

## The effect of adding anthocyanin pigment extracted from cabbage and red cabbage on the chemical composition and microbial properties of frozen lamb meat

Omar Ahmed Saleh<sup>1</sup> and Maysaloon Wael Ibraheem<sup>2</sup>

<sup>1,2</sup>Animal Production Department, College of Agriculture, University of Tikrit, Tikrit, Iraq.

<sup>1</sup>E-mail: [os230106pag@st.tu.edu.iq](mailto:os230106pag@st.tu.edu.iq)

<sup>2</sup>E-mail: [maysaloon2019@tu.edu.iq](mailto:maysaloon2019@tu.edu.iq)

### Abstract.

This experiment was conducted in the laboratories of the College of Agriculture / Tikrit University for the period from 15 November 2024 to 15 May 2025 with the aim of adding anthocyanin pigments extracted from *Hibiscus sabdariffa* and red cabbage to meat. Lamb meat was purchased immediately after slaughtering from the local markets in Al-Dhuluiya district. The thigh cut of the sheep was selected and then placed in the refrigerator for (24) hours to allow the resolution of rigor mortis. After that, the pigment was added to the sheep meat at different proportions 1.0 ,0.5 and 1.5% (w/w) of the weight of the meat. The meat was immersed in the pigment after diluting the proportions in 500 ml of distilled water for 8 hours in the refrigerator at a temperature of 4°C. The meat was removed from the diluted pigment and placed in bags and frozen for two periods of two months and four months by freezing. The study aimed to evaluate the effect of the anthocyanin pigment extracted from *Hibiscus sabdariffa* and red cabbage on the chemical composition and microbial properties. In frozen lamb, the results of the addition treatments showed a significant increase with increasing extract concentration in both moisture and protein content, with a decrease as the storage period progressed. Meanwhile, fat and ash contents decreased. Aerobic and refractory bacterial count also decreased with increasing extract concentration, with bacterial counts increasing as the storage period progressed.

**Keywords.** Microbes, anthocyanins, lamb meat, guava and red cabbage extract.

at appropriate concentrations [1]. Anthocyanins also affect microbial metabolism by depriving organisms of the substrates necessary for their growth. [2] Animal protein is more susceptible to microbial spoilage and the oxidation of lipids and proteins due to its greater contact with surfaces and oxygen. [3] Polyphenolic compounds such as anthocyanins possess antimicrobial activity against a wide range of microorganisms, particularly in inhibiting the growth of foodborne pathogens [4]. Lipid oxidation also leads to a decrease in sensory, physical, chemical, and microbiological properties [5]. The most

### 1. Introduction

The susceptibility of meat and meat products to oxidation and microbial spoilage poses a threat to food quality, safety, and shelf life. The use of bioactive compounds, particularly plant antioxidants, can reduce the rate of autoxidation and microbial growth, thereby extending the shelf life of meat. These bioactive compounds include polyphenols, flavonoids, and anthocyanins, which have antioxidant and antimicrobial properties. They can act as preservatives and improve the sensory, physical, and chemical properties of meat products when added

bucket and dried in an electric oven at 105°C until the weight remained constant [11]. The moisture content was calculated using the following equation.

$$\text{Moisture percentage}\% = \frac{\text{weight of Sample before drying} - \text{weight of Sample after drying}}{\text{weight of Sample before drying}} \times 100$$

### 2.1.2. Crude protein estimation

The crude protein content of meat samples was estimated using a Kjeldahl Semi-micro apparatus, according to the method described in [11]. 0.3 g of minced veal for each treatment was placed in the apparatus' digestion tube. 5 ml of concentrated sulfuric acid (95%) was added to each tube, along with two drops of perchloric acid (HClO<sub>4</sub>). Digestion continued until the solution became clear. The digested samples were then distilled after adding 10 ml of sodium hydroxide (0.1 N). The liberated ammonia was collected in a 50 ml flask containing 25 ml of 2% boric acid with two drops of Bromocresol Green and Methyl Red. The samples were then rinsed with 0.05 N hydrochloric acid (HCl). The amount of acid required to change the color of the indicator from green to red was calculated, and the crude protein content was calculated according to the equation. Next.

$$\text{Crude protein (\%)} = \frac{\text{Amount of HCl consumed (ml)} \times \text{molarity (0.05)} \times 0.014 \times 6.25}{\text{Model weight (g)}} \times 100$$

### 2.1.3. Fat percentage estimation

The fat percentage in minced veal samples was estimated according to the [11] method, using a German-made Gerhardt Soxhlet method. A weight of 0.5 g of dried ground meat was placed in a special container of the device, the weight of which was previously known. 35 ml of solvent (methanol with chloroform) was then added at a ratio of

common form of spoilage caused by a change in chemical composition is the oxidation of proteins containing lipids and iron [6]. Studies have shown [7] that the use of plant extracts with known antimicrobial and antioxidant properties can be of great importance in food preservation, benefiting human health. Many plants are rich sources of flavonoids, phenolic acids, and other organic acids, which act as antioxidants and antimicrobial compounds [8]. Their use has been shown [9] to be more effective in improving the shelf life and sensory, chemical, physical, and microbiological properties of meat. Antioxidants can be used to maintain quality and extend shelf life [10]. Even low concentrations of antioxidants added to meat can slow the oxidation of lipids and proteins, thereby increasing shelf life by protecting them from chemical oxidation. This study aimed to add anthocyanin pigment extracted from some plant sources and to know the extent of its effect on different types of meat and at different storage periods, and to know the effectiveness of anthocyanins on some microbial and chemical characteristics of meat.

## 2. Materials and Methods

This experiment was conducted in the laboratories of the College of Agriculture / Tikrit University for the period from 15 November 2024 to 15 May 2025 with the aim of adding anthocyanin pigments extracted from kajar and red cabbage to sheep meat at different rates 1.0 ,0.5 and 1.5% (w/w)of the meat weight and placing them in bags and freezing them for two and four months.

