Effects of Soy Sauce and Some Other Additives on Lipid Oxidation and Its Related Properties of Minced Beef Meat During Cold Storage

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Abstract

This study was designed to investigate the effects of soy sauce and some other antioxidants on some of important meat and meat products properties in cold storage conditions. Five treatment were demanded. Control group without any kind of adding. NaCl treatment with adding 10% NaCl solution. SS treatment with adding 10% soy sauce solution. NaCl+SS treatment with adding 5% NaCl+5%soy sauce solutions. And the last SS+Asc treatment with adding 10% soy sauce solution+ 0.05% ascorbic acid. samples in all treatment divided to groups and been exposed to five storage period (0,3,6,9,12) days at 4oc. to study the effect of treatments and storage periods on twelve properties (pH, WHC, Drip loss, Mb concentrations, TVN, TBA, P.V, FFAs, moisture percentage, protein percentage, fat percentage and ash percentage). The results showed a benefit in all studied properties related with using soy sauce with or without ascorbic acid. These natural antioxidant material may be considered as an effective natural antioxidants and good replacement instead of synthetic types.

Keywords: Antioxidants, Soy sauce, Ascorbic acid, NaCl, FFAs, TBA, TVN, Lipid oxidation

1. Introduction

In all kind of storage processes including cold storage, the meat would be exposed to multiple kind of deterioration in its nutritional, chemical and sensory properties [1]. One of the most important kind of these changes in meat quality is the oxidation of lipids, which would cause a critical bad changes in meat properties [1,2]. Beef meat is not an exception, and it would be exposed to the same deterioration processes, because of its high contents of lipid, that it may reach to 17% [3,4]. Mincing process has a profound effect on meat characteristics [5], it may enhance the texture, water holding capacity and increase the extraction of myofibrillar protein [6] but it due to an increase in air exposed surface area which would be a reason for acceleration the oxidation of lipids [7].

Min and Ahn [8] defined the lipid oxidation as the main process for reducing the shelf life of meat and meat products. Lipid oxidation affects texture, color, taste, nutritional values and aroma and leads to rancidity [9]. Many researches [10,11] referred to that oxidation begins with phospholipids which become catalyzed by the iron contained in heme proteins immediately after slaughtering. Phospholipids are found mainly in cell membrane and are rich of unsaturated fatty acids, which mincing would set them free to be exposed to iron and starts oxidation process. To keep meat and meat products out of oxidation for longer periods many techniques have been applied, starting from moderations in animals feed programs or improving slaughtering procedures or packaging and storage operations etc.. But the most recently spread all over the world are the processes of adding antioxidant additives whether synthetic or natural types [12,13].

Many researches referred to a low acceptance of consuming meat or meat products with synthetic antioxidant additives, because of risks of toxicity and carcinogenicity which have been identified in some studies [14]. For that, the meat industry converted their interest towards the natural antioxidants which look like out of those risks [11,13]. This study aims to investigate the effect of some of natural antioxidants and their mixtures (sodium chloride NaCl, soy sauce SS, a mixture of the both NaCl+SS, a mixture of soy sauce and Ascorbic acid SS+Asc and no additives at all as a control group) on the oxidation of minced beef meat by studying their effects on some of related meat quality properties.
2. Materials and Methods

2.1. Samples

beef meat was taken from local markets in Baghdad, 24 hour postmortem. femur muscle *biceps femoris* was used in study. The muscle fat has been removed and the lean was exposed to mincing 8mm plate. Then, kidney and pelvic fat from the same carcass was minced under the same conditions and added to the minced lean as 20% constant percentage to 70% lean and mixed together. Then, the mass of this mix was divided into five group for the five treatments.

2.2. Treatments

Five treatments were conducted in this study demanding on soy sauce and NaCl and ascorbic acid and their mixtures in addition to the non-added group as a control. The soy sauce was purchased from local market and diluted in ice water until reaching the final salt concentration 10% (w/v), the NaCl solution was prepared too by adding ice water until reaching the concentration of 10% (w/v), the treatments were as follows:

- Control group (c): 70% lean + 20% fat + 10% water.
- NaCl group (NaCl): 70% lean + 20% fat + 10% NaCl solution.
- Soy sauce group (SS): 70% lean + 20% fat + 10% soy sauce solution.
- NaCl+soy sauce group (NaCl+SS): 70% lean + 20% fat + (5% NaCl+ 5% SS) solutions.
- Soy sauce+ Ascorbic acid group (SS+ASC): 70% lean + 20% fat + 10% SS + 0.05% ASC.

