

## Effect of Partial Replacement with vegetable sources on the microbial content of freeze-stored beef burger

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### Abstract

The study included the preparation of burger beef partial replacement with vegetable sources, including barley powder, oats and wheat bran. The study was conducted on five treatments and the first treatment was the control treatment (without replacement), the second treatment in which meat was replaced with vegetable sources at an average of (5%), and the third treatment in which meat was replaced by (10%) and the fourth treatment was replaced by (15%) and the fifth treatment had the replacement rate (20%). The product was stored in freezing ( $2\pm 18^\circ\text{C}$ ) for 120 days, during which the microbial tests of the prepared burger were followed up, which included the total count of aerobic bacteria and counting. Coliform Bacteria Total Count, Bacteria Psychrophilic of Total Count and Bacteria Staphylococcus of Total Count and the detection of Burger faecal coliform bacteria for periods (0, 30, 60, 90 and 120) days, and the results of the study were that the replacement rates and storage period had a significant ( $P<0.05$ ) effect on the microbial growth, as it was noted that the microbial growth decreased with an increase in the percentage of replacement and the progression of the period of freezing storage.

تأثير الاستبدال الجزئي بالمصادر النباتية على المحتوى الميكروبي لبيركر اللحم البقري المخزن بالتجميد

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المستخلص

تضمنت الدراسة تحضير بيركر اللحم البقري المستبدل جزئياً بمصادر نباتية شملت مسحوق الشعير والشوفان ونخالة الحنطة. أجريت الدراسة على خمسة معاملات وكانت المعاملة الاولى معاملة السيطرة (بدون استبدال) والمعاملة الثانية استبدال اللحم فيها بالمصادر النباتية بنسبة (5%) والمعاملة الثالثة استبدال اللحم فيها بنسبة (10%)، والمعاملة الرابعة تم الاستبدال فيها بنسبة (15%) والمعاملة الخامسة نسبة الاستبدال فيها (20%) خزن المنتج بالتجميد ( $2\pm 18^\circ\text{C}$ ) لمدة 120 يوم تم خلالها متابعة الفحوصات الميكروبية للبيركر المحضر والمتضمنة العد الكلي للبكتيريا الهوائية Bacteria Total Count of Aerobic، والعد الكلي لبكتيريا القولون Bacteria Psychrophilic of Total، والعد الكلي للمكورات العنقودية Bacteria Staphylococcus of Total، والكشف عن بكتيريا القولون البرازية Count والمعد (0 و 30 و 60 و 90 و 120) يوم وكانت نتائج الدراسة ان نسب الاستبدال ومدة الخزن اثرت معنوياً ( $P>0.05$ ) على النمو الميكروبي اذ لوحظ انخفاض النمو الميكروبي بزيادة نسبة الاستبدال وتقدم مدة الخزن بالتجميد.

### Introduction

In recent years, grains and their components have been accepted as functional foods, mainly due to the continuous promotion of dietary fiber, protein, energy, minerals, vitamins, and antioxidants required for human health. It has

been stated that carbohydrate-based fat substitutes from plant sugars such as fiber and starch can retain moisture and provide qualities that are normally provided by fats. These products have generally been produced by reshaping meats by incorporating healthy

components such as a variety of fibers, proteins, and polyunsaturated fatty acids (PUFA). The bioactive plant compounds include phenolic compounds, carotenoids, tocopherols, phytosterols, tocotrienols, and ferulic acid (7) and (20). Burger beef is an attractive meat product that is consumed by different age groups due to its ease of preparation and organoleptic properties which are very popular in many regions around the world. This product contains a high percentage of fat and amounts of sodium chloride in its composition. Excessive intake of fat and sodium is associated with diseases such as obesity, high blood fats, and high blood pressure. Therefore, reducing these components is important in order to produce healthy foods. Although Burger considers meat as a high-calorie food, It is possible to produce beef burger s with good quality raw materials and can be fortified with some components that can offer some functional properties (5 , 19). In view of the relationship between the quantity and quality of fat consumed on the one hand, and the widespread of heart disease, on the other hand, the consumer has become increasingly focused on buying foods that contain low levels of fat, especially meat products, and the abundance of vegetable sources in local markets and their low prices, such as barley, corn, oats, and others, and the large consumption of products Meat such as burger s, sausages, etc., due to the high prices of these products, in addition to the fact that eating too much affects health due to its high content of fat, cholesterol and animal protein, so we decided to conduct this study to manufacture meat products in which meat is partially replaced and in different proportions with some available plant sources and to study the effect of replacement ratios on microbial content during storage.

## Materials and methods

### meat

Beef veal was purchased from the thigh area from the local markets in Basra.

### frying oil

He used sunflower oil (the Aldaar type ) imported specifically for the Iraqi Ministry of Trade, a volume of 1 liter purchased from the local markets in Basra Governorate.

### The plant sources used in the study

In the study, barley grains and oat flakes obtained from local markets in Basrah province were used, and wheat bran obtained from grain mills in Basra province was used.

