Inhibition of Protein and Lipid Oxidation of Beef Patties by Using Sumac Fruit Extract During Freeze Storage

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Abstract

The fruits of sumac Rhus coriaria L. are widely used in traditional folk medicine as spices and flavors, especially in meats, because they are a rich source of natural antioxidants and also their tremendous potential in improving the quality of meat. The study aimed to use different concentrations of sumac fruits extract (SFE) prepared with a concentration of control T0, T1: 0.05% and T2: 0.1% and studying their effect on inhibiting protein and lipid oxidation of beef patties stored in Freeze -18±1 °C for 90 days. The T2 could significantly inhibit an increased carbonyl content, PV and TBA value (P<0.01) and inhibition of pH value at (P<0.01) while the thiol content and water content significantly (P<0.01) decreased finding. The SFE may be used as natural antioxidant compound in helping to extend the period of meat products.

Keywords: Antioxidants activity, Beef patties, Protein and Lipid oxidation, Sumac fruit extract, Inhibition.

1. Introduction

Meat constitutes a large percentage of the food consumed around the world as it is a source of many important nutrients for the human body, such as proteins which are rich in essential amino acids, as red meat (raw muscle) contains approximately 20% protein, and it is a good source of many vitamins, lipid and mineral elements [1]. Due to the structure of meat, it is spoiled rapidly, if left in bad conditions preservation. Therefore, cold and freezing methods have been widely used to increase the shelf life of meat for consumption [2]. However, preservation operations in these methods may lead to decrease in the quality of the meat [3], through the oxidative changes that occur to proteins and lipid, as Lipid oxidation is the main reason for the damage of many food products, but the new focus is on oxidative reactions of proteins [4]. Protein oxidation is characterized as a covalent modification of a protein caused by direct or indirect interactions with reactive oxygen species (ROS) or oxidative stress by-products [5]. Meat proteins are oxidized during storage or processing leads to loss of essential amino acids, formation of carbonyl groups, loss of thiol groups [6], and reduced ability of meat to retain water and tissue formation [7]. It also leads to the inhibition of enzymes activity which is responsible for meat tenderization, cross-links formation and oxidative changes occurring in meat pigments [8]. In order to inhibit the oxidative reactions occurring in the meat, industrial antioxidants were used, such as the use of nitrates known to have an antioxidant effect in meat and its products. However, nitrates have been identified as toxic and carcinogenic [9]; therefore, attention has recently been focused on the use of natural antioxidants that are better accepted by consumers and are safer and some have higher antioxidant capability than synthetic ones. This is in addition to the positive effects of natural antioxidants on the organoleptic properties of meat products [10]. This study investigated the antioxidant activity of (SFE) and the effects of SFE on the quality of beef patties and the role of SFE in the inhibition during freezing storage and protein and lipid oxidation in beef patties.

2. Materials and Methods

2.1. Preparation of sumac fruits Extract

Sumac fruits were obtained from the local market in Basra Governorate, The alcoholic extract of the ground fruits were prepared according to the method described by [11], using the organic solvent of methanol in the extraction process at a ratio of 1: 10 g / ml. The alcoholic extract, and airtight containers were used to placed the mixture in a temperature-controlled water bath at 45 °C for 2 hours. The samples were taken out of the water bath and the extraction process was completed at room temperature for 24 hours. The extract was filtered using Whatman No.1. A rotary evaporator was used to concentrate the filtrate, then, the concentrated extract was dried and stored in the refrigerator until use.
2.2. Measurement of Antioxidant Activity

