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## The Effects of Alcoholic Extract of *Ficus carica* Leaves on Some Chemical and Microbiological Properties of Beef during Refrigerated Storage

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### Abstract:

This study explored the preservative effects of the alcoholic leaves' extract of *Ficus carica* plant on beef refrigerated for 15 days. Phytochemical analysis showed that the plant extract contained terpenoids, flavonoids, tannins, saponins, and alkaloids. Furthermore, the alcoholic extract of plants significantly reduced the total viable counts of psychrotrophic bacteria, pathogenic bacteria (*Proteus*, *Salmonella typhimurum*, *Escherichia coli*, and *Staphylococcus aureus*), and yeasts (*Candida krusei*, *Candida lambica*, and *Zygosaccharomyces*) isolated from meat samples, particularly at 100 and 200 mg/ml concentrations.

The antioxidant activity of the extract was determined by using TBA and TVN values. The results showed that meat samples treated with 100 and 200 mg/ml of the alcoholic extract of *F. carica* had significantly lower TBA values (25, 0.24 mg/kg respectively) on day 5 which became 0.92, 0.53 mg/kg on day 15. Whereas the control values increased from 0.25 to 1.75 mg/kg on day 15, followed by the TVN values of treatment meat samples with 100 and 200 mg/ml 5.57, 5.12 mg N/100 g meat respectively on day 5 which became 12.16, 10.65 mg N/ 100 g meat on day 15, while the TVN in control samples increased significantly ( $p < 0.05$ ) from 7.35 to 15.76 mg N/100 g meat.

All results confirmed that the alcoholic extract of *F. carica*, which is rich in bioactive compounds is more effective as a natural antibacterial, antifungal and antioxidant, than synthetic antioxidants that maintain the quality of beef, compared to the control by reducing lipid oxidation and microbial growth at refrigeration temperatures, especially at 100 and 200 mg/ml concentrations of the plant extract.

**Keywords:** *Ficus Carica*; Antioxidant; Alcoholic extract; Meat; Antimicrobial

تأثير المستخلص الكحولي لاوراق نبات *Ficus carica* على بعض الخصائص الكيميائية  
والميكروبيولوجية للحوم البقر اثناء الخزن بالتبريد

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أوضحت هذه الدراسة تأثير المستخلص الكحولي لاوراق نبات *Ficus carica* على عينات اللحم المخزونة بالتبريد لمدة 15 يوم. أظهر التحليل الكيميائي لمستخلص النبات انه يحتوي على تيربينويد، فلافونات، تانينات، صابونين و قلويدات. علاوة على ذلك فان مستخلص النبات خفض بشكل كبير العدد الكلي لبكتريا المحبة للبرودة *psychrotrophic*، وأنواع البكتريا الممرضة ( *Proteus, Salmonella typhimium, Escherichia coli* و *Staphylococcus aureus* ) و الخمائر ( *Candida kruse, Candida lambica, and Zygosaccharomyces* ) المعزولة من عينات اللحم خاصة عند التركيز 100 و 200 ملغم /مل .

تم تحديد قابلية المستخلص كمضاد للاكسدة بقياس قيم TBA و TVN. اظهرت النتائج ان عينات اللحم المعاملة بتركيز 100 و 200 ملغم /مل من المستخلص الكحولي لنبات *F. Carica* خفضت في اليوم الخامس قيم TBA (25,0.24) ملغم /كغم على التوالي، والتي اصبحت (0.92,0.53) ملغم/كغم في اليوم الخامس عشر. في حين ازادت القيم في عينات السيطرة من 0.25 الى 1.75 ملغم/كغم في اليوم الخامس عشر. اما قيم TVN لعينات اللحوم المعاملة بتركيز 100 و 200 ملغم /مل في اليوم الخامس كانت (5.57,5.12) ملغم لكل 100 غم من اللحم بالتوالي والتي اصبحت (12.16,10.65) ملغم لكل 100 غم من اللحم في اليوم الخامس عشر. بينما زادت قيمة TVN في عينة السيطرة من 7.35 الى 15.76 ملغم لكل 100 غم من اللحم .

