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STUDYING THE ANTIOXIDANT ACTIVITY OF OREGANO AND MAUI ROSE POWDERS AND THEIR EFFECT ON LIPID OXIDATION INDICATORES OF COLD-PRESERVED BEEF LUNCHEON

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Abstract:
This study included the purchase of fresh beef taken from the thigh area and vegetable herbs (oregano *Origanum vulgare* and maui rose *Borago officinalis*) from the local markets of the city of Basrah, and the meat was finely chopped, As for the botanical herbs, they were finely ground and kept in the refrigerator at 4 °C in tightly closed containers until use. Aqueous extracts of the botanical herbs were made and the antioxidant activity was evaluated by the free radical capture method (DPPH) and compared to the with the industrial antioxidant BHT.
The luncheon product was prepared by making seven treatments (a control sample devoid of luncheon spices, vegetable herbal powder, and industrial antioxidant T_1, the sample treated with luncheon mix spices T_2, the sample treated with industrial antioxidant by 0.02% of (BHT) T_3), luncheon treat by 0.3% of oregano T_4, luncheon meat treated with 0.5% of oregano T_5, luncheon meat treated with 0.3% of the maoist rose T_6, luncheon meat treated with 0.5% of the maui rose T_7) , The luncheon treatments were kept cooling at a temperature of 4 C for a period of (0, 3, 6, 9, 12, and 15) days, and studied the antioxidant activity of oregano and maui rose powders and their effect on lipid oxidation indicatores (peroxide value, percentage of fatty acids, and thiobarbutyric acid value) throughout the cooling periods of preservation beef luncheon.

It was noted through the results that The antioxidant activity (DDPH) of oregano powder was higher than that of maui rose powder, and the effectiveness increased by increasing the percentage compared to BHT, whose effectiveness was higher than both, and it was found that there were highly significant effects at the level of probability ($P\leq 0.05$), between the two types of herbs and industrial antioxidant. While a decrease in the peroxide values, percentage of free fatty acids and thiobarbutyric acid values, and these parameters increased with the progression of the preservation period, The statistical results showed that there was a highly significant effect at the level of probability ($P\leq 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the peroxide values, percentage of free fatty acids and thiobarbutyric acid values.

Keywords: Luncheon, oregano, maui rose, antioxidant activity, chemical indicatores, cooling preservation, condiment, peroxide value, percentage of free fatty acids, thiobarbutyric acid value.

Introduction:

Luncheon products are among the most popular meat products that are consumed by millions of people from all on the world, and they are common and favorite foods for adults and children. Luncheon-meat can be defined as a food product cooked from red meat or finely chopped poultry meat, which was treated with sodium nitrite and ascorbate to increase the stability of the color of the product. Starch and salt are added to it, It was cooked or it may be smoked, It was very small pieces pressed into a mold, The luncheon was packed in the form of long sausages or meat-loaf cylinders, and it was cut into thin slices upon consumption and served cold and used to prepared sandwiches with cheese as a snack, or it may be used separately as appetizers or salads (EL-Hadidie *et al*., 2017) and because of the low prices of herbs and spices and their abundance it's production was the alternative to many industrial compounds (Cai *et al*., 2004). Among these herbs was oregano, known as marjoram, which was used in food preservation, in the manufacture of meat products, and in fish packaging because of it's role as an antioxidant that contributes to delaying the occurrence of oxidative rancidity. Likewise, the maui rose, known as borage was used for culinary and medicinal purposes because it's seeds were considered one of the best sources of linoleic acid (Tasset-cuevas *et al*., 2013) and (Oribi, 2017) and because of the increasing demand for natural additives in the food industry, which were natural antioxidants that delay the oxidation of fats and oils in food products, improved their quality and nutritional value, and extend their shelf life, especially meat and it's products. As natural antibiotics were more acceptable and safer for the consumer in terms of health (Camo *et al*.,2008 ;Fasseas *et al*., 2007) In addition, the antioxidants in the diet have many benefits for human health because they protect biologically important cellular components such as nucleic acids, proteins and membrane lipids from the effects of free radicals formed (Su *et al*., 2007), Many studies have been conducted on the effectiveness of spices, herbs and their extracts such as rosemary and oregano to reduced oxidation in meat products (Velasco and Willams, 2011).