The efficacy of the antioxidant extract was tested according to a modified method [12]. Mixing 1 ml of DPPH reagent (1, 1-Dphenyl-2-picyrlhydrazyl) prepared in methanol at a concentration of 0.1 mmol with alcoholic extract at different concentrations ranging between (0.5-5) mg / ml, and is added 2 ml of alcoholic extract. Incubate the mixture in a dark area at the laboratory temperature for 30 min. The control sample was prepared by mixing 2 ml of distilled water with 1 ml of DPPH at a wavelength of 517 nm. The synthetic antioxidant Butylited Hydroxy Toluene (BHT) was used for comparison. The percentage of inhibition of DPPH was calculated (I%) using The following equation[13]:

\[ I\% = \left\{ \frac{(A_o-A_s)}{A_o} \right\} \times 100\]

Ao = Absorbance of the control sample
As = Absorbance of the extraction solution

2.3. Preparation of beef patties

The beef patties were prepared by of meat using an meat grinder in laboratory. The meat was divided into three treatmentes as follows:

T0: The control sample without adding extracts.
T1: Add the alcoholic extract of sumac at a concentration of 0.05 g / 100 g of meat.
T2: add the alcoholic extract of sumac at a concentration of 0.1 g / 100 g of meat.

Meat patties were formed and placed in polyethylene bags and storage in refrigerated at -18±1 ° C for 90 days. They were carried out a set of chemical and physical tests, as well as conducting sensory tests that included color, aroma, tenderness, juiciness and general acceptance during storage periods of (30, 60 and 90) days.

2.4. Chemical analysis

2.4.1. Determination of carbonyl content (COOH)

One gram of the meat was taken and then mixed with 10 ml of Tris-HCL buffer solution 50 nmol, the pH was 7.4 and the solution containing 1 mM of EDTA containing 0.1 g/L butylated hydroxytoluene (BHT) and homogenizer thoroughly, separating the suspensions and used to determine the carbonyl content [14] by the method of -2,4 dinitrophophenyl hydrazine (DNPH) and described before[15,16] By taking 1 ml of the protein suspension and adding 20% TCA to it, and a blank sample was prepared. The tubes were incubated in ice for 5 minutes, then centrifugation was conducted at 10,000 rpm for 5 minutes. Separation of the suspensions and washing of the precipitated protein with 1 ml of 20% TCA and centrifugated to 10,000 rpm for 5 minutes. The suspension was separated and the sediment incubated with 2 ml of 10 mm DNPH prepared in 2 N of HCL and a blank sample was added by 2 ml of 2N of HCl (without adding DNPH ). The samples were mixed with vortex and incubated at laboratory temperature in the dark for 1 hour. The precipitated protein was washed with 1 ml of a mixture of ethanol and ethyl acetate 1: 1. The process was repeated three times to remove the (DNPH) dye. Centrifugation was performed, and the precipitated protein was dissolved in 8 M Urea, the absorbance was measured at a wavelength of 370 nm and the carbonyl content (nmol / mg protein) was expressed according to the following equation [16]:

\[ \text{Carbonyl (nmol / mg protein)} = \frac{A_S - A_b}{\varepsilon} \times 10^9 \]

Where as :

\(A_S\) = absorbance of the sample
\(A_b\) = Planck absorbance
molar factor 22,000 mol / cm = \(\varepsilon\)

2.4.2. Determination of Total Thiol group

Thiol groups were estimated using Ellman’s reagent or 5,5-Dithiobis (z-nitro benzoic acid) (DTNB) following the method described by [17] [18]. Generate 2 gm of meat with 50 ml of buffer Tris 0.10 mol pH 8 containing 5% SDS and add 100 μl of BHT at 0.01% concentration. In a water bath, the mixture was placed at 80 ° C for 30 minutes, exposed to centrifugation at
5,000 rpm for 20 minutes. Filtered and determined the protein content of the suspension using a biuret method using the Biuret Kit. Dilute the filtrate with the buffer solution that was used in the homogenization by mixing 0.5 ml of the filtrate with 2 mL of buffer solution and 0.5 mL of 10 mM reagent (DTNB) was added to it. The samples were incubated in the dark for 30 minutes and the absorbance was measured at a wavelength of 412 nm. A blank sample was prepared using buffer instead of the sample, and the total Thiol Group was expressed according to the thiol content from the following equation, [19].