### Prepare plant sources

The barley grains were cleaned of impurities and ground using the electric grinder 28000 rpm and then sieved several times to get a fine powder. As for the oats, I used the ready-made Quaker oatmeal packets available in the local market. It was ground using an electric grinder until a fine powder was obtained. As for wheat bran, it was obtained from grain mills in Basrah, and it was ground and sieved several times to obtain a fine powder.

### Preparing the beef burger patties

10 kg of beef was minced with an electric chopping machine, its holes are 3 mm in diameter, and salt was added to it at 1.5% of the weight of the meat and a mixture of spices (a special mixture for the Burger product includes cinnamon, cloves, cubeba, nutmeg, ginger, , anise flavor, cardamom, Black pepper, white pepper and dried garlic) by 0.5%, black pepper by 0.5% and garlic by 0.25%. Fat was added at an average of 15% and divided into three sections at a rate of 3 kg for each section:

The first section: has been replaced by barley powder

Section Two: Replaced with oat powder

Section Three: Replaced with wheat bran powder

Each section was divided into five treatments, at an average of 600 g for each treatment, according to the proportions of replacement with plant sources, as follows:

First treatment: 0% meat replacement (control sample)

The second treatment: replace the meat by 5%

The third treatment: Replace the meat by 10%.

Fourth treatment: Replace meat by 15%.

Fifth treatment: Replace the meat by 20%.

Barker tablets were manufactured and then placed in bags of vacuumed polyethylene, separated from one tablet and another piece of wax paper, the bags closed well and stored by freezing ( $-18 \pm (2 \text{ m})$ ) for 120 days, during which the effect on microbial growth was monitored during storage periods 0, 30 and 60 And 90 and 120 days included total aerobic bacteria count, coliform count, staphylococcus count, cryptophilic count, and fecal coliform bacteria.

### **Total Count of Aerobic Bacteria**

The solid medium was used for counting the Nutrient Agar dishes. Transfer 1 ml of the appropriate dilutions to a Petri dish with a sterile pipette, then pour the medium after cooling to 45 °C and stir the dishes quietly for homogeneity and distribution well and left to harden. Then the Petri dish were inverted and incubated at 37 °C for 24 - 48 hours, and the number of bacterial colonies growing in the Petri dish was calculated according to the method (3).

### **Estimation of the number of coliform bacteria**

MacConkey Agar solid medium was used to estimate the number of coliform bacteria. 1 ml of the appropriate dilution was transferred to a Petri dish with a sterile pipette, then the medium was poured after cooling to 45°C. The Petri dish were moved quietly for homogeneity and distribution well and left to solidify, then the Petri dish were

inverted and incubated at a temperature of 37 °C for 24 - 48 hours, and the number of bacterial colonies growing in the Petri dish was calculated according to the method (3).

### **Estimation of the number of psychrophilic bacteria**

Use the Nutrient Agar solid medium. Transfer 1 ml of the appropriate dilutions to a Petri dish with a sterile pipette and pour the medium after cooling to 45°C. The Petri dish were stirred quietly for homogeneity and distribution well and left to solidify, then the Petri dish were inverted and incubated at a temperature of 5 °C for 5-7 days. The colonies growing on the medium were counted according to the method (3).

### **Estimation of the numbers of Staphylococcus bacteria**

Manitol Salt Agar solid medium was used to estimate the number of staphylococcal bacteria. 1 ml of the appropriate dilution was transferred to a Petri dish with a sterile pipette, then the medium was poured after cooling to 45°C and the plates were moved quietly for homogeneity and distribution well and left to harden. Then the Petri dishes were inverted and incubated at 37 °C for 24 - 48 hours, and the number of bacterial colonies growing in the Petri dish was calculated according to the method (3).

### **Bacteria Faecal Coliform of detection**

The Most Probable Number (MPN) method was used, using 3 tubes for each dilution, and inside each one of them was a dirham tube. The result is calculated as positive for the tubes in which gas is formed. MacConkey broth medium was used and incubated at a temperature of 45.5 for a period of 48-24 hours according to method (2).

### **Statistical Analysis**

Factorial Experiment Designing (CRD) was used to analyze all the studied factors as they were

statistically analyzed by means of SPSS Version24 (2016) and these factors were tested using the modified least significant difference test (R.L.S.D) at a probabilistic level ( $p < 0.05$ )).

## Results and discussion

### Total aerobic bacteria count

The results in Table (1) showed that there was a significant effect ( $P < 0.05$ ) of the vegetable source on the total number of aerobic bacteria in Burger beef partially replaced with vegetable sources. The total number of aerobic bacteria decreased in all treatments replaced with plant sources compared to the control treatment. It was also noticed from the statistical results a significant ( $P < 0.05$ ) decrease of the total number of aerobic bacteria by increasing the proportion of replacement, where the total number of aerobic bacteria in Burger meat partially replaced with barley powder (Table 1) decreased from  $15 \times 10^3$  W.m.m/gm in the control treatment. To  $10 \times 10^3$  W.M.M/g at the replacement rate of 5%, then it reached  $8 \times 10^2$  W.M.m/g at the 20% replacement rate.  $13 \times 10^3$  and  $11 \times 10^3$  WM/g at a replacement rate of 5% to  $9 \times 10^2$  and M.M/g at a replacement rate of 20%, respectively. The results agreed with what was mentioned by (27) when they studied the effect of partial substitution of some plant sources on the qualitative and sensory characteristics of burger tablets. The factory-made from the meat of aged goose, where they found that the numbers of aerobic bacteria in the treatments replaced by oats, wheat embryo, and rice reached the logarithm of bacterial numbers 5.1, 4.9, and 5.2 W.m.m/g respectively, where for the control treatment, the logarithm of bacterial numbers reached 4.9 W.m.m/gm. The reason for the increase the number of aerobic bacteria may be due to the role of replacing with plant sources in lowering the acidity of the factory burger and thus increasing the number of bacteria (12)

