Study of physical, sensory and bacteriological properties of cold-preserved luncheon beef meat containing powder of oregano and maui rose

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Abstract:

This study was conducted in order to study the effect of using different proportions of powders (oregano *Origanum vulgare* and maui rose *Borago officinalis*) on the physical, sensory and bacteriological properties of the luncheon product cooling at 4 $^{\circ}$ C for periods of (0, 3, 6, 9, 12, 15) days, compared with an industrial oxidation (Butylated Hydroxy Toluene (BHT)) and control treatment, luncheon product was prepared by making seven treatments (control sample devoid of luncheon spices, herbal powder and industrial antioxidant T_1 , sample treated with luncheon mix seasoning T_2 , sample treated with industrial antioxidant with 0.02 % of The BHT)) T_3 , the luncheon sample treated with 0.3% of oregano T_4 , the luncheon sample treated with 0.5% of oregano T_5 , the luncheon sample treated with 0.3% of the Mao rose T_6 , the luncheon sample treated with 0.5% of the maui rose T_7).

The treatments of the luncheon product were kept at a temperature of 4 C° for (0, 3, 6, 9, 12 and 15) days, and the pH was studied as a physical indicator, sensory properties (colour, flavour, texture, uniformity of shape and chewinees) and bacterial indicators (numbers of total bacteria, psychrophilic, protolytic and lipolytic bacteria) in the luncheon product.

The results showed a decrease in the pH value, in the logarithm of the numbers of total bacteria, psychrophilic, protolytic and lipolytic bacteria, and in the sensory evaluation scores (color, texture, flavor, uniformity of shape, chewiness) in the luncheon product treatments (T_4 , T_5 , T_6 and T_7) compared to samples T_1 , T_2 and T_3 , and the statistical results indicated that there is a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the values of the studied physical and bacterial indicators and in the evaluation scores of sensory properties.

Keywords: luncheon, oregano, maui rose, numbers of total bacteria, psychrophilic, protolytic and lipolytic bacteria, cooling preservation, sensory evaluation of luncheon, luncheon pH.

Introduction:

Processed meat nowadays plays a great importance in the life of the consumer because it provides products that are easy to prepare and have nutritional value. On the other hand, these products are distinguished by their long preservation period, in addition to their flavors and granular texture. However, these products have a high content of fats, salts and preservatives such as nitrates, which they are often unhealthy and cause disease (de Barcellos *et al.*, 2011).

Emulsified meat products such as mortadella, luncheon meat, hot dog sausage, bologna, frankfurter and others are very popular and widespread in various countries of the world. These products belong to the family of high-moisture coated meats with a short shelf life and some to the family of canned meats with a long shelf life (luncheon, canned hot dog sausages), and these products are considered foodstuffs with high nutritional value, as they contain all the essential amino acids necessary for growth, maintenance and building cellular tissues (Ayyash *et al.*, 2018).

Many plants such as fenugreek, castor, henna, coriander, cumin and black seed have been used because of their biological and medicinal effectiveness against bacteria and other pathological fungi. One of 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

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oxidative rancidity, which was responsible for undesirable changes in color, flavor and taste. Also, the maui rose known as borage was used for cooking and medicine purposes because it's seeds are considered one of the best sources of linoleic acid (Tasset-cuevas *et al.*, 2013) and (Oribi, 2017).

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° 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):

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 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):T₁

Figure (2):T₂





Figure (3):T₃

Figure (4):T₄



Figure (5):T₅



Figure (6):T₆

Figure (7):T₇

Then the following examinations were carried out:

- 1- The physical indications of the luncheonette product:
- pH Value:

The pH was estimated using a pH-meter according to the method mentioned by (Mendenhall, 1989).

2- Sensory characteristics of the luncheonette product:

The sensory evaluation (color, flavor, texture, chewing ,uniformity of shape) of luncheon product samples was conducted by fifteen experienced evaluators and graduate students in the Department of Food Sciences / College of Agriculture - University of Basra, according to the evaluation form prepared for this purpose and according to the method shown by Meilgaard *et al.*, (1999).

3- Bacterial indications of the luncheon product:

Sample preparation:

Tests and bacterial culture were conducted under conditions of hygiene and sterilization in order to obtain good results according to the method followed by Andrews (1992) by preparing decimal dilutions by weighing 1 g of the luncheon product sample and adding to 9 ml of 0.1 % sterile peptone water solution that was prepared (with a weight of 0.1 g in 100 ml of distilled water) to prepare the first dilution 10^1 , then the other dilutions 10^2 and 10^3 were prepared by taking 1 ml of the first dilution and transferred to the second dilution and so on by pouring into plates, after which bacterial tests were carried out and the expression of microorganisms growing in the plates in the colony formation unit was carried out C.F.U/g.