Materials and Methods:

Plant preparation:

The plants leaves (oregano *Origanum vulgare* and maui rose *Borago officinalis*) were ground well with an electric mill, then sifted and kept in airtight containers in the refrigerator at 4 C^{0} until use.

Luncheon manufacturing process:

5 kilos of beef taken from the thigh area were brought from the local markets of the Basrah city, The outer fat was removed and finely chopped with a meat grinder. Using a disk with a hole diameter of 1.5 cm, then the grinding was repeated for the second time with a disk with a hole diameter of 0.8 cm, The proportions of the ingredients used in the manufacture of the luncheon are as shown in Table (1):

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Table (1): Percentages of ingredients used in the manufacture of luncheon meats

n.	ingredients	percentage (g)
1	beef	100
2	black pepper	0.1
3	cumin	0.1
4	paprika	0.1
5	onion powder	0.1
6	garlic powder	0.1
7	salt	3
8	Starch	3
9	bread crumbs	3

and followed the method of Abd El-Rahman *et al.* (2019) in the manufacture of luncheon, the following steps were taken with some modifications:

1- The weights of the minced meat samples were prepared.

2- Starch, bread crumbs and salt were added to the minced meat mixture to make seven samples of 100 gm for each sample as follows:

T_1 = control treatment (minced meat only without addition).

T_2 = treatment to which spices (cumin, black pepper, paprika, onion powder and garlic) were added at an average of 0.1.

T_3 = treatment to which the industrial antioxidant (BHT) was added at a concentration of 0.02 %.

T_4 = treatment to which oregano powder was added at an average of 0.3 g per 100 g of meat.

T_5 = treatment to which oregano powder was added at an average of 0.5 g per 100 g of meat.

T_6 = a treatment to which maui rose powder was added at an average of 0.3 g per 100 g of meat.

T_7 = treatment to which maui rose powder was added at an average of 0.5 g per 100 g of meat.

3- The samples were mixed manually using sterile hand gloves in order to achieve homogeneity of the materials and to ensure that the additives were evenly distributed.

4 - The luncheon mixture prepared for the above samples was wrapped using thermal polyethylene bags, giving it a cylindrical shape, and then wrapping it with aluminum foil.

5- Luncheon cylinders were cooked in boiling water for an hour, then left to cool and kept in refrigeration at 4 C^o until lipids oxidation indicators were conducted every period of (0, 3, 6, 9, 12, 15) days.

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Figure (1): The final shape of the luncheonette cylinders

Figure (2):T_2

Figure (2):T_1

Figure (4):T_4

Figure (3):T_3

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Figure (6):T_6

Figure (5):T_5

Figure (7):T_7

Preparation of plant extracts to measure antioxidant activity:

The method of Harborn (1984) based on Al-Mansour (1995) mentioned by Al-Sharifi and Abdel-Amir (2018) was adopted to prepare cold aqueous extracts. As the leaves of oregano and the maui rose were ground well, then 50 g of the powder of these leaves were dissolved in 500 ml of cold distilled water in a flask with a capacity of 1000 ml (one liter).The mixture was mixed using a magnetic sterer for 15 minutes, then left for 24 hours at room temperature, then filtered with two layers of sterile gauze (tulle cloth) to get rid of impurities. After that, filter paper was used, and the filtrate was collected in glass containers and transferred to the centrifuge at a speed of 2000 rpm / min to obtain the clear liquid, and the sediment was discarded.Then the filtrate was dried in the oven at a temperature of 40°C for a period of days until it was completely dry. After that, the powder was collected and weighed on a sensitive scale, packed in sterilized dark containers, and kept in a refrigerator at 4°C until use.