All groups were minced again to a finer degree (3mm plate) and patties were made for 100g to each one. Then, all samples in each group divided to a sub groups and been exposed to a different period of cold storage at 4°C. the five periods of storage were (0, 3, 6, 9, 12) days respectively.

2.3. Properties

- pH values: According to Verma [15] 5g of each sample was homogenized with 50 ml of distilled water for 1 minute and filtered through filter paper No.1, then measuring pH with justified pH meter.
- Water holding capacity (WHC): following Dolatowski and Stasiak [16] with modifications. 25g of each sample homogenized with 25ml of distilled water for 1 minute. The mixture then but in centrifuge at speed of 5000 rpm for 10 minutes at 4°C. WHC percentage was calculated as follows:

  \[ \text{WHC\%} = \left( \frac{\text{added water weight} - \text{water weight after centrifugation}}{\text{sample weight}} \right) \times 100 \]

- Drip loss: 25g of each sample put in a container with separating net to avoid contact between samples and container surfaces. And covered with polyethylene bags and but in refrigerator at 4°C overnight. Each sample then was dried out and weighted [17]. Drip loss was calculated as follows:

  \[ \text{DL\%} = \left( \frac{\text{prior weight} - \text{final weight}}{\text{prior weight}} \right) \times 100 \]

- Myoglobin concentration: according to procedure of Zessin[18] the myoglobin concentration was estimated by taking 10g of each sample and homogenize with 90ml of distilled water. The 10g gram should be taken from this mixture and homogenize it again with 10ml of distilled water. This new mixture was filtered through paper No.1. then test the solution with spectrophotometer at 535-wave as light absorption wave length. Myoglobin concentration will be as follows:

  \[ \text{MG} = \text{absorbability x 2.4/sample weight x 0.452} \]

- Total volatile nitrogen compounds (TVN): 100g of each sample was homogenized with 300ml of tri chloro acetic acid (TCA) 5%. Then filtered and 5ml of clear extraction was transferred into distillation flask (Kildhal). 5ml of 2% of sodium hydroxide was added. With increasing heat the distilled liquid was obtained by flask filled with 15ml of 4% boric acid mixed with little droplets of methylene red and bromocresol green. Finally pipetted with hydrochloric acid 0.01M [19]. TVN was calculated as follows:

  \[ \text{Amount of TVN (mg nitrogen / 100g meat)} = \frac{V \times (300 + MO) \times x14}{500} \]

When:

\[ V = \text{volum of hydrochloric acid ml.} \]
\[ MO = \text{moisture percentage.} \]

- Thiobarbituric acid value (TBA): following Witte et al. [20]. 1g of sample homogenized with 25ml of 20% tri chloro acetic acid (TCA) cold solution, the dissolved in phosphoric acid 2% for two minutes. The mixture transferred flask and the volume completed to 50ml with distilled water. The mixture should be shaken for a while and 25 ml of it was taken and centrifuged at 3000 rpm for 30 minutes. Then filtered through paper No.1. 5ml of supernatant was transferred to the test tube and TBA reagent (5ml of 0.005M) was added and dissolved in distilled water. All tube had been closed,
shacked and stored in dark place for 15-16 hours in room temperature. The light absorbed value was measured at 530 wave length via spectrophotometer.