• Total Plate Count (TPC):

The total number of aerobic bacteria was calculated according to the method mentioned by Andrews (1992), where the dishes were incubated at a temperature of 37 °C for 48 hours.

• Psychrophilic Bacteria Count:

The number of Psychrophilic bacteria was calculated according to the method found in APHA (1992) and mentioned by Hassan and Al-Hubaiti (2018), and the plates were incubated in the refrigerator at a temperature of 4 °C for 7 days.

Protolytic Bacteria:

The number of colonies surrounded by a transparent halo (protein degradation zone) was counted according to the method in APHA (1992) and the plates were incubated at 35 °C for 48 hours.

Lipolytic Bacteria:

The numbers of bacteria with a bright yellow color and a soapy appearance were calculated according to the method in APHA (1992), and the dishes were incubated at a temperature of 35 °C for a period of (24-48) hours.

• Coliform Bacteria Count Total:

Medium was used MacConkey Agar, which was prepared by dissolving (12.875 gm of this medium in 250 ml distilled water), and the dishes were incubated at a temperature of 37 for a period of 48 hours, and the numbers of coliform bacteria that appeared in the form of pink circular colonies were calculated (Andrews, 1992).

Detection of Salmonella and Shigella:

1gm of the luncheon product sample was added to 9 ml of the activation medium Tetrathionate broth, which was previously prepared to activate the salmonella bacteria and ensure their presence in the sample, and the tubes were incubated at a temperature of 35 C for 24 hours, after which 1 ml of the bacterial suspension was planted in dishes then the Salmonella Shigella agar (SSA) culture medium that was prepared by dissolving (15.75 g of this medium in 250 ml of distilled water) was poured without sterilization, and the dishes were incubated at a temperature of 37 for 48 hours, according to the method in (Andrews, 1992).

Results and discussion:

1- The physical indications of the luncheonette product:

• pH Value:

It was found from Figure (8) that there was a decrease in the pH values between 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 pH values in these treatments were (5.26, 5.24, 5.30, 5.27) respectively at the storage period (0) days, and it was found that the pH values decreased with increasing the proportion of

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herbal powder used in the luncheon treatments and for all preservation periods, if compared with the treatments (T_1 , T_2 , and T_3). which contained the pH value (5.44, 5.39, 5.41) respectively at the preservation period (0) days, and after fifteen days of preservation, the pH values increased to reach (7.20, 6.24, 6.51, 6.10, 6.07, 6.14, 6.16) for samples (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

The results showed that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in maintaining the pH values somewhat stable in luncheon treatments during the storage period.

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

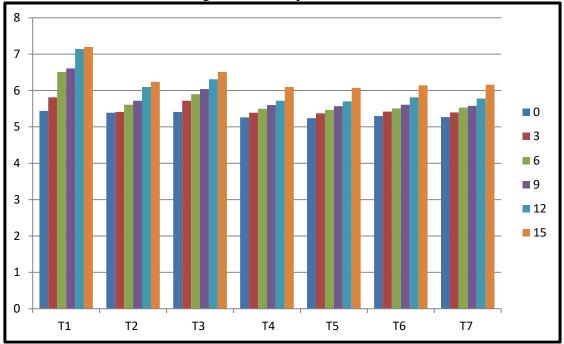


Figure (8): The pH value for luncheon treatments cooling for different periods

- All results in the figure are an average of two replicates
- LSD 0.05 for the effect of the type of treatment on the pH value = 0.02
- LSD $_{0.05}$ for the effect of the duration of preservation in the pH value = 0.04
- \bullet LSD $_{0.05}$ for the effect of the binary interaction between the type of treatment and the duration of preservation in the pH value = 0.06

The results are consistent with (Al-Rubaie *et al.*, 2017), which showed that the increase in the pH over the period of cooling was due to the occurrence of protein decomposition of the meat, which leads to the liberation of nitrogenous bases that raise the pH values. The coolant leads to the activity of some gramnegative Psychrophilic bacteria, such as *Pseudomanas* and *Acinetobacter*, which work to break down protein and release amine groups if it was increased in the diet, it will lead to an increase in the pH values (Rajkumar and Dwivedi, 2011).

And it agreed with Terefe (2017) that it was found that there was a decrease in the pH values when ginger powder was added at (1, 3, 5) %, and it was noted that the decrease decreased with increasing concentration.

As for the reason for the decrease in the pH of the luncheon product, compared to T_1 , T_2 and T_3 , this is because the luncheon treatments contain spices and natural plants that reduce self-decomposition because they contain effective compounds that preserve the luncheon product throughout the cooling period.