### 2.1. Chemical Analysis of Meat

#### 2.1.1 Moisture Estimation

The moisture content of chilled veal leg samples was estimated based on the weight loss before and after drying. A specific weight of 10 g of minced meat samples was placed in a pre-weighed

0.1 ml of appropriate dilutions were transferred to sterile Petri dishes, and Plate Count Agar (PCA) was added to them. The dishes were incubated at 37°C for 24 hours. The number of bacterial colonies in the dishes was counted using a colony counter, and the average for two dishes was calculated. The results were expressed as colony-forming units per gram according to the equation mentioned in [13]:

$$\text{CFU/g} = N \times D1 \times Wt1$$

where CFU represents colony-forming units.

### 2.2.2. Psychrophilic bacteria

The method mentioned in [14] was followed, where 0.1 ml of the appropriate dilutions were transferred to sterile Petri dishes, the culture medium was added, and the dishes were incubated at 7-10°C for 10 days. The number of bacterial colonies in the dishes was counted using a colony counting device, and the average for two dishes was calculated. The results were expressed on the basis of colony-forming units/g according to the equation mentioned in [13]  $\text{CFU/g} = N \times D1 \times Wt1$

### 2.3. Statistical Analysis

The statistical evaluation of the experimental data was performed using a standard statistical software package, as referenced in [15]. The analysis followed a factorial arrangement (2 × 4) within a two-way completely randomized design (CRD), aimed at assessing the individual and interactive effects of the treatment types and storage durations on the measured parameters. To identify significant differences among group means, a post-hoc comparison was conducted using the method outlined in [16]. The applied statistical model was structured as follows:

$$Y_{ijk} = \mu + P_i + T_j + PT_{ij} + e_{ijk}$$

2:1. The extraction process lasted approximately 4–6 hours. The solvent was then collected from the device, the pellet was removed, and placed in an electric oven for half an hour at 80°C to remove any solvent residue from the pellet. The dried pellet was then weighed after cooling, and the fat percentage was calculated according to the following equation:

$$\text{Fat percentage \%} = \frac{\text{Weight of the thimble before extraction} - \text{Weight of the thimble after extraction}}{\text{Sample weight}} \times 100$$

100

### 2.1.4. Estimating Ash Content

The ash content of minced veal samples was estimated by burning a dry meat sample after placing it in a pre-weighed ceramic bowl in a kiln at 525°C for 6 hours. The ash content was calculated using the following equation [11].

$$\text{Ash percentage (\%)} = \frac{\text{Weight of the lid with the sample after burning} - \text{Weight of the lid}}{\text{Sample weight}} \times 100$$

100

### 2.2. Bacterial Testing

Before conducting bacterial testing of meat samples according to Method [12], 1 g of minced meat sample was mixed and placed in a sterile stomacher bag with 9 ml of dilution solution (0.1% peptone). The mixture was then blended for 2 minutes in a blender (stomacher), resulting in a dilution of 1-10. 1 ml of the mixture was then transferred to 9 ml of dilution solution, resulting in a dilution of 10-2, and so on. 0.1 ml of the appropriate dilutions were transferred and placed in sterile Petri dishes, and the appropriate media were poured over them according to the required test.

#### 2.2.1. Total aerobic bacterial count

dye type on moisture content, no significant difference ( $P < 0.05$ ) was observed between the first dye extracted from kale and the second extracted from cabbage. A significant difference ( $P < 0.05$ ) was also observed between the dye concentrations, as the highest concentration, T4 (70.66%), outperformed the other concentrations in moisture content. The lowest level was T1 (68.22%). These results are consistent with [17] when turmeric and cinnamon extracts were added to the effect of their effect on moisture content in ground beef stored in the freezer for different storage periods. All addition treatments were found to be superior to the control treatment in moisture content, and the treatment with the highest concentration recorded the highest moisture content. As for the interaction effect between the period and dye type on moisture content, the results showed a significant difference ( $P < 0.05$ ) between the first dye extracted from kajars and the second dye extracted from red cabbage during both periods. The same table shows the interaction effect between the freezing period and the concentration of the different dyes in the treatments on the moisture content of lamb meat. We note a significant increase ( $P < 0.05$ ) in moisture content (71.10%) for the T4 treatment (1.5 g/kg/meat) during the two-month freezing period, compared to the control treatment, which recorded the lowest moisture content (67.60%) during the four-month freezing period. This means that there are significant differences between treatments and different storage periods. As for the interaction between the dye and the dye concentration, the moisture percentage was at its highest level at the dye concentration (1.5 g of kj/kg/meat), which reached (71.17%), and the lowest level of interaction was at (T1), the control treatment. This is attributed to the active ingredients in natural extracts

Where:

- $Y_{ijk}$  represents the observed value,
- $\mu$  is the overall mean,
- $P_i$ : denotes the effect of the  $i$ -th storage period,
- $T_j$ : indicates the effect of the  $j$ -th treatment type,
- $PT_{ij}$ : is the interaction effect between period and treatment,
- $E_{ijke}$ : refers to the random experimental error associated with each observation.

### 3. Results and Discussion

#### 3.1. Chemical composition of frozen lamb meat

##### 3.1.1. Moisture

The results in Table (1) showed that the moisture content in frozen lamb was clearly affected by the storage period. A significant decrease in moisture content was observed with increasing freezing period. Moisture content was at its highest level at 2 months (69.83%), then began to decline over the storage period until reaching its lowest level at 4 months. The results are consistent with [17] when turmeric and cinnamon extracts were added to the study, which showed a significant increase in moisture content in ground beef stored in the freezer for different storage periods. A significant increase in moisture content was found at the beginning of the freezing period compared to the control treatment, which recorded the lowest moisture content at the end of the freezing period. The results are consistent with [18] which indicated in its study the effect of storage period on various types of meat, including cattle, sheep, buffalo, and chicken and their preservation in the freezer for different storage periods, indicating a significant decrease in moisture content. Regarding the effect of

increase in protein content in minced beef supplemented with turmeric and cinnamon extracts of ground beef stored in the freezer for different storage periods. A significant increase in the protein content was observed at the beginning of the freezing period, with the lowest protein content observed in the control treatment at the end of the freezing period. As for the effect of the dye type on the protein content, there were no significant differences ( $P < 0.05$ ) between the first dye extracted from kale and the second extracted from cabbage, reaching (17.39%) and 17.18%, respectively. A significant increase was observed between the pigment concentrations, as the highest concentration T3 (17.67%) and T4 (17.98%) outperformed the rest of the concentrations in moisture content, while the lowest level was T1 (16.46%). These results were consistent with [17] when turmeric and cinnamon extracts were added to the protein content of ground beef stored in the freezer for different storage periods, and there was a significant effect of the treatment on the protein content, as all addition treatments outperformed the two control treatments in the protein content, and the treatment with the highest concentration recorded the highest protein content.