1- Measuring antioxidant activity using the DPPH method:

The antioxidant activity of the prepared aqueous extracts and the industrial antioxidant (Butylated Hydroxy Toluene (BHT)) was estimated according to the method modified by (Blois, 1958). So, 1 ml of DPPH reagent (2,2 Diphenyl,1-2-picrylhydrazyl) at a ratio of 0.01 molar prepared in methanol 98% was mixed with 2 ml of aqueous extracts with concentrations ranging from (0.5 - 5) mg / ml, then The mixture was incubated in the dark at laboratory temperature (25 °C) for 30 minutes.The control sample was prepared by mixing 1 ml of DPPH with 2 ml of distilled water, and the absorbance was measured at a wavelength of 517 nm. The antioxidant activity (1% DPPH) was calculated using the following equation:

$$\%1\ DPPH = \frac{(As - A0)}{A0} \times 100$$

A_0 = absorbance of the comparison sample
As = absorbance of the extraction solution

2-Measurement of lipids oxidation indicators:

- **Peroxide value:**

According to the method presented in Pearson (1976), the peroxide value was calculated according to the following equation:

Peroxide value = (ml) of sodium thiosulfate needed for flushing xtiter x1000/sample weight (g)

- **Free Fatty Acids (FFA%):**

The acidity value was estimated according to the method mentioned in Pearson (1976), and then the percentage of free fatty acids was calculated according to the method mentioned in (Al-Taie and Al-Mousawi, 1992).

The amount of free fatty acids FFA% = acidity value / 2

Acidity value = 0.1 mL NaOH required for elution x 56.1 / sample weight (g)

- **The value of thiobarbutyric acid:**

The method mentioned in Soltanizadeh and Ghiasi-Esfahani (2015) was adopted, and the TBA value was calculated according to the following equation:

TBA value in mg malonaldehyde/kg = A532 x 5.4

As A532 represents the value of absorption at a wavelength of 532

Results and discussion:

1-Antioxidant activity of aqueous extract of oregano and maui rose (the ability to capture free radicals using DDPH):

Figure (8) shows the results of the estimated antioxidant activity in the aqueous plant extracts of oregano and maui rose and compared with the industrial antioxidant BHT. The effectiveness of the aqueous extract of oregano was (56.00, 65.00, 67.50, 70.95, 77.60, 79.45) and it was higher than the effectiveness of the aqueous extract of mao rose, which was (43.50, 47.70, 54.00, 58.85, 63.75, 68.00) % respectively at concentrations (0.5, 1, 2, 3, 4, 5) mg/ml, The results agreed with Khanum *et al.* (2011) which showed that the activity of the aqueous extract of oregano was higher than the activity of the

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aqueous extract of maui rose, As for the effectiveness of BHT % was (90.90, 91.65, 25.92, 92.35, 93.35, 93.47) % respectively, which was higher than the effectiveness of both plant extracts (oregano and maui rose). The results also showed that the antioxidant activity increased with increasing the concentration of the aqueous extract of the herbs.This is what Al-Saad and Abdel-Karim (2017) found, which showed that the antioxidant activity of the aqueous extract of aloevera at a concentration of (1, 5) mg / ml was (50, 65) %, The reason for this was due to the presence of active compounds in the aqueous extract of herbs, which are due to the high antioxidant activity, which at the same time increases with increasing concentration (Khaing, 2011).

The results showed that the antioxidant activity of the extracts was affected by the nature and proportions of the active compounds in them, as well as the method of extraction and the concentration of the extracts (Abu-Reidah *et al*., 2014).The statistical results showed that there was a highly significant effect at the level of probability ($P \leq 0.05$) for the type of antioxidant and the concentration of the antioxidant and the interaction between them on the ability to capture free radicals for the aqueous extracts of oregano, maui rose and BHT.