\[
\text{SH concentration (μmol/g protein)} = \frac{A_S - A_b}{e} \times D \times 10^9
\]

Where as:

- \( A_S \) = absorbance of the sample
- \( A_b \) = Planck absorbance
- \( e \) = molar factor 13600 mol / cm
- \( D \) = Number of times dilution

### 2.4.3 Peroxide value (PV)

The value of peroxide was calculated using [20] method. One gram of beef Patties was taken and placed in tubes with a tight cover, one gram of potassium iodide and 20 ml of a mixture of organic solvents (ice acetic acid and chloroform in a 2:1 ratio) were taken and the tubes were then put in a water bath. At a boiling temperature for a period ranging for a time 20-30 seconds, Every tube contents were poured into a beaker containing 30 ml of a 5% potassium iodide solution. Tubes were washed twice in distilled water with 25 ml. The iodine that was free had been neutralized by correcting with a 0.002 N sodium thiosulfate solution until reaching before the neutral point (In pale yellow color). Then add 1 ml of 1% starch solution and wipe until the end of the correction point is reached by the disappearance of the blue color. Prepare the control sample by following the previous steps without adding the sample, the peroxide value was calculated as in the following equation:

\[
\text{Peroxide value} = \frac{(B-S) \times N \times 1000}{\text{Sample weight (g)}}
\]

Where as:

- \( S \) = volume of sodium thiosulfate needed for sample (ml)
- \( B \) = volume of sodium thiosulfate required for control (ml)
- \( N \) = sodium thiosulfate titrate

### 2.4.4 Thiobarbituric acid (TBA)

The value of thio-barbituric acid was estimated according to the method [21] which is modified by [22]. By homogenizing 5 gm of meat adding 0.5 ml of BHT (4.2% dissolved in ethanol) with 5 ml of 5% TCA the homogenized mixture was filtered, two ml of the filtrate was taken and mixed with 2 ml of TBA at a concentration of 0.02 M, for 40 minutes, the tubes were incubated in a water bath at boiling temperature, the tubes were cooled and the absorbance was measured at a wavelength of 532 nm. The TBA content was expressed as mg Malone dihydrate / kg meat and the TBA content was calculated from the following equation:

\[
\text{TBA (malone didehyde mg / kg)} = \text{absorption} \times 7.8
\]

### 2.5 Physical analysis

#### 2.5.1 Water content

Determine the water content of the beef patties by drying using an electric air oven at a temperature of 105 °C, measuring the water content on the basis of weight loss of the sample [23] according to the following equation:

\[
\text{Water content} = \frac{W_{\text{sample with a ladle before drying}} - W_{\text{sample with a ladle after drying}}}{W_{\text{sample}}} \times 100
\]
2.5.2. The pH evaluation

A pH-meter was used to estimate the pH by mixing 10 g of ground beef patties with 20 ml of distilled water, leaving the mixture for 5 minutes and then recording the pH reading [24].

2.5.3. Statistical analysis

Factorial experiments were used in a completely randomized design (Factorial Experiment Designing)(CRD) to analyze the factors used and analyzed statistically, by using the ready-made statistical program Genstat Release 12.1, the least significant difference was tested (L.S.D) at a level (P<0.05).