**Table 1.** Effect of adding anthocyanin pigment extracted from cabbage and red cabbage on moisture and protein content (%) in frozen lamb meat for different storage periods. Mean  $\pm$  standard error for frozen lamb meat.

Factors		Moisture (%)	Protein (%)
Storage Periods (P)	P1	69.83 $\pm$ 0.22 a	16.92 $\pm$ 0.17 b
	P2	68.78 $\pm$ 0.24 b	17.65 $\pm$ 0.17 a
Extract Type (L)	L1	$\pm$ 0.27 a 69.37	17.39 $\pm$ 0.221 a
	L2	$\pm$ 0.24 a 69.24	17.18 $\pm$ 0.15 a
Extract Concentration (T)	T1	$\pm$ 0.19 c 68.22	16.46 $\pm$ 0.10 c
	T2	$\pm$ 0.26 b 69.03	17.02 $\pm$ 0.30 b

playing a role in preserving cell membranes from the damage caused by the oxidation process that occurs in the lipids of these membranes, thus preserving their moisture content and nutritional value [19]. These results are consistent with [20], which indicated that adding marjoram extract to minced beef stored in the freezer for (1), 30 and 60 days led to an increase in the moisture content in all addition treatments compared to the control. These results are consistent with [21], which indicated that adding hibiscus cup extract to the chemical composition of broiler chicken led to an increase in the moisture content with increasing levels of concentration of hibiscus cup extract from 0 to 20 g/L used in seasoning broiler chicken.

### 3.1.2. Protein

The statistical analysis in Table (1) showed a significant effect of the protein content in minced meat supplemented with plant extracts increased during storage mainly due to moisture loss resulting in a concentration effect. ( $P < 0.05$ ). The protein content decreased, reaching its lowest level during the 2-month period, then began to increase with the progression of the storage period until it reached its highest level during the 4-month period. The results are consistent with those of [17], showing an

	T3	$\pm 0.28$ b 69.31	17.67 $\pm 0.18$ a
	T4	$\pm 0.304$ a 70.66	17.98 $\pm 0.205$ a
Interaction between period and dye type	L1 X P1	$\pm 0.34$ a 69.79	17.11 $\pm 0.335$ ab
	L2 X P1	$\pm 0.298$ a 69.86	16.73 $\pm 0.112$ b
	L1 X P2	$\pm 0.403$ ab 68.95	17.67 $\pm 0.280$ a
	L2 X P2	$\pm 0.298$ b 68.61	17.62 $\pm 0.219$ a
	T1 X P1	$\pm 0.108$ cd 68.83	16.33 $\pm 0.040$ d
	T2 X P1	$\pm 0.28$ bc 69.55	16.42 $\pm 0.440$ d
	T3 X P1	$\pm 0.41$ b 69.82	17.26 $\pm 0.26$ bc
Interaction between Period and Extract Concentration	T4 X P1	$\pm 0.337$ a 71.10	17.67 $\pm 0.243$ ab
	T1 X P2	$\pm 0.105$ f 67.60	16.60 $\pm 0.192$ cd
	T2 X P2	$\pm 0.360$ d 68.52	17.61 $\pm 0.277$ ab
	T3 X P2	$\pm 0.268$ cd 68.79	18.08 $\pm 0.109$ a
	T4 X P2	$\pm 0.464$ ab 70.22	18.30 $\pm 0.294$ a
	T1 X L1	$\pm 0.295$ d 68.22	16.46 $\pm 0.151$ d
	T2 X L1	$\pm 0.447$ bcd 69.27	16.85 $\pm 0.564$ cd
	T3 X L1	$\pm 0.254$ cd 68.83	17.81 $\pm 0.131$ ab
Interaction between Extract Type and Concentration	T4 X P1	$\pm 0.226$ a 71.17	18.44 $\pm 0.243$ a
	T1 X L2	$\pm 0.295$ d 68.22	16.46 $\pm 0.151$ d
	T2 X L2	$\pm 0.311$ cd 68.80	17.18 $\pm 0.286$ bcd
	T3 X L2	$\pm 0.438$ bc 69.78	17.53 $\pm 0.351$ abc
	T4 X L2	$\pm 0.500$ ab 70.14	17.53 $\pm 0.213$ abc

Dye concentrations, T1: control without addition, T2: 0.5 g/kg meat added, T3: 1 g/kg meat added, T4: 1.5 g/kg meat added, P1: 2-month frozen storage period, P2: 4-month frozen storage period. L1: anthocyanidin dye extracted from guava, L2: anthocyanidin dye

extracted from red cabbage. \*Different letters within a column indicate significant differences.

the 2-month period. It is clear that there was an increase in the protein content of frozen lamb meat for the 4-month period, as treatment T3 and T4 recorded (1 and 0.51), respectively, the highest protein content for that period, reaching 18.08 and 18.30%, respectively. It was followed by treatment (1.5) T4 for the first period and (0.5) T2 for the second period, g/kg meat, as the protein content was 17.67 and 17.26% for the first and second storage periods, respectively, while the control treatment T1 ranked last, and recorded the lowest protein content in the first storage period. 16.33%. The results were consistent with [21], indicating that adding hibiscus calyx extract to the chemical composition of broiler chickens led to an increase in protein content with increasing concentrations of hibiscus calyx extract used in seasoning broiler chickens. The interaction between the dye and the dye concentration and its effect on protein content was highest at the hibiscus calyx dye concentration (1.5 g/kg/meat), reaching 18.44%, and the lowest interaction level was at (T1) without the addition of hibiscus. The increase in crude protein content may be due to two factors. First, it is caused by the protein contribution of the hibiscus calyx extract [22]. The results were consistent with [25], when two concentrations of cinnamon extract (2) and (4) were added by spraying and immersion to minced meat taken from the thigh muscle of one-year-old Awassi lambs, resulting in a significant increase in protein content in favor of the treatment with the higher extract concentration compared to the control treatment.