It also agreed with (26) in their study of the effect of adding wheat bran and dried carrot residues on the qualitative characteristics of chicken patties, as they noticed a decrease in the total number of bacteria in the treatment to which wheat bran was added to 2.50 W.m.m/g compared to the treatment with dried carrot residues added and the control treatment, which was 2.69 and 2.65 mM/g, respectively. It also agreed with (13) when they studied the effect of adding different amounts of rice bran on the qualitative traits of burger meat, including the microbiological quality criteria for different burger meat mixtures. They observed a decrease in the total number of bacteria in burger meat with an increase in the level of replacement with rice bran in the treatments compared to the control treatment. The number of bacteria decreased in the treatments to which rice bran was added at levels of 50 g, 100 g, and 125 g to 4.25, 3.88, and 3.26 Mm/gm for the above three levels, respectively, while in the control treatment it was 4.69 Mm/gm. The results indicated that the storage period had a significant effect ( $P < 0.05$ ) on the total number of aerobic bacteria, where it was noticed that the total number of aerobic bacteria decreased with the progression of the storage period. As the freezing process leads to the destruction of the bacterial cell walls, and thus the number of bacteria decreases and their presence may disappear with the progression of storage period (8 and 17), as it decreased in the control treatment from  $15 \times 10^3$  Wm/gm before storage to  $5 \times 10^2$  Wm/gm before storage. /gm after 120 days of freezing storage, while in Berger partially replaced with barley, oats and wheat bran powder at 5% replacement level, it decreased from  $103 \times 10^3$ ,  $11 \times 10^3$  and  $13 \times 10^3$  W.M/gm before storage and no growth appeared at the end Freezing storage period. She agreed with (1) in his study about the effect of different levels of Moringa seed flour on the quality of beef burger at levels of 2%, 4% and 6%, as they noticed a decrease in the total number of bacteria to 5.45, 4.83, and 3.84 mM/g on the straight after 21 days of

storage. The variation in bacterial growth between the control treatment and the treatments to which Moringa seed flour was added during the storage period could be attributed to the different pH values, which were affected by the flour level of Moringa seeds. It was found that the triple interaction between the plant source, replacement percentages and storage period had a significant effect ( $P < 0.05$ ) on the total number of aerobic bacteria, as it was noticed that the total number of aerobic bacteria decreased with an increase in the replacement rates and the storage period progressed in all treatments.

It also agreed with (26) in their study of the effect of adding wheat bran and dried carrot residues on the qualitative characteristics of chicken patties, as they noticed a decrease in the total number of bacteria in the treatment to which wheat bran was added to 2.50 W.m.m/g compared to the treatment with dried carrot residues added and the control treatment, which was 2.69 and 2.65 mM/g, respectively. It also agreed with (13) when they studied the effect of adding different amounts of rice bran on the qualitative traits of burger meat, including the microbiological quality criteria for different burger meat mixtures. They observed a decrease in the total number of bacteria in burger meat with an increase in the level of replacement with rice bran in the treatments compared to the control treatment. The number of bacteria decreased in the treatments to which rice bran was added at levels of 50 g, 100 g, and 125 g to 4.25, 3.88, and 3.26 Mm/gm for the above three levels, respectively, while in the control treatment it was 4.69 Mm/gm. The results

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**Table (1) Total aerobic bacteria count in burger meat partially replaced with vegetable sources: A-barley, B-oats, C-wheat bran during storage at (2±18-)C for 120 days**