- **TBA value**: A530x5.2mg/kg of meat.
- **Peroxide value (P.V)**: 30ml was taken from a mixture of three part of glacial acetic acid an two part of chloroform and added to 5g of obtained fat then mixed with 0.5ml of saturated potassium iodide and 1cm of starch reagent with 30ml of distilled water. All this was pipetted with 0.01 sodium thio sulphate solution until blue color disappeared [21]. He peroxide value was:
  \[ P.V = \text{number of millimeters of sodium thio sulphate} \times 0.01 \times 1000 / \text{sample weight in gram} \]
- **Free fatty acids (FFA)**: 28 g of obtained fat was added to 50ml of ethanol 95%. Then, droplets of phenolphthaleine reagent were added. All was pipetted with 0.1molar sodium hydroxide until changing the color to the rosy red [21]. Free Fatty acids percentage was:
  \[ \text{FFAs} \% = \text{number of milliliters of NaOH} \times 0.028 / \text{sample weight in gram} \]
- **Moisture Percentage**: according to A.O.A.C [22] 5g has been taken carefully from each sample and entered into an oven at 105°C for 16 hour, the moisture percentage was:
  \[ \text{Moisture} \% = \left[ (\text{weight before drying} - \text{weight after drying}) / \text{weight before drying} \right] \times 100 \]
- **Protein Percentage**: kieldahl procedure was used to estimate protein percentage. 5 ml of concentration Sulfuric acid was added to 0.2 g of each sample in a flask, , a calculated amount of copper sulfate and potassium sulfate mixture was added as well. Heating proses was carried out and digestion operation started until the contents become a clear pale blue liquid. Then converted to instillation flask of keldahl, which contains sodium hydroxide 40%. The flask was conducted with condenser Immersed in another flask filled of 2% boric acid already mixed with some drops of methylene red and Bromocresol blue. An amount of 25 ml of collected liquid was pipetted by using Burette with 1% molar of hydrochloric acid [19]. The protein percentage was:
  \[ \text{Protein} \% = \left( \text{HCL consumed volume} \times \text{molarity} \times 0.014 \times 6.25 / \text{sample weight} \right) \times 100 \]
- **Fat Percentage**: A dried grinded amount of each sample( 0.5g) was converted into the thimble Soxhlet. 150 ml of hexane was added and the extraction process continued over 16 hour. the flask after removing the solvent was put into oven at 80°C for an hour to ensure that no hexane residues could remained [22]. Fat percentage can be obtained as:
  \[ \text{Fat} \% = \text{flask weight before extraction} - \text{flask weight after extraction} / \text{sample weight} \]
- **Ash Percentage**: a specific weight of each sample was burnt up by Muffle furnace at 525°C for 16-18 hour [22]). The ash percentage has been calculated as follows:
  \[ \text{Ash} \% = (\text{ash weight} / \text{sample weight}) \times 100 \]

### 2.4. Statistical Analysis Model

Data were statistically analyzed using factorial experiment design 5x5 (5 treatments X 5 periods) with two replicates. Analysis of variance was performed on all the variable using linear model in SAS statistical package [23]. Duncan’s multiple range tests were used to determine the significance of the differences between means. The analysis was carried out according to the following standard formula:

\[ Y_{ij} = \mu + A_i + B_j + AB(ij) + e_{ij} \]

### 3. Results and Discussion

#### 3.1. Moisture percentage

| Storage Periods (days) | Control | NaCl | SS | NaCl+SS | SS+Asc |
|------------------------|---------|------|----|---------|--------|
| 0                      | 66.10±0.03F | 70.15±0.06A | 67.45±0.05D | 68.85±0.05B | 66.80±0.10E |
| 3                      | 63.92±0.01J | 68.12±0.03C | 65.10±0.10I | 65.35±0.05H | 65.02±0.02I |
| 6                      | 61.10±0.05O | 65.60±0.03G | 62.11±0.01L | 62.90±0.05K | 61.70±0.10M |
| 9                      | 57.67±0.03U | 61.42±0.03N | 58.90±0.15R | 60.70±0.10P | 58.65±0.01S |
| 12                     | 56.25±0.05X | 60.19±0.09Q | 57.20±0.05V | 58.42±0.02T | 56.85±0.05W |

Means ±SE with the same letters are no significant differences (P<0.01).

Table 1 shows the effect of adding types of natural additives and storage periods on moisture contents. The highest value (70.15) was scoring with adding NaCl at the zero storage periods. The last one was belong to the control (56.25) at 12 days as a storage period. All other values distributed significantly between these two values with a few exceptions.
Generally, moisture percentage decreased with elongating storage periods but adding salt may be reduces this process by accelerating the heat transfer from the meat surface and the chilling surrounding media, this would enhance retention water molecules and keep moisture percentage higher than other treatments [24]. Luna et al. [25] referred to that chilling process would improve with adding salt or additives by reducing water temperature below standard and that would increase water retention.

3.2. Ash percentage

Table 2 illustrated the percentage of ash affected with treatments and storage periods. The values graduated from (2.10) which belongs to the control treatment at 12 days storage period as the highest values to (1.35) which belongs to NaCl+SS treatment at the 12 days storage periods as the lowest value. All other values distributed gradually between them with increasing in non-significances among samples almost in the first three storage periods. The augmentation in ash percentage is usually because the decreasing in other contents during the periods of storages, and that explains the relation between this augment and the 12 days storage period [25,26].