This is consistent with the results of [20] when using marjoram extracts and adding them to ground beef stored in the freezer, as this led to a significant increase in the protein content of the treatments to which it was added compared to the control treatment. The use of hibiscus extract in the treatment of beef thigh meat at different concentrations was used. The higher the level of hibiscus extract, the higher the protein content significantly. Increasing the levels of hibiscus extract from 10% to 16% in beef meat increased the protein percentage [22]. This is consistent with the report prepared by [23], which states that the percentage of crude protein is high at higher concentrations of extracts used in meat seasoning. The results of the experiment were consistent with [24], when using freeze-dried hibiscus extract at different levels in beef thigh meat, it increased the protein percentage in beef thigh meat. Regarding the interaction between the period and type of dye on the protein percentage, the results in Table (1) show a significant increase ( $P > 0.05$ ) in the protein percentage in the first dye extracted from the kernels and the second dye extracted from red cabbage for the second period, which amounted to (17.67%) and (17.62%), respectively. The results of the statistical analysis in Table ( ) showed that the storage period and dye concentration had a significant effect on the protein content of frozen lamb meat ( $P > 0.05$ ). The protein content increased with the progress of the storage period until it reached its highest level in the 4-month period, while the lowest protein content was in

different storage periods. The fat percentage of minced beef decreased for the treatment with the highest concentration of turmeric extract at the beginning of the freezing period, and the highest percentage of fat was in the control treatment at the end of the freezing period. The results of the experiment were consistent. [22] When freeze-dried hibiscus extract was used as a feed additive in beef processing at different concentrations, it was found that the higher the hibiscus extract level, the lower the fat content, making the resulting meat product fit for consumption. The results of the experiment were consistent with [24], when freeze-dried hibiscus extract was used at different levels in beef thigh meat, increasing the protein content and reducing the fat content. The results of the experiment were consistent with [21], which showed that the fat content in chicken meat dipped in different concentrations of hibiscus extract decreased with increasing levels of hibiscus extracts used in seasoning chicken meat. This means that fat oxidation and rancidity can be reduced at higher concentrations of hibiscus extract used in meat processing [26].

As for the effect of interaction between the period and type of dye on the fat content of lamb meat for different storage periods, the results in Table (2) showed a significant difference ( $P < 0.05$ ), as the extract dye outperformed. From red cabbage for a period of 4 months, which amounted to 12.26%, compared to the pigment extracted from kajarat for a period of 2 months, which amounted to 10.99%. As for the interaction between the pigment and the pigment concentration, the moisture content was at its highest

### 3.1.3. Fat

The results of the statistical analysis in Table (2) showed a significant effect ( $P > 0.05$ ) of the freezing storage period on the fat percentage in frozen lamb meat for different storage periods. The fat percentage increased with the progression of the storage period, reaching its highest level in the 4-month period, reaching 11.95%, compared to the 2-month period, which reached 11.32%. This means that the progression of the storage period reduces moisture and increases dry matter, which includes protein, fat, and ash. This is consistent with [17]. When turmeric and cinnamon extracts were added to the study, their effect on the fat percentage in ground beef stored in the freezer for different storage periods was found. A significant effect was found on the fat percentage, as the lowest fat percentage was at the beginning of the storage period and its highest level was at the end of the storage period. As for the effect of the dye type on the fat percentage in frozen lamb meat for different storage periods, a significant difference ( $P > 0.05$ ) was found between The two pigments, as the anthocyanin pigment extracted from red cabbage outperformed the anthocyanin pigment extracted from cauliflower, reaching 11.95 and 11.33% respectively. It was found that the pigment concentration had a significant effect ( $P < 0.05$ ) on the percentage of fat in frozen lamb. A decrease in the percentage of fat was observed at the highest concentration, T4 (1.5) g/kg of meat, which reached 10.51% compared to the remaining concentrations. The highest percentage was in the T1 treatment, which reached 12.73%. These results are consistent with [17], when turmeric and cinnamon extracts were added to minced beef at

Some previous studies indicated a decrease in the percentage of fat in treatments added to some medicinal plants or their extracts. This may be due to the addition of natural extracts to the superior treatments containing flavonoids, which act as antioxidants and thus preserve the cell membrane and phospholipids. The results agreed with [27] that lambs fed rosemary extract recorded a significant decrease in the percentage of fat compared to those fed black seed and the control diet. The results agreed with [20], who indicated that adding marjoram to frozen minced beef recorded a significant decrease ( $P>0.05$ ) in the percentage of fat compared to the control treatment. The results agreed with [22], which indicated that the use of freeze-dried guajrat extract led to a decrease in the percentage of fat in the meat, such that it became less than the control treatment, and the treatment with the highest level of guajrat extract showed the lowest percentage of fat compared to the other concentrations.