Treatments	replacement percentage	bacterial numbers				
	Storage period (day)	w.m.m/gm				
		0%	5%	10%	5%1	20%
A	0	$15 \times 10^3$	$10 \times 10^3$	$7 \times 10^3$	$3 \times 10^3$	$8 \times 10^2$
	30	$10 \times 10^3$	$6 \times 10^3$	$2 \times 10^3$	$8 \times 10^2$	$5 \times 10^2$
	60	$3 \times 10^3$	$12 \times 10^2$	$4 \times 10^2$	$2 \times 10^2$	ND
	90	$12 \times 10^2$	$8 \times 10^2$	$2 \times 10^2$	ND	ND
	120	$5 \times 10^2$	$1 \times 10^2$	ND	ND	ND
B	0	$15 \times 10^3$	$13 \times 10^3$	$8 \times 10^3$	$4 \times 10^3$	$9 \times 10^2$
	30	$10 \times 10^3$	$9 \times 10^3$	$2 \times 10^3$	$11 \times 10^2$	$7 \times 10^2$
	60	$3 \times 10^3$	$13 \times 10^2$	$8 \times 10^2$	$4 \times 10^2$	$1 \times 10^2$
	90	$12 \times 10^2$	$9 \times 10^2$	$3 \times 10^2$	$2 \times 10^2$	ND
	120	$51 \times 10^2$	$2 \times 10^2$	ND	ND	ND
C	0	$15 \times 10^3$	$11 \times 10^3$	$8 \times 10^3$	$3 \times 10^3$	$9 \times 10^2$
	30	$10 \times 10^3$	$7 \times 10^3$	$3 \times 10^3$	$7 \times 10^2$	$6 \times 10^2$
	60	$3 \times 10^3$	$16 \times 10^2$	$11 \times 10^2$	$5 \times 10^2$	$2 \times 10^2$
	90	$12 \times 10^2$	$11 \times 10^2$	$4 \times 10^2$	$2 \times 10^2$	ND
	120	$5 \times 10^2$	$3 \times 10^2$	$1 \times 10^2$	ND	ND

**R.L.S.D for the effect of the interaction between the plant source, replacement percentage and storage period: 0.14416 coliform bacteria total**

The results in Table (2) show that the total number of bacteria in the beef burger that was partially replaced with vegetable sources was significantly ( $P < 0.05$ ) affected by the vegetable source, as it was noticed that the total number of bacteria in the burger prepared for all treatments compared to the control treatment, The replacement ratios also had a significant

( $P < 0.05$ ) effect on the total number of colon bacteria, where the number of bacteria in the burger decreased with an increase in the percentage of replacement. When replacing 15%, the total number of bacteria decreased to  $1 \times 10^2$  m.m/g when replaced with barley powder (Table A) and when replacing with oat powder (Table B), it decreased from  $3 \times 10^2$  WMM/g to  $1 \times 10^2$  WMM/g when replaced by 15%. When replaced with wheat bran powder (Table C), it decreased

from  $3 \times 10^2$  mm/gm to  $2 \times 10^2$  mm/g when replaced by 15%, and no bacterial growth appeared at 20% replacement rate in all treatments. In the control treatment, it was  $5 \times 10^2$  W.M.M/g. The reason for the decrease is due to the fact that plant sources contain effective compounds that have anti-microbial activity, such as phenolic compounds, phenolic acids, carotenoids and flavonoids (22 and 14). These compounds reduce the internal pH of microbial cells by ionizing acid molecules and causing a defect in the transport of reactants through changing the cell membrane permeability, deterioration of the cell wall, destruction of the cytoplasmic membrane, and damage to membrane proteins, which leads to interference with the enzymes embedded in the membrane, ultimately leading to cell death (23 and 11) It agreed with (13) when they studied the effect of adding different amounts of rice bran on the qualitative characteristics of burger meat discs, as it was 2.72 W.m.m/g in the control treatment. In the treatments to which rice bran was added at levels of 50 g, 100 g, and 125 g, it was 2.67, 2.52, and 2.28 mm m/g respectively. The decrease in the number of bacteria with an increase in the level of replacement with bran fibers is attributed to the decrease in the free water due to the high water-binding capacity of the bran fibers (18).

The results agreed with (9) when studying the microbial and antioxidant properties of chilled chicken patties treated with Moringa leaf powder at levels 50% and 100%. As they noticed a decrease in the number of *E. coli* bacteria in the sample to which Moringa leaf powder was added at a level of 50%, it was 2.56 W.M.m/gm with its absence in the sample to which Moringa leaf powder was added at a level of 100%. As for the control treatment, the number of bacteria was 2.78 W.m.m/gm, and (27) found in their study the effect of partial substitution of some plant sources on the qualitative and sensory traits of burger tablets made from aged goose meat. A decrease in the number of *E. coli* bacteria in the

partially substituted treatment with wheat embryo to 4.1 W.M.M/gm compared to the treatments replaced with oats and rice, which amounted to 4.2 and 4.6 W.M.M/gm and in the control treatment it was 4.2 W.M.M/gm. The reason for the decrease in the number of bacteria in the treatment replaced with wheat embryos is due to the presence of a high percentage of flavonoid compounds that inhibit the growth of large numbers of bacteria (2). The results also showed that the storage period had a significant effect ( $P < 0.05$ ) on the total number of *E. coli* bacteria. It was observed that the total number of bacteria decreased in all the replaced treatments with the progression of the storage period, and in the control treatment the number of bacteria decreased from  $5 \times 10^2$  m.m.m/gm before storage to  $2 \times 10^2$  for m.m.m/gm after 30 days of freezing storage. After 60 days, no bacterial growth appeared. In the burger replaced with barley, oats and bran powder at 5%, the number of bacteria decreased from  $4 \times 10^2$ ,  $3 \times 10^2$  and  $3 \times 10^2$  mm/g before storage to  $1 \times 10^2$  mm/g after 30 days in table A. As for Tables B and C, no bacterial growth appeared after 30 days, and so on for all replacement percentages. The results agreed with (9) who found that the decrease in the number of bacteria in the samples to which Moringa leaf powder was added at the level of 50% and 100% during the storage period to the active compounds in the powder of Moringa leaves such as polyphenols. The flavonoids and these compounds have antimicrobial activity. The results showed that there was a significant effect ( $P < 0.05$ ) of the interaction between the plant source, replacement percentage and storage period on the total number of colon bacteria, where it was noticed that the total number of colon bacteria decreased with the progression of the storage period and the increase in the replacement average in the partially replaced birker with vegetable sources.