2- Sensory characteristics of the luncheonette product:

• Color characteristic:

Figure (9) showed that there was an increase in the degrees of the color characteristic 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 the treatments T_1 , T_2 and T_3 , as it was the degrees of the color characteristic in these treatments

(22.73, 23.46, 20.06, 21.46) respectively at the preservation period (0) days, and it was found that the degrees of the color characteristic increased with the increase in the proportion of herbal powder used in the luncheon treatments and for all periods of preservation, if compared to the treatments (T_1 , T_2 and T_3), which contained degrees of color (19.7, 21.20, 18.80) respectively at the preservation period (0) days, and after fifteen days of preservatio, the degrees decreased to reach (6.26, 16.93, 15.13, 18.00, 19.00, 17.00, 17.86) for the coefficients (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

The use of oregano powder in two percentages (0.3 and 0.5)% was more efficient in preserving the color degrees, and the reason for the decrease in the color degree with the progression of the preservation period is due to the oxidation of the myoglobin dye and it's conversion to the metamyoclobin dye during the continuation of the preservation period, and thus leads to a decrease in the color degree after cooking.

Statistical results showed that there is a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the in color degree of luncheon treatments that have been cooling for different periods.

As for the reason for the increase in the color of the luncheon samples treated with herbs, because these herbs contain phenolic compounds that maintain the stability of the myoglobin dye and reduce it's oxidation to the metamyoglobin dye, which helps to stabilize the color of the meat in the form of F^{+2} (Al-Adhari, 2017).

The results agreed with Al-Mayali (2018) when adding mixtures of spices to meat patties and cooling them for different periods of time, and the average color was from (22 to 24) and with Tangkham (2020), which indicated that adding turmeric powder at concentrations of (1 and 2)% to sitck beef was the reason for good color scores.

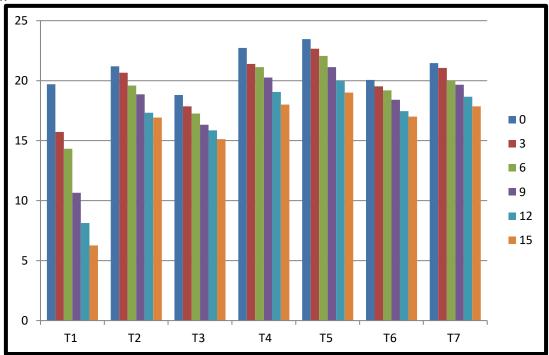


Figure (9): Degrees of the color for luncheon treatments cooling for different periods

- All results in the figure are an average of fifteen replicates
- LSD $_{0.05}$ for the effect of the type of treatment on the degrees of the color = 0.48
- LSD 0.05 for the effect of the duration of preservation in the degrees of the color = 0.76
- LSD $_{0.05}$ for the effect of the binary interaction between the type of treatment and the duration of preservation in the degrees of the color = 1.24

• Flavor (taste and smell) characteristic:

It was observed from figure (10) that there was an increase in the degrees of flavor (taste and smell) among the different luncheon reatments, $(T_4, T_5, T_6 \text{ and } T_7)$ with continuous cooling period (0, 3, 6, 9, 12, 15) days compared to treatments T_1 , T_2 and T_3 , as the scores for evaluating the flavor in these treatments were (24.00, 25.00, 23.40, 23.53) respectively at the storage period (0) days, and it was found that the

evaluation scores for the flavor character increased with the increase in the percentage of herbal powder used in the luncheon treat and for all preservation periods. , if compared with the treatments T_1 , T_2 and T_3 whose degrees were (19.00, 22.26, 20.33) respectively at the preservation period (0) days, and after fifteen days of cooling, the flavor levels decreased to reach (10.00, 17.00, 16.00, 20.00, 21.00, 19.00, 19.33) for the for samples (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

It was concluded from the study that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in preserving the flavor levels during the storage period, and the reason was due to the fact that the botanical herbs (oregano and Maui rose) contain phenolic compounds that give the distinctive smell to the luncheon product.

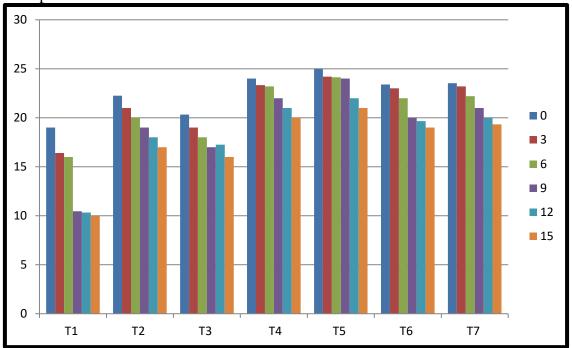


Figure (10): Degrees of the flavor for luncheon treatments cooling for different periods

- All results in the figure are an average of fifteen replicates
- LSD $_{0.05}$ for the effect of the type of treatment on the degrees of the flavor = 0.36
- LSD 0.05 for the effect of the duration of preservation in the degrees of the flavor = 0.65
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the degrees of the flavor = 1.01

Statistical results showed that there is a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the in flavor degree of luncheon treatments that have been cooling for different periods.