جميع النتائج اكدت ان المستخلص الكحولي لاوراق نبات *F. Carica* الغني بالمركبات الفعالة يمكن ان يكون ذات فعالية كبيرة كمضاد طبيعي (مضاد بكتريا ومضاد فطري ومضاد للاكسدة) لتي تحافظ على جودة لحوم البقر بتقليل اكسدة الدهون والنمو المايكروبي عند الخزن بالتبريد وخاصة بعد معاملته بتركيز 100 و 200 ملغم /مل من مستخلص النبات.

## Introduction:

Preservatives are chemicals added in small quantities to food products and used to prolong the life and viability of food nutrients, as well as to protect them from contamination and deterioration by hindering the growth of microbiota or enzymes found in food, delaying and preventing the oxidation of lipids and food oils (antioxidants), and preventing the process of food decay. In recent years, there has been a significant increase in the use of alternative substances such as plant-based food additives, herbs and their extracts. Studies have shown that these extracts have tremendous benefits, are safe, cause no health problems for consumers and are globally classified as additives. It has recently been proven that they do not cause any damage to the product, nor they have any side or toxic effects compared to chemicals and antioxidants that are used as additives for food preservation. Nitrites and nitrate compounds have profound side effects on consumers' health [1]. *Ficus carica* fig tree, highly nutritious tree, belongs to the Moraceae family and is one of the oldest planted fruit trees as well as an important crop worldwide for dry and fresh consumption. It is important for many elements and compounds. The fruits contain proteins and fibers at 5-9%, and vitamins such as B complex, C, and A [2] They have numerous minerals like sodium, calcium, phosphorus, manganese, magnesium, iron, copper, selenium, and zinc. The leaves also contain potassium and calcium. The plant contains several antioxidants and organic compounds like coumarins and rutin that are inhibitory to the growth of a number of microbiota [2, 3]. A recent comprehensive review also reported the clinical use of *F. carica* to treat anemia, cancer, diabetes, liver diseases, paralysis, skin diseases, and anti-oxidative and antidiabetic cases [4]. Many studies evaluated the antibacterial effects of different parts of *F. carica* when used to inhibit activity against two gram-positive bacteria and two gram-negative bacteria, for instance ethanolic extract from leaves of *F. carica* latex had a strong antibacterial effects at different concentrations against *Enterococcus faecalis* in the infected dentinal tubules when used as intracanal medicaments. Whilst another study indicated the inhibiting effectiveness against *Aspergillus oryzae* [5]. Other studies showed that a minimum inhibitory concentration

of ethanolic extract of the leaves of *F. carica* against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae* were noticed at 5% concentration [6, 4].

Current research aimed to study the possibility of using fig leaf extract grown in Iraqi environment as natural antioxidants in preserving refrigerated beef by measuring the thiobarbituric acid value (TBA) and total volatile nitrogen base (TVN) values and determining antimicrobial activity against *psychrotrophic* bacteria, pathogenic bacteria, and yeast isolated from meat samples.

## Materials and Methods

### 1. Collection of *Ficus carica* Leaves:

Leaves of *F. carica* were collected from fig trees planted in Baghdad University Gardens. They were washed with tap water to get rid of any contamination and dust and were then left to dry at room temperature away from sunlight, while flipping every day. Once dried, the leaves were ground and then kept in dark glass bottles till use.

#### 1.1 Preparation Alcoholic Extract from plant Leaves:

Thirty gram of leaf powder was mixed with 300ml of absolute methanol alcohol (99.9%). The mixture was then placed in a Soxhlet Extractor at 80–60°C temperature. Later the mixture was filtered with a Whitman filter. The filtrate was re-filtered through a 0.22 mm bacterial filter, assisted by an electrostatic vacuum device (Pump). The sterile extract was placed in sterile brown sealed bottles and later kept in the refrigerator for preservation till use [7].