**Table (2) Total count of coliform bacteria in burger meat partially replaced with vegetable sources: A-barley, B-oats, C-wheat bran during storage at a temperature of (2±18-)C for 120 days**

Treatments	replacement percentage	bacterial numbers				
	Storage period (day)	w.m.m/gm				
		0%	5%	10%	5%1	20%
A	0	$5 \times 10^2$	$4 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$	ND
	30	$2 \times 10^2$	$1 \times 10^2$	ND	ND	ND
	60	ND	ND	ND	ND	ND
	90	ND	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND
B	0	$5 \times 10^2$	$3 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$	ND
	30	$2 \times 10^2$	ND	ND	ND	ND
	60	ND	ND	ND	ND	ND
	90	ND	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND
C	0	$5 \times 10^2$	$3 \times 10^2$	$2 \times 10^2$	$2 \times 10^2$	ND
	30	$2 \times 10^2$	ND	ND	ND	ND
	60	ND	ND	ND	ND	ND
	90	ND	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND

R.L.S.D for the effect of the interaction between the plant source, replacement percentage and storage period: 0.06993

### Total count of cryptophilic bacteria

The results in Table (3) showed that the plant source had a significant effect ( $P < 0.05$ ) on the total number of cryptophilic bacteria in beef burger, where the total number of cryptophilic bacteria was decreased in the prepared birker and for all treatments compared to the control treatment. The decrease was more obvious when

replacing with wheat bran powder (Table C) due to the presence of phenolic compounds in the wheat bran that have antimicrobial activity (15), and the total number of cryptophilic bacteria significantly ( $P < 0.05$ ) decreased with an increase in the replacement percentage, where the number in the burger decreased. Meat partially replaced with wheat bran powder (Table C) from  $51 \times 10^2$  WMM/gm in the control treatment



(without replacement) to  $32 \times 10^2$  WMM/gm at 5% replacement rate, then it reached  $102 \times 17$  and  $9 \times 10^2$  and  $5 \times 10^2$  W.M.M/gm at the replacement ratios of 10%, 15% and 20%, respectively, followed by the partially replaced burger oatmeal (Table B) which reached  $102 \times 37$ ,  $102 \times 18$ ,  $102 \times 11$  and  $102 \times 7$ . W.M.M/gm and in burger partially replaced by barley powder (Table A), the number decreased to  $41 \times 10^2$ ,  $21 \times 10^2$ ,  $13 \times 10^2$ ,  $9 \times 10^2$ , M.M/g at replacement rates of 5%, 10%, 15% and 20%, respectively. agreed with (26) in their study of the effect of adding wheat bran and dried carrot residues by 6% on the qualitative characteristics of chicken patties. It had dried carrot residues and the control treatment was 1.60 and 1.47 W.m.m/gm. Also, (13) when they studied the effect of adding different amounts of rice bran on the qualitative characteristics of burger meat patties, a decrease in the number of cryophilic bacteria was noticed by an increase in the level of rice bran added compared to the control sample. The logarithm of the number of bacteria decreased in the treatments to which rice bran was added by rates of 50gm, 100gm and 125gm to 3.14, 2.85, and 2.62 Wmm/gm respectively, while in the control treatment it was 3.30 Wmm/gm. It was observed through the results that there was a significant effect ( $P < 0.05$ ) for the storage period on the total number of aerobic bacteria, where it was noticed that the total number of aerobic bacteria decreased with the progression of the storage period, where it decreased in the control treatment from  $102 \times 51$  before storage to  $26 \times 10^2$  and  $13 \times 10^2$  and  $5 \times 10^2$  W.M.M/gm during 30, 60 and 90 days of freezing storage and no growth appeared after 120 days of storage. In the partially replaced burger with wheat bran powder it decreased from  $32 \times 10^2$  before storage to  $10 \times 10^2$  and  $3 \times 10^2$  W.M.M/gm during 30 and 60 days of freezing storage and no growth appeared at 90 and 120 days and in the partially replaced burger with oatmeal powder and barley at 5% replacement rate decreased from  $37 \times 10^2$  and  $41 \times 10^2$  mm/g before storage to  $5 \times 10^2$  and  $7 \times 10^2$  mmm/g after 60 days in a row, and no

growth appeared after 120 days. from storage. The results showed that there was a significant effect ( $P < 0.05$ ) of the triple interaction between the plant source, replacement ratios and storage period on the total number of C.

### Partially with vegetable sources

It was noticed that the total number of cryophilic bacteria decreased with the progression of the storage period and the increase in the percentage of replacement in all treatments.