#### 3.1.4. Ash

The results of the statistical analysis in Table (2) showed that the effect of the freezing storage period on the ash content of frozen lamb meat had a significant effect ( $P>0.05$ ) for the 4-month period compared to the 2-month period. The first 2-month period recorded the lowest ash content, reaching 1.40%, which began to increase with the progress of the storage period until it reached its highest level in the 4-month period, reaching 1.63%. This is normal, as the storage period increases, moisture decreases, and dry matter increases, which includes protein, fat, and ash [27]. The results were consistent with [17] when turmeric and cinnamon

level at the kajarat pigment concentration (1.5 g kajarat/kg/meat), which amounted to (71.17%), and the lowest level of interaction was at (T1), the control treatment. The same table shows the effect of the interaction between the freezing storage period and the concentration of the different pigments for the treatments on the percentage of fat in the lamb meat. A significant decrease ( $P>0.05$ ) was observed in the percentage of fat for the T4 treatment at a concentration of (0.51) for the first and second periods, as it amounted to 10.29% during the 2-month freezing storage period and 10.74% during the 4-month freezing storage period, compared to the control treatment, which recorded the highest percentage of fat during the storage period. 4 months of freezing and reached 13.20% for the second period. There were significant differences between the pigment concentrations in the two periods. The results were consistent with [17] when turmeric and cinnamon extracts were added, and their effect on the fat content of ground beef stored in the freezer for different storage periods. The control treatment achieved the highest fat content among the treatments and for all storage periods. As for the interaction between the pigment and pigment concentration on the fat content in frozen lamb meat for different storage periods, a significant decrease in the fat content was observed with the kajarat pigment at concentrations T3 and T4 (1.5 g kajarat/kg lamb meat), which reached 11.10 and 10%. This was followed by the red cabbage pigment at concentration T4 (1.5 g cabbage pigment/kg lamb meat), which reached 11.03%, compared to the control treatment, which recorded the highest level of fat content, reaching 12.73%.

between the concentrations, as T4 (1.5 g/kg meat) recorded a significant decrease in the ash percentage, which reached 1.35%, while T1 (the control treatment) recorded the highest ash percentage, which reached 1.77%. The results were consistent with [21] that the ash percentage in chicken meat dipped in different concentrations of kajarat extract increases with the increase in the concentration of hibiscus extract used in seasoning chicken meat. The results were consistent with [17] when turmeric and cinnamon extracts were added, and their effect on the fat percentage in ground beef stored in the freezer for periods. Different storage conditions, and a decrease in the ash percentage was observed for the treatment with the highest concentration over all treatments, with the highest ash percentage being in the control treatment.

extracts were added to the effect of the effect on the fat content of ground beef stored in the freezer for different storage periods. It was found that the storage period had an effect, as the lowest ash content was at the beginning of the storage period and its highest level was at the end of the storage period. This is normal, as moisture decreases and dry matter increases as the storage period increases. The results also showed the effect of the dye type on the ash content. In frozen lamb meat, no significant differences ( $P>0.05$ ) were observed in the ash percentage of the two dyes extracted from kajarat and extracted from red cabbage. Studies have previously indicated a decrease in the ash percentage in treatments to which some medicinal plants or their extracts were added compared to the control treatment [28]. As for the effect of the dye concentration, it was found that there were significant differences

**Table 2.** Effect of adding anthocyanin pigment extracted from cabbage and red cabbage on the percentage of fat and ash (%) in frozen lamb meat for different storage periods. Mean  $\pm$  standard error for frozen lamb meat.

Factors		Fat (%)	Ash (%)
Storage Periods (P)	P1	11.32 $\pm$ 0.20 a	1.40 $\pm$ 0.035 b
	P2	11.96 $\pm$ 0.27 b	1.63 $\pm$ 0.044 a
Extract Type (L)	L1	11.33 $\pm$ 0.273 b	1.50 $\pm$ 0.048 a
	L2	11.95 $\pm$ 0.200 a	1.53 $\pm$ 0.045 a
Extract Concentration (T)	T1	12.73 $\pm$ 0.219 a	1.77 $\pm$ 0.048 a
	T2	11.91 $\pm$ 0.346 b	1.47 $\pm$ 0.048 b
	T3	11.41 $\pm$ 0.222 b	1.47 $\pm$ 0.049 b
	T4	10.51 $\pm$ 0.253 c	1.35 $\pm$ 0.049 c
Interaction between period and dye type	L1 X P1	10.99 $\pm$ 0.337 b	1.39 $\pm$ 0.053 b
	L2 X P1	$\pm$ 0.190 ab 11.651	1.42 $\pm$ 0.049 b
Interaction	L1 X P2	11.66 $\pm$ 0.423 ab	1.62 $\pm$ 0.066 a
	L2 X P2	12.26 $\pm$ 0.338 a	1.64 $\pm$ 0.062 a
	T1 X P1	12.26 $\pm$ 0.189 ab	1.61 $\pm$ 0.011 b

between Period and Extract Concentration	T2 X P1	11.53 ±0.286 bc	1.37 ±0.054 de
	T3 X P1	±0.285 bcd	1.38 ±0.059 de
		11.21	
	T4 X P1	10.29 ±0.381 d	1.26 ±0.059 e
	T1 X P2	13.20 ±0.296 a	1.93 ±0.011 a
	T2 X P2	12.30 ±0.621 ab	1.58 ±0.055 bc
	T3 X P2	11.61 ±0.347 bc	1.57 ±0.059 bc
	T4 X P2	10.74 ±0.340 cd	1.45 ±0.059 cd
	T1 X L1	12.73 ±0.325 a	1.77 ±0.072 a
	T2 X L1	11.48 ±0.580 bc	1.50 ±0.055 b
Interaction	T3 X L1	11.10 ±0.278 c	1.45 ±0.058 b
between Extract	T4 X P1	10.00 ±0.313 d	1.29 ±0.085 b
Type and	T1 X L2	12.73 ±0.325 a	1.77 ±0.072 a
Concentration	T2 X L2	12.34 ±0.343 ab	1.44 ±0.082 b
	T3 X L2	11.71 ±0.321 abc	1.49 ±0.083 b
	T4 X L2	11.03 ±0.279 c	1.41 ±0.045 b

Dye concentrations, T1: control without addition, T2: 0.5 g/kg meat added, T3: 1 g/kg meat added, T4: 1.5 g/kg meat added, P1: 2-month frozen storage period, P2: 4-month frozen storage period. L1: anthocyanidin dye extracted from guava, L2: anthocyanidin dye extracted from red cabbage. \*Different letters within a column indicate significant differences.