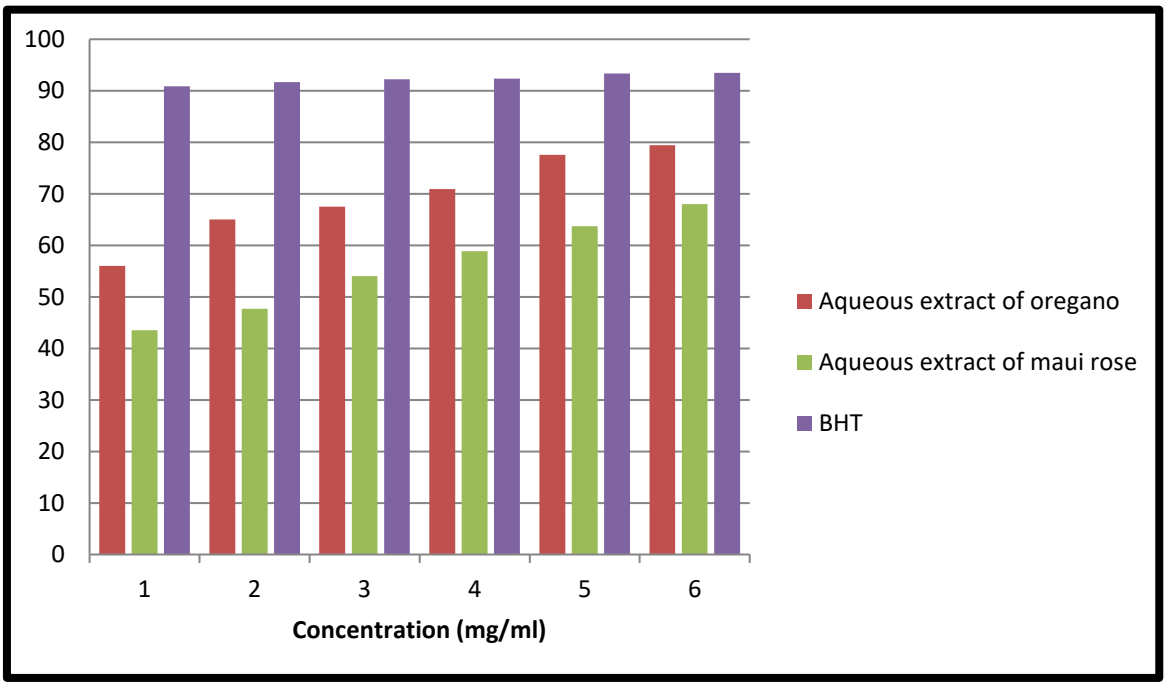

Figure (8): The ability to capture free radicals (%) of aqueous extracts of Oregano and Maui rose compared with BHT

- **All results in the figure are an average of two replicates**
- **LSD $_{0.05}$ for the effect of antioxidant type on the ability to capture free radicals = 13.4**
- **LSD $_{0.05}$ for the effect of the antioxidant concentration on the ability to capture free radicals = 2.05**

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• LSD $_{0.05}$ for the effect of the binary interaction between the type of antioxidant and its concentration = 15.45

2-Measurement of lipids oxidation indicators:

- **Peroxide value:**

Figure (9) showed a decrease in the peroxide values among the different luncheon treatments (T_4, T_5, T_6, T_7), with a continuous cooling period of (0, 3, 6, 9, 12, 15) days compared to treatments (T_1, T_2, and T_3). As the peroxide value in these treatments was (0.71, 0.70, 0.73, 0.72) Meq/kg respectively at the storage period (0) days, It was found that the peroxide values decreased with the increase in the percentage of herbal powder used in the luncheon treatments and for all storage periods When compared with the treatments (T_1, T_2 and T_3) that contained peroxide values (0.91, 0.70, 0.80) Meq /kg respectively at a storage period of (0) days, After fifteen days of preservation, the peroxide values increased to reach (6.51, 4.46, 5.66, 3.41, 3.33, 4.31, 4.14) Meq /kg for the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) respectively.

The reason for the decrease in the treatments of the luncheon product treated with vegetable herbs (oregano and maui rose) compared to T_1, T_2 and T_3 was due to the fact that they contain phenolic compounds that work as antioxidants that slow down the formation of free radicals (peroxide radicals) and thus disrupt the oxidation process in fats.

Also, the reason for the increase in the peroxide values with the progression of the storage period was due to the formation of peroxides, albeit in a small amount. However, treatment with herbal spices and herbs that have antioxidant activity reduces the formation of these compounds. Therefore, we find that the peroxide values during this study were lower in treatments containing vegetable powders of oregano and maui rose these reasons agree with what was indicated by (Al-Qatifi, 2019).

The results showed that the use of oregano powder in two percentages (0.3 and 0.5%) was more efficient in preserving the peroxide value in luncheon treatments during the storage period.

The statistical results showed that there was a highly significant effect at the level of probability ($P \leq 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the peroxide values of luncheon treatments that have been cooling for different periods.

The current results are consistent with Al-Alwani (2017) who showed a significant decrease in the peroxide value of fresh, chilled ground beef treated with concentrations of carnosic acid and rosemary, compared with the control sample and the sample treated with BHA, The peroxide values increased with the advancing period of preservation for a period of 21 days and he explained that the permissible limits for the peroxide value should not exceed 10 Meq/ kg of fat according to the standard specification.The obtained results also agreed with Karam and Al-Mosawi (2016) and with Shelbaya *et al.* (2014) as they showed that the use of herbal powders (marjoram, rosemary and cumin) can delay oxidation in meat products.