Some studies showed that the degree of flavor was higher for luncheon product samples treated with extracts (green tea and thyme oil) than for the comparison sample (Abu-salem *et al.*, 2011), which is consistent with the current study.

• Texture characteristic:

Figure (11) showed a rise in texture levels among the different luncheon treatments, namely $(T_4, T_5, T_6 \text{ and } T_7)$ with a continuous cooling period of (0, 3, 6, 9, 12, 15) days, which maintained the degrees of consistency compared to other treatments T_1 , T_2 and T_3 , as the grades in these treatments were (19.13, 20.00, 17.00, 18.00) respectively at the storage period (0) days, and it was found that the grades of texture increased with the increase in the proportion of herbal powder used in the luncheon treatments and for all storage periods, if compared with the treatments $(T_1, T_2 \text{ and } T_3)$ that contained degrees of texture (17.06, 18.80, 18.00) respectively at the preservation period (0) days, and after fifteen days of preservation, the values of texture decreased to reach (7.66, 15.00, 14.00, 16.60, 17.00, 15.00, 15.13) for the treatments $(T_1, T_2, T_3, T_4, T_5, T_6 \text{ and } T_7)$ respectively.

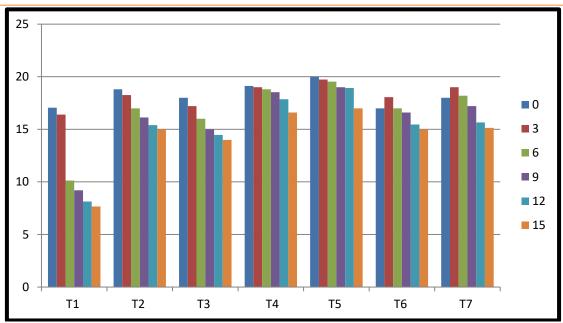


Figure (11): Degrees of the texture for luncheon treatments cooling for different periods

- All results in the figure are an average of fifteen replicates
- LSD $_{0.05}$ for the effect of the type of treatment on the degrees of the texture = 0.224
- LSD 0.05 for the effect of the duration of preservation in the degrees of the texture = 0.047
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the degrees of the texture = 0.291

The reason for the decrease in the degrees of texture is due to the decomposition of meat proteins and the liberation of nitrogenous bases that lead to the deterioration of the character of the texture and also due to the activity of proteolytic bacteria, but in general it was noted that the luncheon treatments (T_4 , T_5 , T_6 and T_7) maintained their texture during the preservation period and this is due to the fact that the botanical herbs (oregano and maui rose) act as materials that prevent self-degradation because they contain active compounds with biological activity, and thus have preserved the luncheon product (Rubio *et al.*, 2013).

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

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

The results agree with Shelbaya *et al.*, (2014) who studied the sensory characteristics including the texture in beef kofta to which marjoram powder was added, and noted that the scores for the texture quality decreased with the passage of the preservation period.

• Chewing characteristic:

Figure (12) indicated that there was an increase in the degree of chewiness among the different luncheon treatments (T_4 , T_5 , T_6 and T_7) with a continuous cooling period of (0, 3, 6, 9, 12, 15) days, compared to T_1 , T_2 and T_3 , as it was the scores for these treatments are (9.40, 10.00, 9.00, 9.33) respectively at the preservation period (0) days, and it was found that the degrees of chewiness increase with the increase in the percentage of herbal powder used in the luncheon treat and for all storage periods, if compared to the treatments (T_1 , T_2 and T_3) in which the degrees of chewing were (8.26, 8.93, 8.46) respectively at the preservation period (0) days, and after fifteen days of preservation, the degrees of chewing decreased to reach (4.00, 7.26, 5.00, 7.00, 8.00, 6.40, 6.90) for the treatments (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

It was found that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in maintaining the evaluation scores of chewing characteristics during the preservation period, due to the fact that the botanical herbs (oregano and maui rose) contain active compounds that give the product the characteristic of softness and ease of chewing in the mouth.