treatment for the second period recorded a significant difference, as it reached the highest ash percentage, which was 1.93%, and the lowest ash percentage level was reached at the highest concentration of T4, T3, and T2 pigments, with a concentration of (0.50, 1, (1.5), which reached values of 1.58, 1.57, and 1.45%, respectively, during the 4-month storage period. The results were consistent with [17] when turmeric and cinnamon extracts were added, and their effect on the fat percentage in ground beef stored in the freezer at different storage periods was observed, as a decrease in the ash percentage was observed for the treatment with the highest concentration at the beginning of the freezing storage period, and the highest ash percentage was observed in the control treatment at the end of the freezing storage period. The table

As for the interaction effect between the period and dye type on the ash percentage, the results showed a significant increase ( $P < 0.05$ ) in the ash percentage for the first two dyes extracted from kajars and the second extracted from red cabbage in the second 4-month period, reaching 1.62 and 1.64%. The same table shows the interaction effect between the freezing storage period and the concentration of the different dyes on the ash percentage of frozen lamb meat. A significant decrease ( $P < 0.05$ ) was observed in the ash percentage for the T4, T3, and T2 treatments at concentrations of 0.50, 1, and 1.5, reaching 1.37, 381, and 261%, respectively, during the 2-month storage period compared to the control treatment, which recorded the highest ash percentage during the 2-month storage period. A month of freezing, reaching 1.61%, while the first

level of bacterial counts was at the beginning of the storage period and the highest level at the end of the storage period. The table shows the effect of the dye type on frozen lamb. There was no significant effect ( $P>0.05$ ) on bacterial counts for either dye. As for the effect of dye concentration, there were significant differences between the concentrations, as T4 recorded a significant decrease in bacterial counts. Aerobic bacteria, which reached 4.46, while the T1 control treatment recorded the highest percentage of bacterial counts, reaching 4.93. The results of this experiment were consistent with [29], who observed a decrease in the total colony count when adding 2 and 3% of guava extract to beef compared to the control treatment. The results of this experiment were consistent with [21], who obtained a higher percentage of bacterial count reduction in chicken meat samples dipped in guava extract compared to the control treatment. The results of this experiment were also consistent with [20], which stated that adding green tea extract to minced beef led to a decrease in the total bacterial count with an increase in the colony count in the control treatment. They were consistent with [17], who added different concentrations of turmeric and cinnamon extracts, which affected the bacterial count in minced beef stored in the freezer for different storage periods, as they observed a decrease in the addition treatments compared to the two control treatments. As for the effect of the interaction between the period and the type of dye on the number of aerobic bacteria in lamb meat. The results showed a significant ( $P<0.05$ ) increase in the number of aerobic bacteria for the two dyes in the second 4-month period compared to the first 2-month

shows the effect of the interaction between the pigments and the concentration of the different pigments on the ash percentage of frozen lamb meat, as a significant decrease ( $P>0.05$ ) was observed in the ash percentage for the T3 and T2 concentrations. T4 T1 (0.5, 1, 1.5) g/kg meat for both dyes. The control treatment recorded the highest ash content for both dyes, reaching 1.77%. The addition of hibiscus calyx extract to the chemical composition of broiler chicken led to an increase in the ash content with increasing concentration levels of hibiscus calyx extract from 0 to 20 g/L used in broiler seasoning. [21] The results were consistent with [17] when turmeric and cinnamon extracts were added to the meat, and their effect on the fat content of ground beef stored in the freezer for different storage periods was observed. A decrease in the ash content was found in the addition treatments, which had the lowest ash content, while the control treatment had the highest ash content.

### 3.2. bacterial tests

#### 3.2.1. Total aerobic plate counts

The results of the statistical analysis in Table (3) showed a significant effect ( $P>0.05$ ) of the freezing storage period on the number of aerobic bacteria in frozen lamb. It was observed that the lowest level of aerobic bacterial counts was at the 2-month period, then increased with the progress of the storage period until reaching its highest level at the 4-month period. This was consistent with [] when different concentrations of turmeric and cinnamon extracts were added to the effect of the effect on bacterial counts in ground beef stored in the freezer at different storage periods. A significant superiority was observed, as the lowest

the interaction effect of dyes and the concentration of different dyes on the aerobic bacterial count of frozen lamb meat. A significant decrease ( $P>0.05$ ) was observed in the aerobic bacterial count for all concentrations and for both dyes. The lowest level was at the T4 concentration (5.1) for the kajarat dye, which reached 4.29, and the highest value was at the T1 concentration for both dyes, reaching 4.93. The aerobic bacterial count decreased at the remaining dye concentrations, 794 and 784 CFU/g of meat. Polyphenolic compounds, such as anthocyanins, have antimicrobial activity against a wide range of microorganisms, particularly in inhibiting the growth of foodborne pathogens [4]. The results are consistent with [30], where two concentrations of kajarat extract were used in beef to prevent the development of bacterial colonies, showing a decrease in Colony forming unit (CFU) after 60 minutes of addition and 24 hours of storage. Samples treated with guava extract showed a decrease of about 3-5 log on the surface of the bacteria-inoculated samples [31]. The results are in agreement with [32], as samples with guava extract added showed significantly lower numbers of microbes compared to the control treatment. The results are in agreement with [33], when grape seed extract was used at different concentrations in minced beef during refrigerated storage at 4°C for 10 days, which resulted in a significant decrease in bacterial counts compared to the control samples, thus enhancing shelf life.