#### 1.2 Preparation of Stock Solution:

A stock solution was prepared by taking 2 ml of the alcoholic extract of the leaves and dissolved it in 10 ml of sterile distilled water to obtain 200 mg/ml concentration of the stock solution. Later on, the concentrations of the stock solution were prepared at 100, 50, and 25 mg/ml. As for the control treatment, it was represented by sterile distilled water [8].

## 2. Detection of Active Plant Compounds:

According to some other studies, several reagents were used to investigate the active compounds of the alcoholic extract of *F. carica* leaves, with two reagents for each group of the following compounds [9, 10, 11]:

### 1. Detection of Alkaloids:

A- Mayer's reagent was used to identify alkaloids present in the plant's leaves. Several drops of the reagent were added to 1 ml of the alcoholic extract of *F. carica* leaves extract.

B. A few drops of Wachner's reagent were added to 1 ml of the alcoholic extract of *F. carica* leaves.

### 2. Detection of Saponins:

Added 5.0 ml of mercuric chloride to 5.1 ml of alcoholic extract until a white precipitate appeared which was a sign of a positive result.

### 3. Detection of Terpenoid:

Five milliliters of the alcoholic extract and 2 ml of chloroform were added to a test tube. 3 ml of concentrated sulfuric acid were gradually added to it until a reddish-brown layer was formed which was a sign of terpenoids' presence.

### 4. Detection of Flavonoids:

Several droplets of concentrated sulfuric acid were added to 1 ml of fig leaf extract. Magnesium crystals were added to 1 ml) of alcoholic extract. Then several drops of concentrated hydrochloric acid were added.

#### 5. Detection of Tannins:

Five milliliters of each plant extract were taken and 1% of lead acetate were added first, followed by 1% of ferric chloride solution. Appearance of the blue color was evidence of its presence.

#### 6. Detection of Phenolic:

Three milliliters of plant extract were added to 2 ml of ferric chloride to obtain a bluish green color which was an evidence of its presence.

### 3. Preparation of Meat Samples:

Ninety samples of minced beef were collected in sterile plastic bags from butcher shops indifferent areas of Baghdad (Three repeaters per sample). Then, transported the samples to the laboratory in icebox, as soon as possible (12).

### 4. Microbial Analysis:

#### 4.1 Bacterial Isolates from Minced Beef Samples:

A 25 g of freshly minced beef was added to 225 ml of sterile media (nutrient broth). Ten- fold serial dilutions were prepared from the mixture by taking 10 ml of it to 90 ml of nutrient broth and therefore for the other serial dilutions. Then, 1ml of fourth and fifth dilution ( $10^{-4}$  and  $10^{-5}$ ) was withdrawn and plated on Nutrient Agar and Mac Conkey agar inoculated at 37°C for 24 h. (12).

Based on the appearance of colonies, Gram staining and some biochemical tests ,bacterial colonies were identified (5)

To isolate *Salmonella* bacteria, it was inoculated in *tetrathionate* or selenite broths media for 24 hours at 37°C. (0.1 ml) of this microbial culture were placed in Dextrose Lysine for 24 hours at 37°C.

#### 4.2 Isolating Yeasts from Fermented Meat Products:

Portions of fermented meat products weighing 10gm were taken randomly and were then cut and grated under sterile conditions in the laboratory before being homogenized for 2 minutes with 90 ml of sterile peptone water. Ten-fold serial dilutions of samples were made. The yeast population was determined by taking 0.1 ml of each of the dilutions and spreading them on a potato dextrose agar (PDA) plate which were then incubated at 28°C for 48 hours. Colonies were isolated from the highest dilutions on plates using sequential transferring to carry the purification process of isolates out of PDA media to be incubated at 30°C for 5 days [13]. All identifications of individual yeast were diagnosed with a Vitek-2 yeast identification card, following the manufacturer's instructions. And then compared the final profile results with the database to obtain the identification of the unknown organism [11].