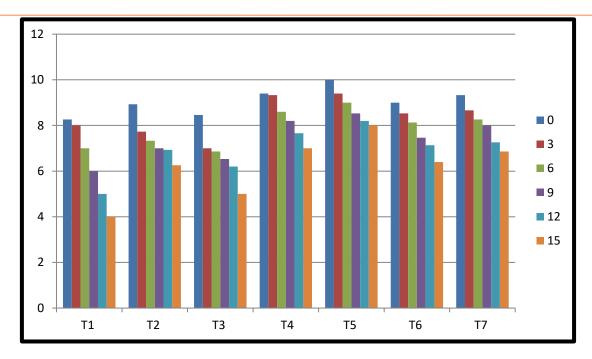


Figure (12): Degrees of the chewing for luncheon treatments cooling for different periods

- All results in the figure are an average of fifteen replicates
- LSD 0.05 for the effect of the type of treatment on the degrees of the chewing = 0.28
- LSD 0.05 for the effect of the duration of preservation in the degrees of the chewing = 0.47
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the degrees of the chewing = 0.75

The statistical results showed that there is a highly significant effect at the level of probability ($P \le 0.05$) for the type of treat and the duration of preservation and the interaction between them in the evaluation scores of the chewing characteristic of luncheon treats that have been refrigerated for different periods.

The results are consistent with (Al-Mayali, 2018) when using mixtures of spices to meat patties and cooling them for different periods of time, and the average degree of chewing was within (7-9) degrees.

• Uniformity of shape characteristic:

Figure (13) showed that there was an increase in the regularity of shape among the different luncheon treatments, namely (T_4 , T_5 , T_6 and T_7) with a continuous cooling period of (0, 3, 6, 9, 12, 15) days, which maintained the regularity of shape compared to other treatments T_1 , T_2 and T_3 , as the degrees of regularity of shape in these treatments were (19.00, 19.20, 18.33, 18.40) respectively at the storage period (0) days, and it was found that the degrees of regularity of shape increased with the increase in the percentage of herbal powder used in the luncheon treatments and for all durations Preservation, if compared to the treatments (T_1 , T_2 and T_3) in which the degrees of uniformity of shape were (17.06, 18.00, 17.40) respectively at the preservation period (0) days, and after fifteen days of preservation the degree of uniformity of shape decreased to reach to (9.40, 14.00, 13.00, 16.73, 17.00, 16.00, 16.40) for the for the treatments (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

The reason for this was due to the physical breakdown of the fleshy tissues due to the enzymatic and microbial action (Hamid, 2020).

The use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in maintaining the degrees of uniformity of shape.

The statistical results showed that there is a highly significant effect at the level of probability ($P \le 0.05$) for the type of treat and the duration of preservation and the interaction between them in the evaluation scores of the uniformity of the shape of the luncheon treats that were cooling for different periods.

The results are consistent with Al-Mayali (2018) when using mixtures of spices in the manufacture of meat patties and cooling them for different periods of time.

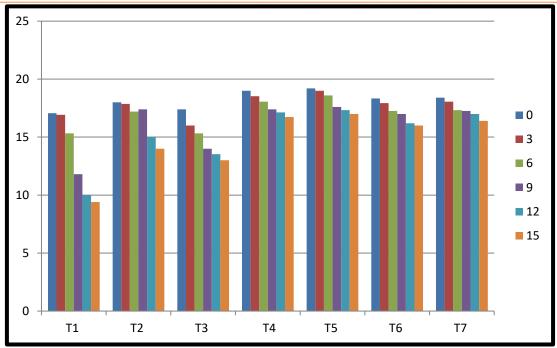


Figure (13): Degrees of the uniformity of shape for luncheon treatments cooling for different periods

- All results in the figure are an average of fifteen replicates
- LSD $_{0.05}$ for the effect of the type of treatment on the degrees of the uniformity of shape = 0.28
- LSD 0.05 for the effect of the duration of preservation in the degrees of the uniformity of shape = 0.43
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the degrees of the uniformity of shape = 0.71
 - 3- Bacterial indications of the luncheon product:

• Total Plate Count (TPC):

Figure (14) showed a decrease in the logarithm of the total number of bacteria among the different luncheon treatments (T_4 , T_5 , T_6 and 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 logarithm of the total number of bacteria in these treatments was (3.70, 3.69, 3.72, 3.71) C.F.U / g respectively at the storage period (0) days, and it was found that the logarithm of the total number of bacteria decreases with the increase in the percentage of herbal powder used in the luncheon treatments and for all periods of preservation, if compared with the treatments (T_1 , T_2 and T_3) that contained logarithms of the total number of bacteria (3.82, 3.73, 3.75) C.F.U / g respectively at the preservation period (0) days, and after fifteen days from preservation, the logarithm of the total number of bacteria increased to reach (5.38, 4.96, 5.13, 4.94, 4.93, 4.98, 4.95) C.F.U / g for the treatments (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

It was observed that there was a significant decrease in the luncheon treatments $(T_4, T_5, T_6 \text{ and } T_7)$ compared to the treatments $(T_1, T_2 \text{ and } T_3)$ due to the fact that plant spices and herbs are considered antioxidants because they contain phenolic compounds (Makki, 2021).