### 3.2.2. Total psychrophilic count

The results in Table (3) indicate a significant effect ( $P>0.05$ ) of the

period. The aerobic bacterial counts for the second 4-month period for the anthocyanin dye extracted from kale and the second dye extracted from red cabbage were 5.06 and 5.11 colony-forming units per gram of meat, respectively. The lowest aerobic bacterial counts were recorded for the first 2-month period for the anthocyanin dye extracted from kale and the dye extracted from red cabbage, at 4.34 and 4.40 colony-forming units per gram of meat, respectively. The effect of using kale and red cabbage extracts on the total bacterial count was demonstrated. A significant ( $P<0.05$ ) increase in the total number of aerobic bacteria was observed for the control treatment as the storage period progressed. The control treatment T1 recorded the highest values, reaching 5.33 CFU/g of meat for a storage period of 4 months, while the treatments to which the extracts of cauliflower and red cabbage were added recorded lower values for the total bacterial count compared to the control, and the treatment T4 with a concentration of 5.1 recorded the lowest values, as the logarithm of the total bacterial count reached 4.14 CFU/g of meat for a storage period of 2 months, followed by the treatment T4 with a concentration of 5.1, as the logarithm of the total bacterial count reached 4.77 CFU/g of meat for a storage period of 4 months. It agreed with [17] when different concentrations of turmeric and cinnamon extracts were added, and their effect on the number of bacteria in minced beef stored in the freezer for different storage periods, as it was noted that there was a significant decrease in the total bacterial count for the treatments to which turmeric extracts were added when compared to the control treatment. The table shows

ability to inhibit and destroy the cell walls of microorganisms, thus penetrating the cell and disrupting the function of its organelles [34]. These results are consistent with [35], which indicated that Cinnamon and turmeric plant extracts have proven antimicrobial and antifungal activity, which was attributed to the role of the active ingredients in the extracts that have a toxic effect on microscopic organisms. This was consistent with [36] when the amount of Psychrotrophic bacterial count was determined in steak slices sprayed with hibiscus extract and stored for 10 days. It was found that the control treatment contained higher numbers of Psychrotrophic bacterial count, and the number of bacteria decreased with increasing hibiscus extract, achieving the lowest number of bacteria. These results are consistent with [37] who observed that hibiscus extract reduced the number of Psychrotrophic bacterial count in contaminated minced meat. [38] achieved lower numbers of intermediate Psychrotrophic bacterial count in beef stored for 10 days at 4°C, when spraying 500 mg/L of hibiscus extract, compared to control samples. This was consistent with [17] when adding different concentrations of turmeric extract. Cinnamon has an effect on the number of cold-loving organisms in frozen minced beef at different storage periods. A significant decrease was observed for the treatments with the highest concentration, which recorded the lowest value compared to the two control treatments. As for the interaction effect between the period and the type of dye on the number of Psychrotrophic bacterial count in frozen lamb meat, the results showed a significant ( $P < 0.05$ ) superiority in the

freezing storage period on the number of Psychrotrophic bacterial count

in frozen lamb meat. It was observed that the number of Psychrotrophic bacterial count was at its lowest level during the 2-month period, reaching 4.34 colony-forming units/g of meat, and then increased with the progress of the storage period to its highest level during the 4-month period, reaching 5.20 colony-forming units/g of meat. This is consistent with [17], when different concentrations of turmeric and cinnamon extracts were added to the effect on the number of Psychrotrophic bacterial count in ground beef stored in the freezer for different storage periods. A significant increase in the number of Psychrotrophic bacterial count was observed with the progress of the storage period. The effect of the type of dye on frozen lamb meat was shown to have a significant effect ( $P > 0.05$ ) on the number of bacteria, as the dye recorded The first dye extracted from cabbage showed a significant decrease in bacterial counts, reaching its lowest level of 4.66 CFU/g of meat, while the second dye extracted from red cabbage recorded its highest level compared to the first dye, reaching 4.88 CFU/g of meat. As for the effect of dye concentration, there were significant differences between the concentrations. T4 recorded a significant decrease in the number of Psychrotrophic bacterial count, which reached 4.39, while T2 treatment (0.5 concentration) recorded the highest percentage in the number of Psychrotrophic bacterial count, which reached 5.05. There was a significant difference between all concentrations. This effect may be due to the mechanism of action of nanoparticles in affecting microorganisms and their

storage period of 4 months. These results are consistent with [36], who achieved lower numbers of Psychrotrophic bacterial count in beef stored for 10 days at 4°C by spraying concentrations of guava extract compared to control samples. These results are consistent with [30], who found the effect of adding guava extract at concentrations of (0.5, 1, 1.5) % on Psychrotrophic bacterial count in chicken kofta stored in the refrigerator. It was observed that increasing concentrations of guava extract reduced the number of Psychrotrophic bacterial count. The number of Psychrotrophic bacterial count continued to increase significantly in the control sample on the seventh day, and was excluded. This may be attributed to the presence of phenolic compounds in guava, which act as antioxidants and antibacterial. Adding onion peel extract to beef patties can prevent the growth of microorganisms and slow down the spoilage process [40]. The effect of using concentrations of Different cinnamon and turmeric extracts affected the number of Psychrotrophic bacterial count. A significant decrease was observed in the number of Psychrotrophic bacterial count for the treatments to which nano-cinnamon and turmeric extracts were added. The treatment with the highest concentration recorded the lowest number of Psychrotrophic bacterial count for all storage periods compared to the control treatment [17]. The table shows the effect of the interaction between the dyes and the concentration of the different dyes on the Psychrotrophic bacterial count of frozen lamb meat. A significant decrease ( $P>0.05$ ) was observed in the aerobic bacteria count for all

number of Psychrotrophic bacterial count for the two dyes in the second period of 4 months compared to the first period of 2 months. The number of Psychrotrophic bacterial count for the second period of the anthocyanin dye extracted from cauliflower and the second dye extracted from red cabbage for the 4-month period reached 5.09 and 5.31 log CFU/g. The lowest levels of Psychrotrophic bacterial count were recorded for the first period of 2 months for the anthocyanin dye extracted from cauliflower and the first dye extracted from red cabbage for the 2-month period, at 4.23 and 4.45 colony-forming units per gram of meat, respectively. The effect of using The extracts of the guava and red cabbage plants in the total number of Psychrotrophic bacterial count. The results showed a significant increase ( $P>0.05$ ) in the number of Psychrotrophic bacterial count for the control treatment with the advancement of the storage period, as the control treatment T1 and T2 (0 and 0.5) g/kg meat recorded the highest values and reached 5.40 and 5.48 bacterial colony forming units/g of meat for storage periods of 4 months, while the treatments to which the extracts of guava and red cabbage were added recorded lower values for the total number of Psychrotrophic bacterial count compared to the control, and the treatment T4, T3 concentration (1 and 5.1) recorded the lowest values, as the logarithm of the total number of Psychrotrophic bacterial count reached 4.24 and 3.96 bacterial colony forming units/g of meat for storage period of 2 months, followed by the treatment T4 concentration 5.1, as the logarithm of the total number of Psychrotrophic bacterial count reached The bacterial count was 4.82 CFU/g of meat for a