### Total count of staphylococcus bacteria

The results in Table (4) indicate that the total number of staphylococcal bacteria in burger beef partially substituted with vegetable sources was significantly ( $P < 0.05$ ) affected by the vegetable source.

The total number of bacteria decreased in all treatments in which meat was partially replaced compared to the control treatment, and the decrease was more pronounced when replacing with wheat bran (Table C). The results also showed that the replacement rates had a significant effect ( $P < 0.05$ ) on the total number of staphylococcus bacteria, as the number of bacteria decreased with an increase in the percentage of replacement in the partially replaced burger with wheat bran powder (Table C) from  $68 \times 10^2$  m.m.m/g in the treatment The control reached  $45 \times 10^2$  and  $17 \times 10^2$  W.M.M/gm at the replacement rates of 5% and 10%, then it reached  $8 \times 10^2$  and  $3 \times 10^2$  W.M.M/gm at the replacement rates of 15% and 20%, respectively and in burger partially replaced by oatmeal (Table B), it decreased from  $54 \times 10^2$  W.M.M/g at the replacement rate of 5% to  $29 \times 10^2$  and  $15 \times 10^2$  and  $20 \times 10^2$ , respectively, but in burger partially replaced by barley powder (Table A), they were  $63 \times 10^2$ ,  $40 \times 10^2$ ,  $19 \times 10^2$ ,  $12 \times 10^2$ , and m/g at the replacement rates of 5%, 10, and 15 % and 20%, respectively, The results agreed with (16) when they studied the microbial and antioxidant properties of

**Table (3) Total count of Cryophilic bacteria in burger meat partially replaced with vegetable sources: A-barley, B-oats, C-wheat bran during storage at a temperature of (2±18-)C for 120 days**

Treatments	replacement percentage	bacterial numbers				
	Storage period (day)	w.m.m/gm				
		0%	5%	10%	5%1	20%
A	0	$51 \times 10^2$	$41 \times 10^2$	$21 \times 10^2$	$13 \times 10^2$	$9 \times 10^2$
	30	$26 \times 10^2$	$16 \times 10^2$	$11 \times 10^2$	$6 \times 10^2$	$4 \times 10^2$
	60	$13 \times 10^2$	$7 \times 10^2$	$4 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$
	90	$5 \times 10^2$	$1 \times 10^2$	ND	ND	ND
	120	ND	ND	ND	ND	ND
B	0	$51 \times 10^2$	$37 \times 10^2$	$18 \times 10^2$	$11 \times 10^2$	$7 \times 10^2$
	30	$26 \times 10^2$	$13 \times 10^2$	$8 \times 10^2$	$5 \times 10^2$	$2 \times 10^2$
	60	$13 \times 10^2$	$5 \times 10^2$	$3 \times 10^2$	$1 \times 10^2$	ND
	90	$5 \times 10^2$	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND
C	0	$51 \times 10^2$	$32 \times 10^2$	$17 \times 10^2$	$9 \times 10^2$	$5 \times 10^2$
	30	$26 \times 10^2$	$10 \times 10^2$	$7 \times 10^2$	$5 \times 10^2$	$1 \times 10^2$
	60	$13 \times 10^2$	$3 \times 10^2$	$2 \times 10^2$	ND	ND
	90	$5 \times 10^2$	ND	ND	ND	ND
	120	ND	ND	ND	ND	ND

**R.L.S.D for the effect of the interaction between the plant source, replacement ratios and storage period: 0.15073**

refrigerated chicken patties combined with moringa leaf powder at levels 50%, 100% that the numbers of bacteria *S. auerus*. Significantly affected by treatments and storage period, as the logarithm of the number of *S. auerus* bacteria in the control treatment was significantly higher than the sample added to Moringa leaf powder at the level of 50%, it reached 2.72 W.m.m/g. As for the treatment to which Moringa leaf powder

was added at the above level, it was 2.52 W.M.M/gm, noting the absence of these bacteria in the treatment to which Moringa leaf powder was added at the level of 100%. It was shown (6) that Moringa leaves contain effective compounds that limit the growth of *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Enterobacter aerogenes*. The total number of staphylococcal bacteria was significantly

( $P < 0.05$ ) affected by the storage period, where it was noticed that the total number of bacteria decreased with the progression of the storage period. Burger partially replaced with barley powder, oats and wheat bran decreased to  $9 \times 10^2$ ,  $5 \times 10^2$ ,  $3 \times 10^2$  and m.m/g after 90 days of freeze storage, respectively. The results of the statistical analysis indicated that there was a significant effect ( $P < 0.05$ ) of the interaction

between the plant source, replacement ratios and storage period on the total number of *Staphylococcus* bacteria in Burger meat Bovine partially replaced with vegetable sources, as it was noticed that the total number of bacteria decreased with the progression of the storage period and the increase in the percentage of replacement in all treatments.