The study showed that the use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in reducing the logarithm of the total number of bacteria in luncheon treatments.

The statistical results showed that there was a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the overlap between them in the logarithm of the total number of bacteria for luncheon treatments that were cooling for different periods.

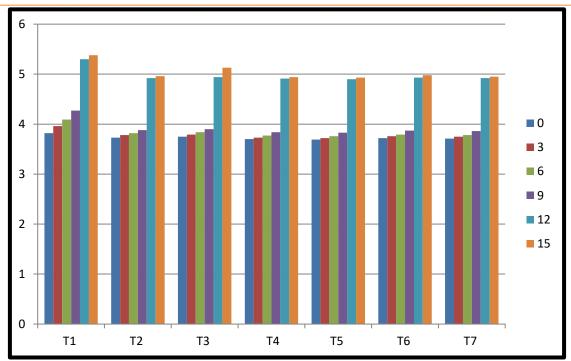


Figure (14): Logarithm of the total number of bacteria for luncheon treatments cooling for different periods

- All results in the figure are an average of two replicates
- \bullet LSD $_{0.05}$ for the effect of the type of treatment on the logarithm of the total number of bacteria = 0.006
- LSD 0.05 for the effect of the duration of preservation in the logarithm of the total number of bacteria = 0.050
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the logarithm of the total number of bacteria = 0.056

The results are consistent with Banon *et al.* (2012) who showed that the permissible limit of total bacterial numbers is 10⁷ C.F.U / g and with Al-Alwani *et al.* (2017) who noticed a significant decrease in the logarithm of the total number of bacteria, which increased with the progression of the preservation period compared to the control sample, and with Mashau *et al.* (2021) who added oleivera leaf powder to the minced meat and noticed a significant decrease in the total number of bacteria, and the number of bacteria increased with the increase in the period of refrigeration, as agreed with Terefe (2017) who showed an effect of adding ginger powder to the cooled minced beef, and noted a significant decrease occurred in the total number of bacteria in all treatments compared with the control sample, and the number of total bacteria decreased with the increase in the concentration of the powder, and one of the researchers indicated that the estimate of the total number of bacteria in meat and it's products was an indicator of the quality and quality of meat during storage and handling (Siraj, 2011).

• Psychrophilic Bacteria Count:

Figure (15) indicated that there was a decrease in the logarithm of psychrophilic bacteria among the different luncheon treatments (T_4 , T_5 , T_6 and 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 logarithm of the total number of bacteria in these treatments was (2.41, 2.40, 2.58, 2.56) C.F.U / g respectively at the storage period (0) days, and it was found that the logarithm of the psychrophilic bacteria decreases with the increase in the percentage of herbal powder used in the luncheon treatments and for all periods of preservation, if compared with the coefficients (T_1 , T_2 and T_3) that contained logarithms of thermophilic bacteria (2.72, 2.61, 2.64) C.F.U / g respectively at the preservation period (0) days, and after fifteen days from preservation, the logarithms of psychrophilic

bacteria increased to reach (4.12, 3.91, 3.92, 3.85, 3.83, 3.91, 3.90) C.F.U / g for the treatments (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

It was observed that there was a significant decrease in the number of psychrophilic bacteria for luncheon treatments (T_4 , T_5 , T_6 and T_7) compared to treatments (T_1 , T_2 and T_3) because the plant spices and herbs are characterized by their effectiveness as antioxidants and inhibitors of microbial growth (Govaris *et al.*, 2007).

The use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in reducing the logarithm of the psychrophilic bacteria in luncheon treatments.

The statistical results showed that there was a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatments and the duration of preservation in the logarithm of psychrophilic bacteria, while the interaction between them was insignificant in the effect of luncheon treatments that were kept in cooling for different periods.

The results are consistent with Karam and Al-Mosawi (2016) when they added rosemary and cumin powders to minced meat that had been refrigerated at 4 °C for periods of (0, 3, 6, 9, 12) days, and noticed a significant decrease in the numbers of psychrophilic, which increased with the progression of the preservation period.