3.3. Protein percentage

Protein percentages in this experiment differed from each other’s obviously. The highest value (21.06) was recorded in NaCl treatment at 12 days storage period. And the lowest one was recorded in control group at the zero storage period. It were high significant differences among all records with an exception in few groups. Generally, protein percentages started to be more and more gradually with increasing in storage periods in all treatment even control. This increment was at the contrast with many researchers [25,26], who recorded a decreasing in protein percentage with progressing storage period. This contrast may due to the difference in longevity of storage period between their experiment and ours. They demanded storage period reaches to 60 days in comparison to our period that reaches to 12 days as a maximum. With elongating the storage period the protein exposed to a sever oxidation processes and degradation in its wholeness profile which may due to a decreasing in its total percentages [27,28]. Table three shows the profile of protein percentage in this experiment.

| Table 2. The effects of interactions between treatments and periods of storage on ash percentage in meat samples. |
| --- |
| Treatment + SE  % | Storage Period (days) | Control | NaCl | SS | NaCl+SS | SS+Asc |
| --- | --- | --- | --- | --- | --- | --- |
| 0 | 1.65±0.05D | 1.63±0.03D | 1.77±0.03D | 1.70±0.05D | 1.62±0.02D |
| 1.73±0.03D | EFGHI | 1.70±0.00D | 1.65±0.05D | 1.61±0.01D | 1.75±0.05D |
| 3 | BCDEFG | 1.75±0.05D | 1.75±0.05D | 1.57±0.02D | 1.53±0.03D | 1.76±0.03D |
| 6 | BCDEFG | 1.85±0.05D | 1.81±0.01D | 1.48±0.02D | 1.47±0.02D | 1.80±0.02D |
| 9 | B  | 2.10±0.10A | 1.85±0.05D | 1.42±0.02D | 1.35±0.05D | 2.05±0.05D |
| 12 | A  | B  | KL  | L  | A  |

Means ±SE with the same letters are no significant differences (P<0.01).

| Table 3. The effects of interactions between treatments and periods of storage on protein percentage in meat samples. |
| --- |
| Treatment + SE  % | storage Periods (days) | Control | NaCl | SS | NaCl+SS | SS+Asc |
| --- | --- | --- | --- | --- | --- | --- |
| 0 | 15.72±0.08P | 17.30±0.05K | 16.35±0.05N | 16.95±0.00M | 16.90±0.05N | 16.10±0.05O |
| 3 | 16.90±0.05M | 18.15±0.05J | 17.05±0.05LM | 17.20±0.00KL | 16.95±0.05M |
| 6 | 18.35±0.05I | 19.53±0.07G | 18.35±0.05I | 19.12±0.02H | 17.98±0.02J |
| 9 | 20.40±0.05CD | 20.97±0.02AB | 20.13±0.03E | 20.28±0.02DE | 19.92±0.08F |
| 12 | 20.55±0.07C | 21.06±0.16A | 20.50±0.05C | 20.83±0.03B | 20.40±0.10CD |

Means ±SE with the same letters are no significant differences (P<0.01).
3.4. Fat percentage

Fat percentages differed from (20.12) as a highest value belongs to control treatment at the 12 days storage period to (10.32) as a lowest one belongs to NaCl treatment at the zero storage period. High significant differences were shown among all recorded values with very little exception between NaCl+SS treatment at 3 days storage period (14.77) and SS+Asc treatment at zero days storage period (14.55), as what are shown in table 4. The results in general exhibited an obvious increment in fat contents with the progress of storage period, this may due to the decreasing in moisture percentage in fact, more than a true augmentation in fat contents. It was at the contrast with what was found by Luna et al. [25] and Jihad et al. [26]. The reason may be the same reason behind the contrast in protein contents which is the difference in length of storage period between their experiments and ours.