#### 4.3 Preparation of Microorganisms Inoculum:

A 0.2 ml of microbial cultures were taken from all the cultures (18–24 h) and dispensed into 20 ml of normal saline. They were absorbed at 580 nm and diluted to attain a viable cell count of  $10^7$ CFU/ml, which corresponds to 0.5 McFarland standards by using a spectrophotometer [11].

## 5. Effects of the Concentration of Alcoholic Extract on Isolated Microorganisms

Well diffusion method was applied to study the effects of the concentration of alcoholic extract on the isolated bacterial species and yeasts [14, 15]. A 0.1ml of inoculum was spread on Mueller-Hinton (bacteria sample) and PDA agar (yeasts) plates. The Petri dishes were labeled according to bacterial samples.

Five wells were punched into the agar medium with a sterile cork-borer (4 mm) and then were filled with 0.1 ml of each plant extract concentrate using a micropipette. Sterile water was used as a control. The plates were incubated with bacteria at 37°C for 24–48 h and the plates with yeast at 28 ± 2°C for 72 h. The antibacterial and antifungal activity screening was evaluated by measuring the zone of inhibition (mm). The experiment was set up in triplicate using a ruler around each hole.

### 5.1 Total *Psychrotrophic* Bacterial Count:

Total psychrotrophic bacterial count was estimated using the plate count agar method [16], taking 1 ml of the Aten- fold Serial dilutions ( $10^{-4}$  and  $10^{-5}$ ), previously mentioned in paragraph 4.1. And were inoculated at 4±1 °C for 7 days.

## 6. s Chemical Analysis:

The remaining minced beef samples were mixed and divided into four equal parts. The first part was used to make meat tablets without adding the plant extract (positive control) by six tablets weighing 30 g. Whereas, the remaining three parts were used in preparing meatballs containing the alcoholic extract of *F. carica* leaves (25, 50, 100, 200 mg/ml), in a(w/v) 1:1 ratio (meat: alcoholic extract). By 6 g/ tablets for weight 30 g. The tablets were placed in sterile bags and stored in the refrigerator at 4 c, they were examined for 15 days (1st, 10th and 15th) for chemicals analysis.

### 6.1 Moisture Content Determination:

The method described in [17] the percentage of moisture in one batch as controlling (without alcoholic extract), and four beef samples were treated with alcoholic extract (25, 50, 100, and 200) mg/ml. Took 50 g of meat, then dried the samples at 105° C until the weight was constant. The percentage decrease in weight was expressed as moisture content by the following equation:

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

The average of moisture content was taken for all meat treatments as the moisture content of the product for 20 days.

### 6.2 Total Volatile Nitrogen Base (TVN):

According to [18], TVN was calculated during the storage stages of meat samples. A 10 g of sample was weighed with 0.01 g accuracy and then added straight into the distillation bottle. Later 2 g of MgO were added along with 300 ml of distilled water and silicone as anti-foam material using glass balls and mixed well. A distillation flask was arranged in the distillation apparatus. A collection flask with 25 ml of boric acid (2%) was placed under the tube from the cooling coil with drops of the detector (methyl red). Distillation was started in the collection flask with 0.1 N H<sub>2</sub>SO<sub>4</sub>. The appearance of light pink (indicative of the point of the end) was calculated by the following equation:

$$\text{TVB-N mg N/100g} = \frac{(\text{ml} \times \text{N} \times 14.01)}{\text{g} \times 100}$$

ml = ml of H<sub>2</sub>SO<sub>4</sub> titrated, N =strength of H<sub>2</sub>SO<sub>4</sub>, g = weight of sample (10g) and 14.01 molecular weight of Nitrogen

### 6.3 Thiobarbituric Acid Value (TBA):

According to [19], 5 g of meat samples were homogenized with distilled water in a distillation flask, then 2.5 ml of 4 N HCl (pH = 1.5) was added. The mixture was heated in a boiling water bath for 35 minutes and then 5 ml of TBA reagent (0.2884 g/100 ml glacial acetic acid 90%) was added. The absorbance was measured at (538 nm) using a spectrophotometer after cooling the mixture to the ambient temperature. The blank out was carried on in the same manner, using 5 ml of distilled water. It showed the TBA value as (mg malonaldehyde/kg) sample by using the following equation:

TBA value (mg /kg) = absorbance × 7.8.