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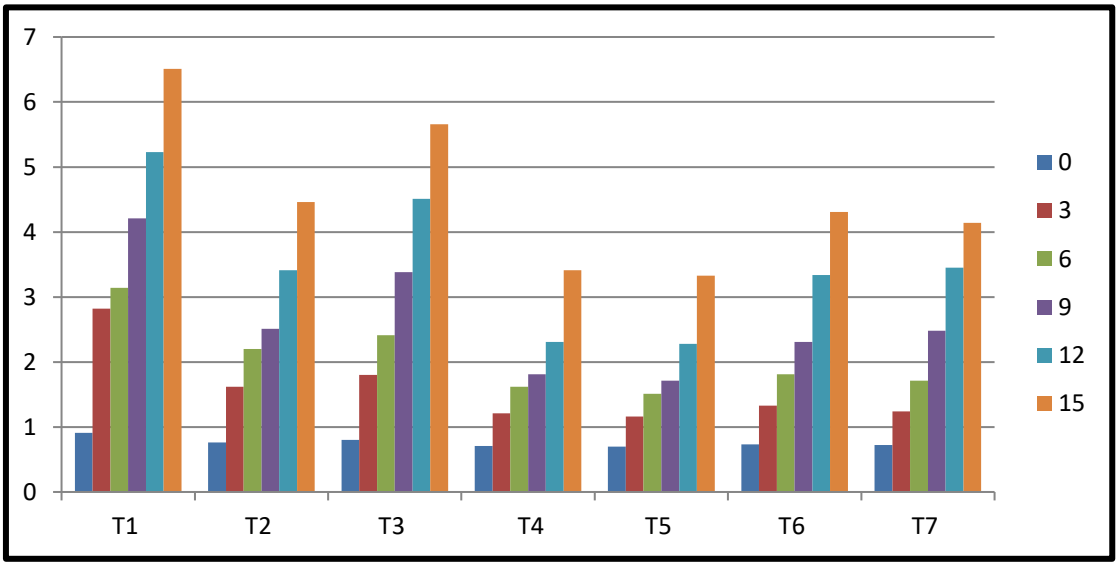

Figure (9): The value of peroxide Meq/ kg for luncheon treatments cooling for different periods

- **All results in the figure are an average of two replicates**
- **LSD $_{0.05}$ for the effect of the type of treatment on the peroxide value = 0.01**
- **LSD $_{0.05}$ for the effect of the duration of preservation in the peroxide value = 0.45**
- **LSD $_{0.05}$ for the effect of the binary interaction between the type of treatment and the duration of preservation in the peroxide value = 0.46**
- **Free Fatty Acids (FFA%):**

Figure (10) showed a decrease in the percentage of free fatty acids among the different luncheon treatments, namely (T_4, T_5, T_6, T_7), with a continuous cooling period of (0, 3, 6, 9, 12, 15) days compared to treatments T_1, T_2 and T_3. As it was in the percentage of free fatty acids in these treatments (0.22, 0.21, 0.24, 0.23) % respectively at the preservation period (0) days, and it was found that the percentage of free fatty acids decreased with the increase in the percentage of herbal powder used in the luncheon treatments and for all periods of preservation when compared with the treatments (T_1, T_2 and T_3) that contained free fatty acids (0.27, 0.25, 0.26) % respectively at a storage period of (0) days, After fifteen days of preservation, the percentage of free fatty acids increased to reach (2.71, 1.02, 1.03, 0.93, 0.90, 0.97, 0.95) % for the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) respectively.

It was found that as the concentration increases the percentage of free fatty acids decreases, and this was consistent with (Terefe, 2017) which showed a decrease in the acidity values in the cooled minced meat samples with an increase in the concentration of ginger powder.

It was observed that the values were close to each other at the beginning of preservation and then began to increase after fifteen days, but the increase was less in the treatments (T_4, T_5, T_6 and T_7) compared to the treatments T_1, T_2 and T_3 due to the fact that spices

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and plant herbs contain phenolic compounds and thus act on breaking the reaction chain by giving a hydrogen atom to the fatty acid and free radicals (Al-Rubaie *et al.*, 2017).