| Storage Periods (days) | Control  | NaCl  | SS  | NaCl+SS  | SS+Asc  |
|------------------------|----------|-------|-----|---------|---------|
| 0                      | 15.73±0.07K | 10.32±0.17R | 13.27±0.25N | 12.03±0.03P | 14.55±0.05M |
| 3                      | 16.50±0.05I | 11.50±0.10Q | 15.55±0.02K | 14.77±0.02LM | 15.75±0.05K |
| 6                      | 17.90±0.05F | 13.27±0.02O | 17.10±0.10H | 15.70±0.03K | 17.63±0.03G |
| 9                      | 19.10±0.05C | 14.90±0.05L | 18.30±0.05E | 16.51±0.01I | 18.65±0.05D |
| 12                     | 20.12±0.12A | 16.10±0.05J | 19.48±0.02B | 17.90±0.02F | 19.70±0.02B |

Means ±SE with the same letters are no significant differences (P˂0.01).

It’s good to be known that the majority of fat contents in meat is as triglycerides and phospholipids. Triglycerides forms the majority of the intramuscular fat and it directly related with the fat contents of meat that would be recorded in experiments. The important other types of fat is the phospholipids, which are just a small amounts of fat in comparison with triglycerides. Phospholipids contribute in forming cell membrane, so, the increasing in fat contents means an increasing in triglycerides in fact, while the phospholipids contents still constant since the number of cell membranes constant too [4,29,30]. Many of researches referred to the fact that the phospholipids is the part of fat which responsible for oxidation development in lipids, because the facilities that offered by the arrangement of lipids in cell membrane to start the oxidation initiation phase, and the highest amounts of polyunsaturated fatty acids in comparison with triglycerides [4,11,29,31]. So the contents of fat in sample is just a secondary factor in oxidation process, while the most important factor is the types of these fats [32]. Therefor a conclusion of Cheng [33] referred to that in lean with very low intramuscular fat and high contents of phospholipids there is a high susceptibility for oxidation process in comparison with the contrast situation.

3.5. Drip loss

Drip loss can be defined as the lost fluid from fresh meat by exudation. Usually expressed as a percentage between sample before and after exudation [34]. The highest drip loss in our results was recorded from control treatment at zero days storage period (1.85), and the lowest one was from NaCl treatment at 12 days storage period (1.15), all records exhibited slightly significant differences and distributed between them. Drip loss always follows protein oxidation and moisture contents [35] because oxidation reduces water holding capacity WHC and set much water content free from molecules bonds [36]. Storage period may cause oxidation which would be a cause -in its turn- to an increment in drip loss. On the other hand, storing may decreases the moisture contents (as in our case) and makes the drip loss at the end of storage period lower than that in the beginning in spite of oxidation process, because of the lake in water in the whole meat texture[37,38].

3.6. Water holding capacity WHC

Figure 1 illustrated the profile of WHC values. It can be shown that all treatment declined in WHC values with progressing of storage period. The lowest WHC values were belong to the control group at the beginning or the end of storing. At the 12 days storage period control group recorded the lowest value at all (35.62). While NaCl treatment overlapped all other in WHC value at the beginning or end of storing. It scored (60.10) at zero days storage period and (46.92) at the 12 days storage period. The treatment of NaCl+SS came at the second stage in this trait.
Figure 1. Effects of interaction between treatments and storage periods on WHC values.

It’s obvious from results that, the effect of NaCl on WHC have the lion share. That because the most water in muscle is held within the myofibrils matrix [34]. The changes in the volume of myofibrils is the reason behind the changes in WHC [39,40]. Myofibrils situation depend upon myofilaments, and these have an electrostatic repulsion among each other’s determines the water holding capacity WHC of myofibrils [41]. Increasing filaments net charges leads to increasing WHC, and that may be achieved by adding NaCl [42]. Another effect of NaCl on increasing WHC, may be due to the association of Cl with myosin (one of the two main filaments proteins) which may be absorbed (the chloride ion) to a deep part of myosin and makes it hydrophilic instead of hydrophobic, and finally enhance WHC at the whole vision [42].

3.7. pH values

PH values differed from each other’s significantly. The highest value (5.86) was from NaCl treatment at the 3 days storage period. The lowest one (5.45) was from control group at 6 days storage period. NaCl treatment overlapped on other treatment in this trait and the NaCl+SS treatment came the second. The preferred pH value was belong to the treatment of soy sauce addition (SS) which was from (5.75) at zero days storage period to (5.62) at the 12 days storage period. The treatment of adding soy sauce and ascorbic acid (SS+Asc) was at the preferred range too, ranged from (5.66) to (5.58). this results was similar to what mentioned by Kim et al. [43] who referred to that frozen beef meat with soy sauce additive had a lower pH value than that with NaCl. The major reason behind decreasing in pH values was the accumulation of lactic acid produced by lactic acid bacteria, which may be enhanced with adding soy sauce and inhibited with NaCl [44,45].