## 7. Statistical Analysis:

The data was analyzed using the Statistical Analysis System-SAS [20] to evaluate the effects of different treatments in properties studied on the storage period in a random design (CRD). The least significant difference (LSD) was used to compare treatment means.

## Results and Discussion:

### 1. Chemical Detection of Active Substances in *F. carica* Leaf Extract:

The phytochemical analysis showed that the alcoholic extract of *F. carica* contained terpenoid, flavonoids, tannins, saponins, alkaloids (Table 1).

**Table 1:** Active compounds in *F. carica* leaf extract

Active Compounds	Reagent	Result
Alkaloids	Mayer's reagent	+
	Wachner's reagent	+
Saponins	Appearance of foam	+
	Mercuric chloride	+Whiteprecipitate
Terpenoid	Chloroform+ H <sub>2</sub> SO <sub>4</sub>	+Reddish-brown layer
Flavonoids	Mg+ H <sub>2</sub> SO <sub>4</sub>	+Red precipitate
Tannins	Lead acetate +Ferric chloride	+Blue color precipitate
Phenolic	Ferric chloride	+ Green color

#### +positive test

These results agree with Abdel-Aziz *et al.*[21] who reported leaf extract of *F. carica* of bioactive compounds (flavonoids, steroids, tannins, saponins, and alkaloids). As well as, these results were consistent with Souhila *et al.* [22] who showed that the methanolic extracts of fig leaves contained the highest amounts of phenolic and flavonoids compounds and contained coumarins and saponins.

### 2. Bacterial and Yeast Isolations from Meat Samples:

Four types of bacteria were isolated from 90 fresh minced beef samples (*Proteus*, *Salmonella typhimurum*, *Escherichia coli* and *Staphylococcus aureus*). (Table 2)

Based on the appearance of colonies and their interaction with Gram stain and biochemical tests [3], referral to the Central Health Laboratory confirmed the bacteria diagnosis.

These results were consistent with Hamzah *et al.*[23] who isolated nine bacterial species in fresh sheep and cow meat collected from various areas of Baghdad, where the results showed contamination with *Staphylococcus* at the highest rate and *salmonella* in 18.5% of that meat. While the *Escherichia coli* and *proteus* bacteria were 18.5% and 3.7%. The most common cause of the difference in the isolation ratios of different bacteria may be due to the extent to which the health conditions followed in the slaughterhouses were non-compliant

with the least healthy measures. The cause may be contamination of the butchers hands or rinsing of carcasses after the removal of the infarction.

Under a microscope, three types of yeasts were isolated, as well from 30 fermented meat products samples (*Candida kruse*, *Candida Lambica* and *Zygosaccharomyces*) as shown in Table 2, based on the colony morphology, shape, color, and diameter and dependence on the Vitek two-identification system [24].

These results were consistent with Cocolin *et al.*[25] who isolated many yeast species from Italian fermented sausages and fermented meat products. Samples collected from various areas of Baghdad showed contamination with *Candida* species and *Penicillium* sp. There were fewer cases of finding *Debaryomyces hansenii*, *Candida zeylanoides* and *Zygosaccharomyces* isolated during meat product fermentation.

**Table 2:** Identification, number and percentage of the bacterial and yeasts isolated from samples (Minced beef and fermented meat products)

Isolates	Minced Beef (90 samples)		Fermented Meat Products (30 Samples)	
	Number	Percentage	Number	Percentage
<i>Proteus</i>	8	7.2%	-	-
<i>Salmonella typhimurium</i>	15	13.5%	-	-
<i>Escherichia coli</i>	18	16.2%	-	-
<i>Staphylococcus aureus</i>	35	31.5%	-	-
<i>Candida kruse</i>	-	-	10	3%
<i>Candida Lambica</i>	-	-	14	4.2%
<i>Zygosaccharomyces</i>	-	-	5	1.5%
<b>total</b>	76		29	

### 3.1 Effects of the Concentrations of Alcoholic Extract on Bacterial Samples:

The results of the antibacterial activity showed that all concentrations of alcoholic extract of *F. carica* leaves inhibited the growth of all bacterial isolates that have been examined in Table 3.