**Table (4) Total count of *Staphylococcus aureus* in Burger meat partially replaced with vegetable sources: A-barley, B-oats, C-wheat bran during storage at a temperature of ( $2 \pm 18$ -)C for 120 days**

Treatments	replacement percentage	bacterial numbers				
	Storage period (day)	w.m.m/gm				
		0%	5%	10%	5%1	20%
A	0	$68 \times 10^2$	$63 \times 10^2$	$40 \times 10^2$	$19 \times 10^2$	$12 \times 10^2$
	30	$38 \times 10^2$	$32 \times 10^2$	$20 \times 10^2$	$11 \times 10^2$	$8 \times 10^2$
	60	$19 \times 10^2$	$15 \times 10^2$	$12 \times 10^2$	$6 \times 10^2$	$3 \times 10^2$
	90	$11 \times 10^2$	$9 \times 10^2$	$3 \times 10^2$	$2 \times 10^2$	ND
	120	$5 \times 10^2$	$3 \times 10^2$	ND	ND	ND
B	0	$68 \times 10^2$	$54 \times 10^2$	$29 \times 10^2$	$16 \times 10^2$	$9 \times 10^2$
	30	$38 \times 10^2$	$25 \times 10^2$	$18 \times 10^2$	$8 \times 10^2$	$5 \times 10^2$
	60	$19 \times 10^2$	$12 \times 10^2$	$9 \times 10^2$	$3 \times 10^2$	$1 \times 10^2$
	90	$11 \times 10^2$	$5 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$	ND
	120	$5 \times 10^2$	$1 \times 10^2$	ND	ND	ND
C	0	$68 \times 10^2$	$45 \times 10^2$	$17 \times 10^2$	$8 \times 10^2$	$3 \times 10^2$
	30	$38 \times 10^2$	$18 \times 10^2$	$10 \times 10^2$	$3 \times 10^2$	$1 \times 10^2$
	60	$19 \times 10^2$	$7 \times 10^2$	$3 \times 10^2$	$1 \times 10^2$	ND
	90	$11 \times 10^2$	$2 \times 10^2$	$1 \times 10^2$	ND	ND
	120	$5 \times 10^2$	ND	ND	ND	ND

## Detection of Faecal Coliform Bacteria

The study showed the absence of *Escherichia coli* in the control treatment and the partially replaced birker with vegetable sources, including barley powder, oats and wheat bran during periods of 0, 30, 60, 90 and 120 days of frozen storage. The results agreed with (21) when they studied the microbial properties of some Meat products, as it was noted that bacteria were not detected in chicken luncheon meat and meat Burger , as it agreed with what was found (25) and (10).

## Conclusions

The results showed that the microbial growth decreased with an increase in the replacement rates and the storage period progressed, and no growth of fecal coliform bacteria appeared.

## References

- 1- Al-Juhaimi, F.; Ghafoor, K.; Hawashin, M.D.; Alsawmahi, O.N. and Babiker, E. (2015). Effects of different levels of Moringa (*Moringa oleifera*) seed flour on quality attributes of beef burgers. *CyTA-Journal of Food*. <http://dx.doi.org/10.1080/19476337.2015.1034784>
- 2- Amali, U. ; Alahakoon, D. ; Dinesh, Y. ; Hae, I. ; Young, B. ; Jin, K. H. ; Sung, M. S. ; Kyung, L. H. and Cheorun, J. (2014). Effects of different natural antimicrobial agents on marinated chicken breast during storage at different temperatures. *Korean J. Food & Nutr*. 27(2):164-174.
- 3- Andrews, W. (1997). Manuals of food quality control. 4. Microbiological analysis. FAO: Food and Nutrition paper 14/4. Rev. 1, Rome, Italy. 338p.
- 4- A.P.H.A. (1992). American Public Health Association. Compendium of Methods for the Microbiological Examination of Food. 3rd ed., Washington, D. C. New York.
- 5- Bhat, Z.; Morton, J. D.; Mason, S. L. and Bekhit, A. E. D. A. (2019). Pulsed electric field improved protein digestion of beef during *in-vitro* gastrointestinal simulation. *LWT – Food Science and Technology*, 102: 45–51.
- 6- Bukar, A. ; Uba, A. ; and Oyeyi, T.I. (2010). Antimicrobial profile of *Moringa oleifera* lam. Extracts against some food-borne microorganisms. *Bayero Journal of Pure and Applied Sciences*. 3: 43–48. doi:10.4314/bajopas.v3i1.58706.
- 7- Carvalho, L.T. ; Pires, M.A. ; Baldin, J.C. ; Munekata, P.E.; Carvalho, F.A.; Rodrigues, I.; Polizer, Y.J.; Mello, L.M.; Lapa-Guimarães, J. and Trindade, M.A. (2018). Partial replacement of meat and fat with hydrated wheat fiber in beef burgers decreases caloric value without reducing the feeling of satiety after consumption. *Meat Science*, doi:10.1016/j.meatsci.2018.08.010.
- 8- Charoenrein, S. (2018). Microstructural changes and their relationship with quality and stability of frozen foods. In *Food microstructure and its relationship with quality and stability*, (pp. 123–138). Elsevier.
- 9- Elhadi, D.A.; Elgasim, E.A. and Ahmed, I.A. (2017). Microbial and oxidation characteristics of refrigerated chicken patty incorporated with moringa (*Moringa oleifera*) leaf powder. *CYTA – JOURNAL OF FOOD*. 15 (2): 234–240.
- 10- FSAI (2013). Food Safety Authority of Ireland: Microbiological safety of raw minced beef and beef burgers on retail sale in Ireland. (11NS1).
- 11- Haque, F.; Rahman, M.H.; Habib, M.; Alam, M. S.; Monir, M. M. and Hossain, M. M. (2020). Effect of different levels of orange peel extract on the quality and shelf life of beef muscle during frozen storage. *IOSR*