Figure 2. Effects of interaction between treatments and storage periods on pH values.
3.8. Myoglobin concentrations (Mb)

Myoglobin is the pigment that cause the red color of meat [1]. After slaughter and manufacturing this pigment begin to altering into metmyoglobin. The concentrations of this compound increase gradually with decreasing in myoglobin concentrations [46]. So, the concentrations of myoglobin may be a good indicator for meat color stability which in its turn an indicator of freshness [1]. Our results showed a highest concentrations of myoglobin recorded from treatment of adding soy sauce and ascorbic acid (SS+Asc) (5.34) at the zero days storage period. The second highest value was belong to this same treatment (SS+Asc) at the 3 days storage period (5.10). the same treatment (SS+Asc) still keeps an adequate myoglobin concentrations even at 12 days storage period (4.71) in comparison with other treatments. The lowest value was from NaCl treatment at the 12 days storage period. The treatment of adding soy sauce (SS) recorded a moderated value at the top half of all values, ranged from (4.91) to (4.57). Sanchez-Escalante et al. [47] illustrated that the decreasing in redness is associated with myoglobin oxidation and which is affected with adding or not adding anti-oxidants. The results showed obviously that adding soy sauce with or without ascorbic acid will enhance meat color by keeping myoglobin concentration out of oxidation for longer period. This results were accorded with that of [1].

3.9. Peroxide value (P.V)

Determination of peroxide value (P.V) is one of the most important tests to exploring oxidation and rancidity [48]. It’s a measurement of peroxides and hydroperoxides which may occurred in unsaturated double bands lipids in initial stage of oxidation and [49].The results in this study showed a significant differences among values of P.V. Started from (9.15) for the control group at 12 days storage period to (1.30) for the treatment of adding soy sauce with ascorbic acid (SS+Asc). All the records that belong to 12 days storage period were at the first half of the wholeness with the highest value. And the records that were obtained from treatments with soy sauce addition (SS, NaCl+SS, SS+Asc) were at the last half of the wholeness with the lowest value. This results were similar to those of Lee et al. [50] which reported that adding various anti-oxidant can delay or inhibit the conformation of P.V. The oxidation process have three main stages (initiation, propagation and termination). Initiation stage would be started with forming free radicals [51], the soy sauce additives have an inhibition effects on this stage in particular, by restrict the activities of free radical or \( \text{H}_2\text{O}_2 \) prevention and stopping lipids oxidation in its primary stages [1]. Our results showed a similar effect of soy sauce additives with or without ascorbic acids.

3.10. Free fatty acids (FFAs)

Free fatty acids percentages have a special importance (in association with myoglobin concentrations) in evaluation the shelf life of meat and its products, for their responsibility for meat flavor[52]. The type of free fatty acid and its amount determines the final flavor of meat, and it is related directly with the fat composition in the intact animal before slaughter [53]. Species, nutrition regime, age and gender would be a critical factor determine the intact animal fat composition and then type of free fatty acid released [53].

![Figure 3. Effects of interaction between treatments and storage periods on FFAs values.](image-url)
Figure 3 illustrated the changes in FFAs according to storage periods and treatments. The highest value was collected from control group (1.55) at the 12 days storage period and NaCa group at the same period as the nearest group with no significant differences between them. While the lowest one was collected from the treatment of adding soy sauce and ascorbic acid (SS+Asc) (0.18) at zero days storage period. No significant differences were observed between control group and NaCl treatment along all the storage periods with an exception in 6 days period which exhibited a kind of that. The SS+Asc treatment exhibited the lowest started value and the lowest final one. Followed by the treatment of adding soy sauce alone SS, which stated with (0.19) and ended at (1.1). In general, all treatment with adding soy sauce (SS, NaCl+SS, SS+Asc) scored the lowest value in all storage period. [1,54] reported that soy sauce contains organic acids like acetic acid, lactic acid pyroglutamic acid and butyric acid, all these compound can share its electrons and end the radical serial reaction. Our results showed a decreasing in FFAs with adding soy sauce, and it accorded with theoretical explanation, in spite of that they came at the contrast of the results of Kim et al. [1] who found an increment with FFAs with adding soy sauce. Many researches referred to that the effect of antioxidants would be more effective on the initiation phase of oxidation to preventing the forming of free radicals from the beginning, and have a secondary role in the propagation phase [8,51]. Thus the soy sauce may play a role by preventing producing free radical in the initial phase and reducing the composition of secondary products in the propagation phase of oxidation process [1,55].