**Table 3:** Effects of alcoholic extract on bacterial samples:

Concentration mg/ml	Inhibition Zone (mm)			
	<i>Staphylococcus aureus</i>	<i>Proteus</i>	<i>Salmonella typhimurium</i>	<i>E. coli</i>
200	25±0.15	28 ±0.08	40 ±0.11	35 ±0.07
100	12 ±0.04	25 ±0.10	33 ±0.08	25 ±0.04
50	5 ±0.07	18 ±0.06	38 ±0.15	20 ±0.04
25	0.00 ±0.00	0.00 ±0.00	28 ±0.07	0.00 ±0.00
Control	0.00	0.00	0.00	0.00
LSD	1.058*	1.147 *	1.078 *	0.893 *
*(P≤0.05)				

The data presented as the average of three replicates ± standard error

The highest significant ( $p < 0.05$ ) inhibition diameters of compartments with control at the concentration of 200 mg/ml against *S. aureus*, *Proteus*, *Salmonella* and *E. coli* were 25, 28, 40, 35 mm respectively and the concentration of 100 mg/ml was 12, 25, 33, 25 mm respectively. The concentration of 50 mg/ml showed the least significant inhibition ( $p < 0.05$ ) against *S. aureus*, *Proteus*, *Salmonella* and *E. coli* which were 5, 18, 38, 20 mm respectively. While the concentration of 25 did not show significant inhibition ( $p < 0.05$ ) against all types of bacteria.

This result was in league with [26]. He used the disc diffusion method to determine the antibacterial activity against *S. aureus* and *E. coli*. The results showed that methanolic extracts of *F. carica* leaves displayed an antimicrobial effect against these bacteria. This was consistent with another study [27] which reported that methanolic extract of *F. carica* leaves have strong activity against oral bacteria. Findings of the current study clearly indicated that *Ficus carica* leaves had antimicrobial properties.

### 3.2 Effect of Alcoholic Extract on Meat Isolated Yeasts

The results of the activity of alcoholic extract on isolated yeasts are shown in Table 4.

**Table 4:** Effects of alcoholic extract on isolated yeasts

Concentration mg/ml	Inhibition Zone (mm)		
	<i>Candida kruse</i>	<i>Candida Lambica</i>	<i>Zygosaccharomyces</i>
200	20±0.07	35 ±0.12	<b>25 ±0.07</b>
100	15 ±0.08	30±0.15	<b>15 ±0.09</b>
50	0.00 ±000	0.00 ±000	<b>0.00 ±000</b>
25	0.00 ±000	0.00 ±000	<b>0.00 ±000</b>
Control	0.00	0.00	<b>0.00</b>
LSD	1.205 *	1.196 *	<b>1.081 *</b>

The data presented is the average of three replicates  $\pm$  standard error. At 200 and 100 mg/ml concentrations, the most significant inhibition zones ( $p < 0.05$ ) were recorded (mm) against *Candida kruse*, *Candida Lambica* and *Zygosaccharomyces*. The concentration of 200 mg/ml was recorded at 20, 35, and 25 mm respectively, and the concentration of 100 mg/ml was recorded at 15, 30, and 15 mm respectively. Whereas 50 and 25 mg/ml didn't show inhibition against purification yeasts.

These results agree with Abdel-Aziz *et al.* (21) who recorded that the ethanolic extracts showed good inhibitory effects against most yeast strains with inhibitions diameters zones.

### 3.3 Total Psychrotrophic Bacteria Count:

The results showed the logarithmic that the growth of psychrotrophic bacteria in control samples was 6.88 log per g, after 7 days of storage. Meat samples treated with alcoholic extract had shown lower logarithmic growth of psychrotrophic bacteria than those in control of the same storage period under similar conditions.