- Journal of Agriculture and Veterinary Science**, 13: 43-56 pp.
- 12- **Hutton , M .T. ; Koskinen, M . A .and Hanlin, J. H . (1991).** Interacting effects of pH and NaCl on meat resistance of bacteria spores . **J.Food Sci .**, 56 : 821-824 .
  - 13- **Ibrahim, M.I.; Hegazy, A.I. and El-Waseif, M.A.(2015).** Effect of Replacing Beef Fat with Flaxseed Oil and Rice Bran on Nutritional Quality Criteria of Beef Burger Patties. **Middle East Journal of Applied Sciences**. 645-655.
  - 14- **Junejo, S.A.; Zhang, L.; Yang, L.; Wang, N.; Zhou, Y.; Xia, Y. and Wang, H.(2018).**Anti-hyperlipidemic and hepatoprotective properties of wheat bran with different particle sizes. **J. Sci. Food Agr.**, 99:1990-1996.
  - 15- **Junejo, S.A ; Geng, H; Li, S; Kaka,A.K; Rashid,A and Zhou, Y.(2019).** Superfine wheat bran improves the hyperglycemic and hyperlipidemic properties in a high-fat rat model. **Food Sci Biotechnol**. <https://doi.org/10.1007/s10068-019-00684-8>.
  - 16- **Mahmoud,M.H.; Abou-Arab, A.A. and Abu-Salem, F.M.(2017).**Quality Characteristics of Beef Burger as Influenced by Different Levels of Orange Peel Powder.**American Journal of Food Technology**.12(4):262-270.
  - 17- **Mohammed,H.H.H.; He,L.; Nawaz,A.; Jin,G.; Huang, X.; Ma,M.; Abdegadir,W.S.; Elgasim,E.A.and Khalifa,I.(2021).** Effect of frozen and refrozen storage of beef and chicken meats on inoculated microorganisms and meat quality. **Meat Science**, 175 :108453. DOI: 10.1016/j.meatsci.2021.108453.
  - 18- **Oroszvári, B. K.; Sjöholm, I. and Tornberg, E. (2006).**The mechanisms controlling heat and mass transfer on frying beef burgers. 1. The influence of the composition and comminution of meat raw material. **J. Food Eng**, 67: 499 - 506.
  - 19- **Pietrasik, Z.; Sigvaldson, M.; Soladoye, O.P. and Gaudette, N.J.(2020).** Utilization of pea starch and fibre fractions for replacement of wheat crumb in beef burgers. **Meat Science**. <https://doi.org/10.1016/j.meatsci.2019.107974> .
  - 20- **Prasadi, N.V.P. and Joye, I.J.(2020).** Dietary fibre from whole grains and their benefits on metabolic health. **Nutrients**. 3045(12):2-20. doi:10.3390/nu12103045.
  - 21- **Ragab, M. M.; Toliba, A.O.; Galal,G .A. and Abo Elmaaty, S.M.(2019).** Physicochemical and microbiological proprieties of some meat products in Sharkia Governorate,Egypt. **Zagazig J. Agric. Res.**, 46 (1):81-90.
  - 22- **Schieber, A.; Stintzing, F.C.and Carle, R.(2001).** By-products of plant food processing as a source of functional compounds recent developments. **Trends in Food Science and Technology**, 12:401–13.
  - 23- **Shan, B.; Cai,Y.; Brooks,J. and Corke, H. (2007).**The in vitro antibacterial activity of dietary spice and medicinal herb extracts. **Int.J. Food Microbiology**, 117: 112 - 119.
  - 24- **SPSS (2016).** Statistical package for social science, version 24. Users guide for statistical, Chicago. USA.
  - 25- **Stagnitta, P.V.; Micalizzi, B. and de Guzman, A.M.S. (2006).** Prevalence of some bacteria Yeasts and Molds in meat foods in San Luis, Argentina. **Cent. Eur. J. Publ. Health**, 14 (3):141–144.
  - 26- **Yadav, S.; Pathera , A.K.; Ul Islam, R.; Malik, A.K. and Sharma, D.P.(2018).** Effect of wheat bran and dried carrot pomace addition on quality characteristics of chicken sausage. **Asian-Australas J. Anim. Sci.**,31( 5):729-737.
  - 27- **Zangana,B.S.R.and Al-Shamri,J.S.H.(2016).** Effect of partial substitution of some plant sources on the qualitative and sensory characteristics of burger made from aged goose meat. **Iraqi Journal of Agricultural Sciences**, 47(4): 1089-1100.