from the outer membrane of bacteria. Anthocyanins also affect microbial metabolism by depriving the organism of the substrates necessary for its growth [2]. In general, the antimicrobial and antioxidant activities of plant extracts are attributed to their phenolic content. The results are consistent with [30], where two concentrations of guava extract were used in beef to significantly inhibited bacterial growth, showing a decrease of up to 4 log In colony forming unit after 60 minutes of addition and 24 hours of storage time. Anthocyanins show antimicrobial activity by destroying the bacterial wall when using 2% gal powder in beef as an antimicrobial, gal addition was found to be lower in bacterial counts (4-5 log CFU/g of meat), after 6 days compared to the control treatment 6–7 CFU/g of meat [41].

concentrations and for both dyes. The lowest level was at concentration T4 (5.1) for the first dye extracted from cabbage and the second extracted from red cabbage, which amounted to (4.21 cabbage and 4.57 cabbage), respectively. The highest value was recorded at concentration T2 (5.0) for the second dye extracted from red cabbage, which amounted to 5.06. While the count of Psychrotrophic bacterial count decreased at the remaining concentrations of T3 and T2 for the first two dyes, 0.35 and 424. CFU/g of meat, and at the concentrations of T4 and T3 for the second dye, 934 and 574. CFU/g of meat, respectively. The antimicrobial activity is also attributed to the ability of anthocyanins to destroy the cell wall, cell membrane, and intercellular matrix, and the ability of anthocyanins to release polysaccharide molecules

**Table 3.** Effect of adding anthocyanin pigment extracted from cabbage and red cabbage on the Logarithm of total aerobic plate counts (colony-forming unit/gram of meat) and the Logarithm of the total number of Psychrophilic bacteria (colony-forming units/g of meat) in frozen lamb meat for different storage periods. Mean  $\pm$  standard error for frozen lamb meat.

Factors		Logarithm of total aerobic plate counts (colony-forming unit/gram of meat)	Logarithm of the total number of Psychrophilic bacteria (colony-forming units/g of meat)
Storage Periods (P)	P1	4.37 $\pm$ 0.042 b	4.34 $\pm$ 0.065 b
	P2	5.09 $\pm$ 0.053 a	5.20 $\pm$ 0.065 a
Extract Type (L)	L1	4.70 $\pm$ 0.093 a	4.66 $\pm$ 0.116 b
	L2	4.76 $\pm$ 0.083 a	4.88 $\pm$ 0.099 a
Extract Concentration (T)	T1	4.93 $\pm$ 0.120 a	4.97 $\pm$ 0.130 b
	T2	4.80 $\pm$ 0.118 b	5.05 $\pm$ 0.133 a
	T3	4.72 $\pm$ 0.112 b	4.67 $\pm$ 0.151 c
	T4	4.46 $\pm$ 0.118 c	4.39 $\pm$ 0.142 d
Interaction between period	L1 X P1	4.34 $\pm$ 0.072 b	4.23 $\pm$ 0.108 b

and dye type	L2 X P1	4.40 ±0.047 b	4.45 ±0.062 b
	L1 X P2	5.06 ±0.090 a	5.090 ±0.108 a
	L2 X P2	5.11 ±0.061 a	5.31 ±0.06 a
	T1 X P1	4.54 ±0.021 d	4.54 ±0.021 d
Interaction between Period and Extract Concentration	T2 X P1	4.43 ±0.054 d	4.62 ±0.049 cd
	T3 X P1	4.37 ±0.051 d	4.24 ±0.116 e
	T4 X P1	4.14 ±0.101 e	3.96 ±0.089 f
	T1 X P2	5.33 ±0.015 a	5.40 ±0.021 a
Interaction between Extract Type and Concentration	T2 X P2	5.168 ±0.073 ab	5.48 ±0.049 a
	T3 X P2	5.07 ±0.061 b	5.10 ±0.116 b
	T4 X P2	4.77 ±0.103 c	4.82 ±0.089 c
	T1 X L1	4.93 ±0.179 a	4.97 ±0.193 ab
	T2 X L1	4.79 ±0.170 ab	5.03 ±0.194 ab
	T3 X L1	4.788 ±0.167 ab	4.42 ±0.196 bc
	T4 X P1	4.29 ±0.154 b	4.21 ±0.196 c
	T1 X L2	4.93 ±0.179 a	4.97 ±0.193 ab
Concentration	T2 X L2	4.81 ±0.180 ab	5.06 ±0.202 a
	T3 X L2	4.66 ±0.162 ab	4.93 ±0.192 ab
	T4 X L2	4.62 ±0.162 ab	4.57 ±0.196 abc

Dye concentrations, T1: control without addition, T2: 0.5 g/kg meat added, T3: 1 g/kg meat added, T4: 1.5 g/kg meat added, P1: 2-month frozen storage period, P2: 4-month frozen storage period. L1: anthocyanidin dye extracted from guava, L2: anthocyanidin dye extracted from red cabbage. \*Different letters within a column indicate significant differences.

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## Conclusions.

From the results obtained from this study, we can conclude that adding anthocyanin pigments extracted from kale and red cabbage at concentrations of 0.5, 1, and 5.1 g/kg of meat to stored lamb meat resulted in a significant increase in moisture and protein content and a decrease in fat and ash content compared to the control treatment. Adding anthocyanin pigments to frozen lamb meat also contributed to a logarithm reduction in the total number of aerobic and psychrophilic bacteria, which cause meat spoilage in both stored and frozen meat.

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