3. Results and Discussion

3.1. DPPH radical scavenging activity of SFE

Table (1) show the scavenging activity of SFE against DPPH radicals, which were used at three concentrations of 5, 10 and 15 mg / ml. The SFE had a strong DPPH radical scavenging activity. At concentrations of 10 and 15 mg/ml, the scavenging rates were 82.63%, 86.86% respectively, The lowest value was obtained at a concentration of 5 mg/ml 77.74%. The DPPH radical scavenging activity increased as the concentration increased, because sumac contains flavonol-rich compounds that have been able to serve as an antioxidant by donating hydrogen or electron to DPPH, Transforming it into more secure roots.

| Concentrations mg / ml | Antioxidant activity % |
|------------------------|------------------------|
| 5                      | 77.74                  |
| 10                     | 82.63                  |
| 15                     | 86.86                  |

3.2. Effect of SFE on protein oxidation of beef patties

3.2.1. Carbonyl content

The results in Figure (1) show that the carbonyl content in beef patties increased significantly (P<0.05) during Freeze storage. The initial carbonyl content of Freeze T0, T1 and T2 were 1.52, 1.44 and 1.37 nmol / mg protein respectively. The T2 showed the highest inhibition on carbonyl content from the initial storage of Freeze (P<0.05) until 60 days of storage, the carbonyl contents at the final period T1 1.88 and T2 1.74 nmol / mg protein, respectively compared with T0 (control) 1.93. The antioxidant role of the SFE in this study was due to the radical scavenging and metal chelating activities of their phenolic compounds [25]. Research results agree with [26] where it was found that carbonyl content lowing At Protein oxidation in Atlantic mackerel (Scomber scombrus) during frozen storage.

3.2.2. Total thiol content

The results of Figure (2) show that the total thiol content was significantly reduced (p <0.05) during Freeze storage. It was successively observed that the thiol content of the pretreatment T0 decreased significantly compared to that of T2 and T1 during freeze storage. The primary thiol content of freeze T0, T1, and T2 were 58.28, 59.58 and 61.42 nmol / mg protein.

![Figure 1](image1.png)

**Figure 1.** Effect of SFE on carbonyl content of beef patties during Freeze storage.

**Figure 2**

![Figure 2](image2.png)

**Figure 2.** Effect of SFE on total thiol content of beef patties during Freeze storage.
respectively, in the final period of freeze T1 55.80 and T2 57.94 nmol / mg protein, respectively, compared to T0 52.48 . The reason for the oxidation of protein in the thiol groups is due to the formation of disulfide bonds through oxidation [26]. In addition of the SFE extract reduced the formation of the disulfur bonds in the meat patties because it contains compounds that inhibit oxidation, thus preserving the thiol groups from loss. This research is in agreement with [28] who studied the effect of adding kebabs on delaying the oxidation of proteins and fats in mackerel fish slices stored by freezing for 100 days, and it was noticed that the content of thiol groups decreased during the period of storage by freezing.

Figure 2. Effect of SFE on thiol content of beef patties during Freeze storage.

3.3. Effect of SFE on lipid oxidation of beef patties

3.3.1. Peroxide values

The results of Figure (3) show that the lipid oxidation significantly increase (p<0.05) in peroxide values of beef stored in freeze temperature. The initial of PV in period freeze T0,T1 and T2 were 1.37, 1.32 and 1.27 mEq/kg respectively, observed T2 treatment had higher inhibition effected on lipid oxidation in all storage period, it was recorded after 60 day 2.57 mEq/kg than the T0 andT1 3.16 and 2.72 mEq/kg respectively. The inhibition effect of SFE on lipid oxidation may be due to its ability to prevent the formation of free radicals. Thus, it works to reduce the development of the oxidation process and protect the meat patties from oxidatives [28]. Furthermore, during storage, oxidation occurs for lipid and thus peroxides are formed, but it is the treatment by extracts that will reduce the formation of peroxides because they contain compounds that have inhibitory activity against the formation of peroxides.

This study is in agreement with the [29] that used a group of plant extracts and studied their effect on the values of the peroxide of beef patties stored in Freezing. The study also showed that the peroxide values of the beef patties treated with different concentrations of plant extracts were lower compared to the control treatment patties and that these values increased with the increase in the period of Freezing storage depending on the concentration used.

Figure 3. Effect of SFE on Peroxide values of beef patties during Freeze storage.