As for the reason for the increase in the percentage of free fatty acids it was due to the action of lipolytic enzymes (lipases and phospholipases) and thus the fats in the meat are exposed to decomposition and the production of many compounds such as hydroperoxides, aldehydes and ketones that increase the presence of fatty acids responsible for the formation of unwanted odor in meat and meat products during storage (Al-Rubeii *et al.*, 2009).

The results showed that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in maintaining the percentage of free fatty acids in luncheon treatments during the storage period.

The statistical results showed that there was a highly significant effect at the level of probability ($P \leq 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the percentage of free fatty acids for luncheon treatments that were cooling for different periods.

The results are consistent with Karam and Al-Mousawi (2016) as he noticed that the percentage of free fatty acids decreased significantly in minced meat to which rosemary and cumin powders were added, and increased with the progression of preservation, and with Abu-salem *et al.* (2011) when manufacturing the luncheon product to which natural extracts were added, Free fatty acids decreased and increased with the progression of the preservation period.

Al-Rubaie *et al.* (2017) stated that the percentage of free fatty acids in fresh chilled beef to which carnosic acid and rosemary were added should not exceed (1-1.5) % according to the standard specification and it was among the results of the study.

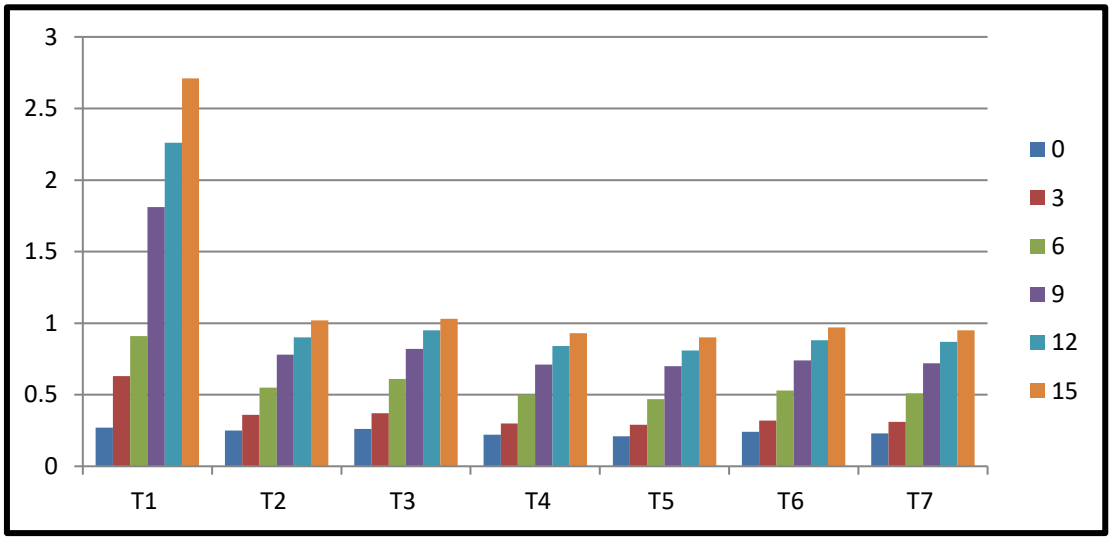

Figure (10): Percentage of free fatty acids for luncheon treatments that have been cooling for different periods

• All results in the figure are an average of two replicates

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• LSD $_{0.05}$ for the effect of the type of treatment on the percentage of free fatty acids = 0.013

• LSD $_{0.05}$ for the effect of storage time on the percentage of free fatty acids = 0.130

• LSD $_{0.05}$ for the effect of the binary interaction between the type of treatment and the duration of preservation in the percentage of free fatty acids = 0.143

The value of thiobarbutyric acid:

Figure (11) showed that there was a decrease in the value of thiobarbituric acid among the different luncheon treatments (T_4, T_5, T_6 and T_7) with continuous cooling period (0, 3, 6, 9, 12, 15) days compared to treatments T_1, T_2 and T_3, The values of thiobarbituric acid in these treatments were (0.21, 0.20, 0.23, 0.22) mg of malonaldehyde / kg of meat respectively at the preservation period (0) days.It was found that the values of thiobarbituric acid decreased with increasing the percentage of herbal powder used in the luncheon treatments and for all storage periods when compared to treatments (T_1, T_2 and T_3) that contained the value of thiobarbituric acid (0.30, 0.28, 0.33) mg malonaldehyde / kg meat respectively at storage period (0) days, After fifteen days of preservation the thiobarbituric acid values increased to reach (4.81, 2.20, 2.33, 1.94, 1.84, 2.15, 2.10) mg of malonaldehyde / kg of meat for the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) respectively.