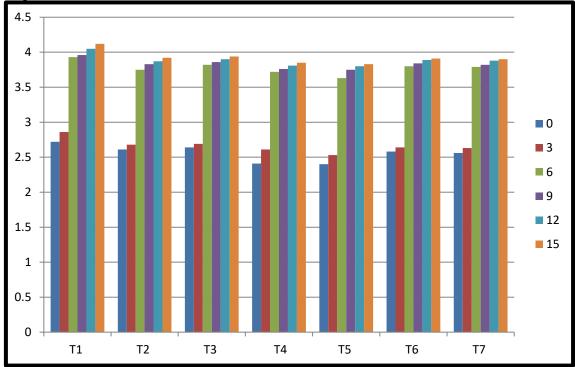


Figure (15): Logarithm of psychrophilic bacteria for luncheon treatments cooling for different periods

- All results in the figure are an average of two replicates
- LSD 0.05 for the effect of the type of treatment on the logarithm of psychrophilic bacteria = 0.004
- \bullet LSD 0.05 for the effect of the duration of preservation in the logarithm of psychrophilic bacteria = 0. 054
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the logarithm of psychrophilic bacteria = 0.058
 - Protolytic Bacteria:

Figure (16) illustrated the logarithm of protolytic bacteria among the different luncheon treatments, which are $(T_4, T_5, T_6 \text{ and } T_7)$ with a continuous cooling period of (0, 3, 6, 9, 12, 15) days compared to treatments $(T_1, T_2 \text{ and } T_3)$, as the logarithm of protolytic bacteria in these treatments were (3.76, 3.75, 3.78, 3.77) C.F.U / g respectively at the storage period (0) days, and it was found that the logarithm of the protolytic bacteria decreases with the increase in the percentage of herbal powder used in the luncheon treatments and for all preservation periods. if compared with the coefficients $(T_1, T_2 \text{ and } T_3)$ that contained logarithms for the number of proteolytic bacteria (3.79, 3.80, 3.81) C.F.U / g respectively, at the preservation period (0) days, and after fifteen days of preservation By cooling, the numbers of proteolytic bacteria increased to reach their

logarithms of (4.35, 4.07, 4.16, 3.95, 3.94, 3.98, 3.97) C.F.U / g for the treatments $(T_1, T_2, T_3, T_4, T_5, T_6)$ and $(T_1, T_2, T_3, T_4, T_5, T_6)$ and $(T_2, T_3, T_4, T_5, T_6)$ and (T_3, T_4, T_5, T_6) and (T_4, T_5, T_6, T_6) are respectively.

From the results, it was found that there was a significant decrease in the number of protolytic bacteria in the luncheon product treatments, due to the fact that spices and plant herbs contain effective compounds against bacteria that reduce their growth and inhibit their activity, and that the reason for the increase in the number of protolytic bacteria was the progression of the preservation period, as they are loving cold microorganisms, as most of these bacteria are the main cause of spoilage in refrigerated products, and they are able to grow and multiply in these conditions, causing spoilage and deterioration of products (Hamid, 2020).

The psychrophilic bacteria have an important role in raising the value of total volatile nitrogen during the preservation process as a result of their secretion of proteolytic enzymes, which leads to the decomposition of the proteins of the muscle tissue of meat and it's products (Al-Halfi *et al.*, 2017).

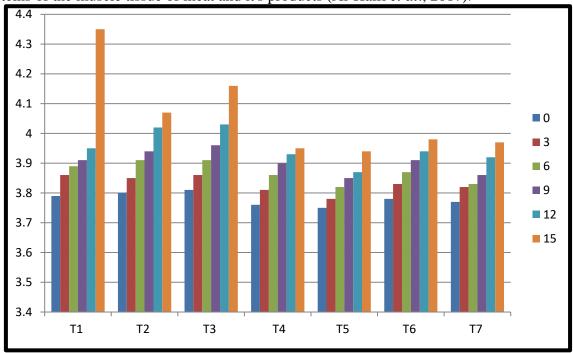


Figure (16): Logarithm of proteolytic bacteria for luncheon treatments cooling for different periods

- All results in the figure are an average of two replicates
- LSD $_{0.05}$ for the effect of the type of treatment on the logarithm of proteolytic bacteria = 0.002
- LSD 0.05 for the effect of the duration of preservation in the logarithm of proteolytic bacteria = 0.035
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the logarithm of proteolytic bacteria = 0.037

The use of oregano powder in two percentages (0.3 and 0.5) % was more effective in reducing the number of proteolytic bacteria in the cold-preserved luncheon product.

The statistical results showed that there was a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the logarithm of the numbers of proteolytic bacteria for luncheon treatments that were kept for different periods.

The results are consistent with Kumar and Tanwar (2011) who used clove powder in the preparation of chicken nuggets and kept them in refrigeration for periods of (0, 5, 10, 15) days, and noted a significant decrease in the number of proteolytic bacteria with an increase in their number with the progression of the preservation period.