3.11. Thiobarbituric acid (TBA)

In contrast with FFAs test or P.V, which focused on evaluate lipids oxidation in its initial and mediate phases. The TBA values give ideas of lipids oxidation in its termination phase [48]. TBA values may be increased with increasing temperature or storage periods and have a high related with lipolysis enzymes in muscle itself and the microorganisms enzymes too[48]. The effect of interactions between treatment and storage period on TBA values was shown in figure 4. It can be seen that the highest TBA values were obtained from NaCl treatment in general and from control group in second place for all storage period. No significant differences were observed between them (control and NaCl) but an exception in the 3 days storage period. The effect of adding soy sauce was clear in the three other groups. The lowest TBA value was belong to SS+Asc treatment. The adding of soy sauce and ascorbic acid reduced the production of TBA significantly. The value of zero days storage period was (0.2) and the value of 12 zero storage period was (0.5). this value followed directly by those of SS treatment, which was scoring (0.21) at zero days storage period and (0.6) at 12 days storage period. The values of NaCl treatment were slightly above them with significant differences among them all and with other two residuals groups. This results were in agreement with the many studies [43,56] who studying the effect of soy sauce on TBA values in frozen beef patties and seasonal pork. Also our result had an agreement at the point of using ascorbic acid as an antioxidant against producing TBA with the study of Ladikos and Lougovois [57]. Moon and Cheigh [58] referred to that soy sauce contains natural antioxidants like phenolic compounds and melanoidin, which is a brown compounds produced by maillard reaction during fermentation have a strong antioxidant effect because of its hydroxyl and amine groups. The results of this study accorded with the suggestion of that the soy sauce with and without ascorbic acid limits the formation of secondary oxidation products like malondialdehyde in beef meat under storage conditions [1,43,59].

3.12. Total volatile nitrogen compounds (TVN)

The products of lipids oxidation process may act as a protein oxidation initiators. Thus when lipids oxidation occurs, protein oxidation may be occurred subsequently [27]. In general, all amino acids can be oxidized and made many of side compounds [60]. Total volatile nitrogen concentrations (TVN) can be a real indicator to investigate a farther oxidation progress in meat and meat products [61], because it depends upon ammonia content which may be produced at the final stage of amino acids oxidation. The oxidation initiators may be came from lipids oxidation products or bacterial enzymes or proteolytic enzymes in muscle itself [61,62].
Figure 4. Effects of interaction between treatments and storage periods on TBA values

In this study, figure 5 illustrated the profile of the effects of treatment and storage periods on TVN values. The highest one was came from control group at the 12 days storage periods (18.25), all values in control group were above others in this trait. The NaCl treatment came in the second rank and have the second highest value (15.62) at the 12 days storage period. The lowest value at the 12 days storage period was came from SS+Asc treatment (10.53), followed by SS treatment (11.95) at the same storage period. The lowest value in results at all was belong to the SS+Asc group (2.15) at the zero days storage period. The results suggested clearly that adding soy sauce have an obvious effect on decline TVN concentrations, and adding soy sauce with ascorbic acid would enhance this results. And that was accorded with what was found by Hassanin et al. [61], who referred to that ammonia is the main spoilage product that responsible for the off odor and bad flavor in spoiled meat. Ammonia is a main indicator of amino acids degradation at its final phases and antioxidant may prevent that by its effect on the initiation and propagation phases of oxidation process [61,63].

Figure 5. Effects of interaction between treatments and storage periods on TVN values.

Conclusion

Adding soy sauce with or without ascorbic acid enhance the meat products by enhancing redness and prevent metmyoglobin production. Soy sauce additives greatly inhibits initiation and propagation phases of oxidation process, which would prevent the lipids oxidation products of being formed and keep protein out of co-oxidation as a sequences. Mix soy sauce with ascorbic acid makes all benefit be more significant.
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