The values of thiobarbituric acid were close to each other at the beginning of preservation and then began to increase after fifteen days, but the increase was less in the parameters (T_4, T_5, T_6 and T_7) compared to treatments T_1, T_2 and T_3 because they contain phenolic compounds that slow down oxidation and remove the free radicals formed because they contain a phenolic OH hydroxyl group and form relatively stable compounds (Liu *et al.*, 2015).

The reason for the increase in TBA values during cooling preservation was due to the increase in the concentration of malonaldehyde due to the breakdown of peroxides, which are final products of fat oxidation in meat and meat products (Alwani *et al.*, 2017).

The results showed that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in preserving the value of thiobarbituric acid in luncheon treatments during the storage period.

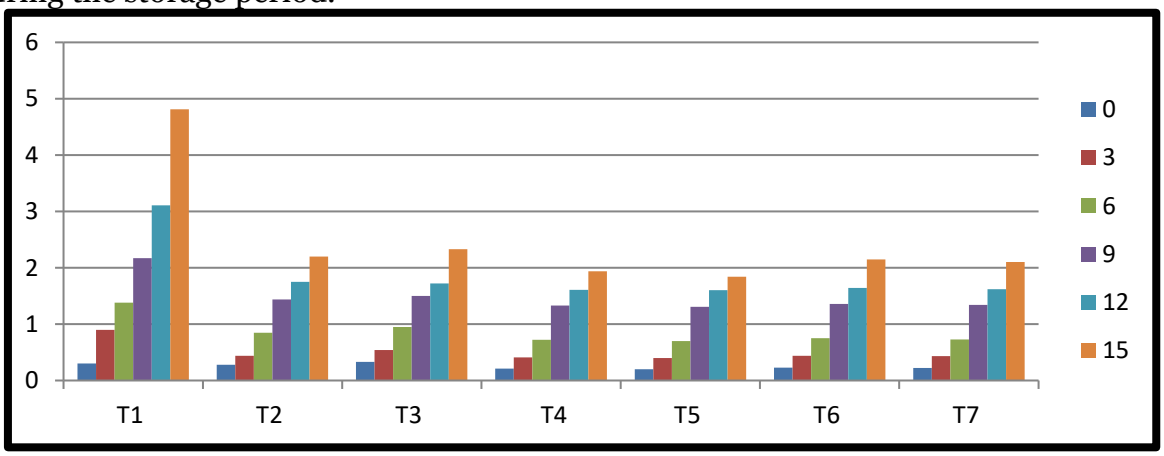

Figure (11): The value of thiobarbutyric acid for luncheon treatments that have been preservation for different periods.

• All results in the figure are an average of two replicates

• LSD $_{0.05}$ for the effect of the type of treatment on the value of thiobarbutyric acid = 0.021

• LSD $_{0.05}$ for the effect of shelf life on the value of thiobarbutyric acid = 0.257

• LSD $_{0.05}$ for the effect of the binary interaction between the type of treatment and the duration of preservation in the value of thiobarbutyric acid = 0.278

The statistical results showed that there was a highly significant effect at the level of probability ($P \leq 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the values of thiobarbituric acid for luncheon treatments that were refrigerated for different periods.

The results are consistent with Shelbaya *et al*., (2014) who noted a significant decrease in the TBA values with an increase in the preservation period when marjoram powder was added to the beef kofta, and with Al-Alwani *et al*. (2017) who indicated a significant decrease in the TBA values, which increased with the advancement of the preservation period. When he added carnosic acid powder, rosemary and industrial antioxidant to fresh beef and showed that the permissible limits for TBA values are 2 mg malonaldehyde / kg meat, which was among the results of the study.

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