• Lipolytic Bacteria:

Figure (17) shows that there was a decrease in the logarithm of lipolytic bacteria among the different luncheon treatments are $(T_4, T_5, T_6 \text{ and } T_7)$ 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 logarithms of the lipolytic bacteria in these treatments were (2.62, 2.60, 2.64, 2.63) C.F.U / g respectively at the storage period (0) days, and it was found that the logarithm of the number of lipolytic bacteria decreases with the increase in the percentage of herbal powder used in the treatment. Luncheon and for all periods of preservation, if compared to the treatments (T_1 , T_2 and T_3) that contained (3.68, 2.71, 2.74) C.F.U / g, respectively at the preservation period (0) days, and after fifteen days of refrigeration the logarithms of lipolytic bacteria increased to reach (4.05, 3.63, 3.94, 3.20, 2.87, 3.54, 3.03) C.F.U / g for the treatments (T_1 , T_2 , T_3 , T_4 , T_5 , T_6 and T_7) respectively.

The use of oregano powder in two percentages (0.3 and 0.5) % was more efficient in reducing the logarithms of the numbers of lipolytic bacteria for cold-preserved luncheon treatments, as the botanical herbs (oregano and maui rose) reduced the numbers of lipolytic bacteria because they contain active compounds that inhibit the activity of lipolytic bacteria. For fat in the secretion of lipolytic enzymes (lipases), while the reason for the increase in the number of lipolytic bacteria with the progression of the preservation period was due to the fact that lipolytic bacteria are psychrophilic organisms that grow at low temperatures (Al-Fadhli, 2009).

The statistical results showed that there was a highly significant effect at the level of probability ($P \le 0.05$) for the type of treatment and the duration of preservation and the interaction between them in the logarithm of the numbers of lipolytic bacteria for luncheon treats that were kept in refrigeration for different periods.

The results correspond with Al -Marazanya *et al.* (2009) and with Kumudavally *et al.* (2005) who concluded that spice powders (cloves, cinnamon and turmeric), ascorbic acid and acetic acid inhibited and reduced the number of lipolytic bacteria during refrigeration.

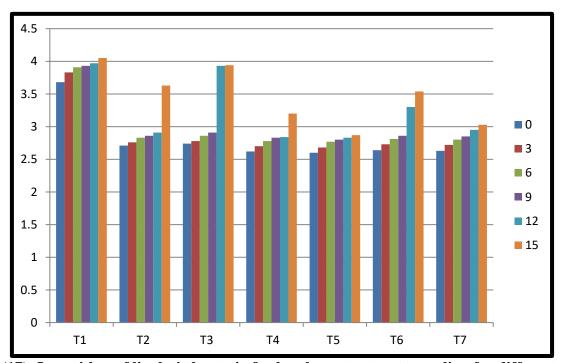


Figure (17): Logarithm of lipolytic bacteria for luncheon treatments cooling for different periods

- All results in the figure are an average of two replicates
- LSD 0.05 for the effect of the type of treatment on the logarithm of lipolytic bacteria = 0.005
- LSD 0.05 for the effect of the duration of preservation in the logarithm of lipolytic bacteria = 0.049
- LSD 0.05 for the effect of the binary interaction between the type of treatment and the duration of preservation in the logarithm of lipolytic bacteria = 0.054

• Total Coliform Bacteria Count:

The results in the current study showed that there was no growth of coliform bacteria in all treatments of the luncheon product and the control treatment for all preservation periods of (0, 3, 6, 9, 12, 15) days by refrigeration, and the results explain that the lack of growth of these bacteria in the luncheon product was

evidence of cleanliness and following the health conditions in the manufacturing and production process, which will positively affect the shelf life and quality of the product (Hamid, 2020).

The results agree with Al-Mayali (2018) when using powders of different flavors in refrigerating meat patties for different periods.

Detection of Salmonella and Shigella:

The results in the current study indicated that there was no growth of Salmonella and Shigella bacteria in all luncheon treatments and the control treatment for all preservation periods of (0, 3, 6, 9, 12, 15) days by refrigeration. These results explain that the lack of growth of these bacteria in the luncheon product was evidence of follow hygiene conditions during the manufacturing process, as well as the heat treatment used in cooking the luncheon product can be a reason for the absence of these microorganisms in the product, as these bacteria (Sammonella and Shigella) are among the types of bacteria that cause diseases to humans and lead to many problems when they are present in food.

The results are consistent with El-Hadidie *et al*, (2017) when manufacturing the luncheonette product, to which lupine seed flour was added in three proportions (30, 60, 100) % and noted that there was no growth of these types of bacteria as an indication of following good health and hygiene conditions during manufacturing.

The spices and vegetable herbs used when preserving or manufacturing meat products have a clear role in inhibiting the types of bacteria (Coli, Salmonella, and Shigella) that can grow on them because of the effective substances they contain, which are phenolic compounds of all kinds, which work to tear the cell membranes of microorganisms by forming a complex with protein or increase the activity of enzymes responsible for metabolic reactions by interfering with the protein and thus leading to its mutation and thus the inability of the bacteria to grow (Badi *et al.*, 2016).

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