3.3.2. Thiobarbituric acid (TBA)

Figure (4) shows that the occurring lipid oxidation significantly increase (p<0.05) in TBA value during Freeze storage. First period TBA value of freeze T0,T1 and T2 were 2.13, 2.08 and 1.96 mg malone didehyde / kg respectively ,the samples treated with SFE had low TBA value compared with control during freeze storage. This leads to the SFE effectively
inhibiting the lipid oxidation, the T2 having higher inhibition effect on lipid oxidation with in 90 days of storage it was recorded 5.53 than T0 and T1 5.53 and 5.40 mg malone didehyde / kg.

In general, the reason for the increase in the oxidation values in all samples indicates the continued formation of aldehydes during storage [30]. This study is in agreement with the [29] that used a group of plant extracts and studied their effect on TBA of beef patties stored in freezing. The study also showed that TBA of the beef patties treated with different concentrations of plant extracts were lower compared to the control treatment patties and that these values increased with the increase in the period of Freezing storage depending on the concentration used.

![Figure 4](image)

**Figure 4.** Effect of SFE on Thiobarbituric acid of beef patties during Freeze storage.

### 3.4. Effect of SFE on Physical Tests of beef patties

#### 3.4.1. Water content

The results of Figure (5) show the water content in beef during the Freeze storage period. Significant differences (p<0.05) in the moisture content values were observed as a result of statistical analysis. First period moisture content value of freeze T0,T1 and T2 were 58.33%  ,60.16% and 61.80 % respectively. The samples treated with SFE had saved moisture content compared with control during cold storage. This leads to the SFE effectively inhibiting the lipid oxidation. However, the T2 having higher inhibition effected on lipid oxidation at 90 days of storage it was recorded 55.67% than T0 51.04% and T1 54.10 %. The effective inhibition of SFE to lipid oxidation may be due to the fact that the extract keeps the lipid from oxidation in the meat patties, thus the lipid act as an insulator to prevent water from escaping from the space inside the cells and thus reduce water loss from the surface of tissue [26]. This is in agreement with [30] where it was observed that adding red pepper seed powder reduced the decrease in the water content of the meat sauces.

![Figure 5](image)

**Figure 5.** Effect of SFE on water content of beef patties during Freeze storage.

#### 3.4.2. pH value

Figure (6) shows the results of the pH values of beef during the 90 day freeze storage period. The results of the statistical analysis show that there are significant differences (p<0.05) for the pH values. During freeze storage, The pH values of T2
treated samples were found to be significantly decreased compared with lower than those of T0 and T1. The initial pH values T0, T1 and T2 were 6.10, 6.05 and 5.96 respectively. While at the final period T1 6.30 and T2 6.23, respectively compared with T0 6.34. The reason for preserving the PH values of the T2-treated beef Patties is due to the concentration of the extract and the containment of the extract on the phenolic compounds that in turn work to bind the protein and thus work to keep the pH values from increasing [30,31]. The cause of the high pH may be due to such accompanying factors during pregnancy. Storage also involves analyzing protein and isolating amine groups, which increase the pH values. This is in agreement with [26] which showed that the pH of the meat patties treated with different concentrations of plant extracts was lower compared to the control treatment patties and that these values increased with the increase in the duration of storage in freezing areas depending on the concentration used, and the increase in the treated samples was less than that of the control sample.

![Figure 6. Effect of SFE on pH value of beef patties during Freeze storage.](image)

**Conclusion**

We conclude through the study that adding sumac fruit extract (SFE) to beef patties at certain concentrations (0.1 and 0.05%) lead to an increase in the preservation period of the product and inhibited the protein and lipid oxidation of beef patties, especially at a concentration of 0.1%, which gave the best result for preserving the product. Plant extracts have been used because they are a safe alternative to industrial food additives that can be used in specific concentrations and doses without adverse health effects on human health. A concentration of 0.05% and 0.1 was used through the research.

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