile Nitrogen (TVN)
Table (2) shows the effect of adding aqueous extract of green tea leaves to minced meat stored by refrigerating to the significant differences (P≤0.01) between the different treatments in the value of TVN. There was no significant differences between the treatments during the 0 day period of the experiment, values which were 7.25, 7.24, 7.23, 7.25 and 7.24 mg/100g meat minced for T1, T2, T3, T4 and T5 respectively.
The results in Table (2) showed significant differences ($P \leq 0.01$) among treatments at the same periods in TVN value 3, 6, 9, and 12 days, showed addition treatments the lowest values of the TVN compared to the control treatment that recorded the highest value and for all periods.

The results in Table (2) showed TVN value of the control treatment was the highest among the treatment (9.58, 11.97, 14.19 and 18.72) mg/100g meat minced, while the treatment T5 (2%) recorded the lowest value (7.86, 8.05, 9.59 and 12.01) mg/100g meat minced, for the periods 3, 6, 9 and 12 days.

The results agree with [14] when using rosemary and green tea extracts to extend shelf life and improve of meat quality during cold storage found decrease in the TVN value. The reason for the decrease in the TVN value of the addition treatments compared to the control treatment, because found active compounds in green tea aqueous extracts such as (phenolic compounds, catechins, and caffeine) [18]. Increase of TVN value during the storage period, due to the It is attributed to the role of microorganisms and enzymes, degradation of proteins, increase in the value of TVN [19].

Table 2. Effect of different concentration of green tea leaves extract on TVN mg/100g meat of sheep meat minced stored at cold temperature (Mean±S.E).

| Treatments | Storage period (days) | 0 | 3 | 6 | 9 | 12 |
|------------|-----------------------|---|---|---|---|----|
| T1(0%)     |                       | 7.25±0.01 | 9.58±0.005 | 11.97±0.01 | 14.19±0.01 | 18.72±0.01 |
| T2(0.5%)   |                       | 7.24±0.01 | 8.22±0.01 | 9.86±0.008 | 10.24±0.005 | 14.20±0.008 |
| T3(1%)     |                       | 7.23±0.01 | 8.02±0.01 | 9.23±0.008 | 10.02±0.01 | 13.75±0.008 |
| T4(1.5%)   |                       | 7.25±0.003 | 7.93±0.01 | 8.67±0.01 | 9.86±0.008 | 12.29±0.01 |
| T5(2%)     |                       | 7.24±0.008 | 7.86±0.008 | 8.05±0.005 | 9.59±0.02 | 12.01±0.008 |

| P. value   | N.S** | 0.01 | 0.01 | 0.01 | 0.01 |

* Means large letters among treatments for each period are significantly different ($P \leq 0.01$)
** Non significant

3.3 Peroxide value (P.V)

Table (3) shows the effect of adding aqueous extract of green tea leaves to minced meat stored by refrigerating to the significant differences ($P \leq 0.01$) between the different treatments in the value of P.V.

There was no significant differences between the treatments during the 0 day period of the experiment, values which were 1.57, 1.56, 1.58, 1.57 and 1.56 meq./Kg for T1, T2, T3, T4 and T5 respectively. The results in Table (3) showed significant differences ($P \leq 0.01$) among treatments at the same periods in P.V value 3, 6, 9, and 12 days, showed addition treatments the lowest values of the P.V compared to the control treatment that recorded the highest value and for all periods.

The results in Table (3) showed P.V value of the control treatment was the highest among the treatment (2.95, 5.26, 5.88 and 6.31) meq./Kg, while the treatment T5 (2%) recorded the lowest value (1.91, 3.37, 3.90 and 4.22) meq./Kg, for the periods 3, 6, 9 and 12 days.

The results agree with [15] found decrease in PV value of beef with the addition of the aqueous extract of green tea and stored in the cold, The reason for the decrease in the peroxide value may be due to the addition of the aqueous extract (catechins and phenols), it work to reduce fat oxidation [20].
Table 3. Effect of different concentration of green tea leaves extract on P.V meq./Kg meat of sheep meat minced stored at cold temperature (Mean±S.E).

| Treatments | Storage period (days) |
|------------|-----------------------|
|            | 0     | 3     | 6     | 9     | 12    |
| T1(%)0     | 1.57±0.01 | 2.95±0.02 | 5.26±0.02 | 5.88±0.01 | 6.31±0.008 |
| T2(%)0.5   | 1.56±0.02 | 2.02±0.01 | 3.67±0.01 | 4.17±0.01 | 5.20±0.01 |
| T3(%)1     | 1.58±0.01 | 1.98±0.01 | 3.53±0.01 | 4.04±0.01 | 4.69±0.01 |
| T4(%)1.5   | 1.57±0.01 | 1.95±0.006 | 3.43±0.008 | 3.98±0.008 | 4.33±0.01 |
| T5(%)2     | 1.56±0.02 | 1.91±0.008 | 3.37±0.01 | 3.90±0.01 | 4.22±0.01 |

P. value N.S** 0.01 0.01 0.01 0.01 0.01

Means large letters among treatments for each period are significantly different (P≤0.01)
** Non significant

3.4 Free Fatty Acid (F.F.A)

Table (4) shows the effect of adding aqueous extract of green tea leaves to minced meat stored by refrigerating to the significant differences (P≤0.01) between the different treatments in the value of F.F.A.

There was no significant differences between the treatments during the 0 day period of the experiment, values which were 0.32, 0.34, 0.32, 0.32 and 0.32 % for T1, T2, T3, T4 and T5 respectively. The results in Table (4) showed significant differences (P≤0.01) among treatments at the same periods in F.F.A value 3, 6, 9, and 12 days, showed addition treatments the lowest values of the F.F.A compared to the control treatment that recorded the highest value and for all periods.

The results in Table (4) showed F.F.A value of the control treatment was the highest among the treatment (0.72, 1.13, 2.02 and 2.84) %, while the treatment T5 (2%) recorded the lowest value (0.33, 0.40, 0.61 and 4.22) %, for the periods 3, 6, 9 and 12 days.

This result may be connected with fat oxidation, role of Enzymes in increase the number of bacteria that Decomposition fat [21].

Table 4. Effect of different concentration of green tea leaves extract on F.F.A % meat of sheep meat minced stored at cold temperature (Mean±S.E).

| Treatments | Storage period (days) |
|------------|-----------------------|
|            | 0         | 3         | 6         | 9         | 12        |
| T1(%)0     | 0.32±0.01 | 0.72±0.01 | 1.13±0.01 | 2.02±0.01 | 2.84±0.005 |
| T2(%)0.5   | 0.34±0.01 | 0.49±0.005 | 0.54±0.006 | 0.80±0.008 | 1.21±0.008 |
| T3(%)1     | 0.32±0.01 | 0.41±0.005 | 0.46±0.005 | 0.73±0.008 | 1.10±0.003 |
| T4(%)1.5   | 0.32±0.008 | 0.36±0.005 | 0.43±0.005 | 0.67±0.003 | 1.04±0.01 |
| T5(%)2     | 0.32±0.01 | 0.33±0.005 | 0.40±0.005 | 0.61±0.008 | 0.95±0.005 |

Means small letters among treatments for each period are significantly different (P≤0.01)
** Non significant
Conclusions
With these results, green tea leaf extract can be used as a natural antioxidant to store meat for long periods in cold storage or to extend the shelf life in term of TBA, TVN, FFA and PV.

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