Treatment meat samples with alcoholic extract of *F. carica* leaves (25, 50, 100, 200 mg/ml) had a lower bacteria count 6.20, 4.22, 3.89, and 2.65 log per g respectively, after 7 days of storage period compared to the control samples. These findings indicated that *F. carica* alcoholic extract had a significant effect on the growth of psychrotrophic bacteria.



The current results are consistent with Abdel-Aziz et al. (21) who reported that treating pasteurized milk with aqueous fig leaves extract has the highest antimicrobial activity against Gram-negative psychrotrophic bacteria that cause post-pasteurization contamination after a long cooling exposure period (over 15 days).

Based on the microbial analysis shown in Tables 2, 3 and 4, and the phytochemical screening of fig leaf extracts in this study, *F. carica* leaf extracts were confirmed to be antibacterial against *S. aureus*, *Proteus*, *Salmonella*, *E. coli*, psychotrophic and Yeasts. However, all the microorganisms tested in this study were lower in beef meat samples treated with alcoholic extract of *F. carica* leaves at concentrations of 200 and 100 mg/ml. They could be used to delay bacterial growth and prolong the shelf life of beef meat.

Numerous research using *F. carica* extracts as antibacterial activity has indicated that the phytochemicals contain a few phenolic compounds, which have pharmacological properties, specifically flavonoids such as rutin, quercetin, and luteolin, phenolic acids such as ferrulic acid, furanocoumarins such as psoralen and bergapten, and phytosterols such as taraxasterol (28 and 29).

Simultaneously, terpenoid is also used to inhibition bacteria. It was noted in the membrane disruption triggered by the lipophilic compounds (30).

#### **4. Chemical Analysis:**

##### **4.1 Moisture Content:**

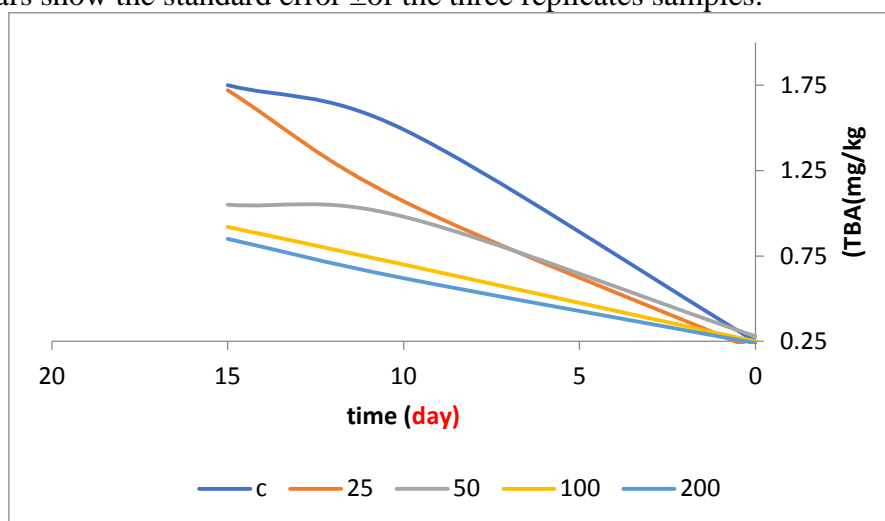
Moisture is a major component of meat products. In all the meat samples (treated and control), the percentage gradually decreased significantly ( $p < 0.05$ ) during the storage period shown in figure 1. At the beginning of storage treatment, meat samples and control 43.85% and 43.79 % of moisture content were recorded. Whereas after 20 days of storage, 35.92 and 35.62 percent of moisture content were recorded. However, the moisture content loss was significant ( $p < 0.05$ ) in the treated meat samples compartment compared with the control (non-treated meat).

This decrease in moisture content during the storage period could be due to evaporation of meat moisture in refrigeration, and the results agree with a study on raw beef which reported that the moisture content decreased in all the meat samples during the storage period (31).

##### **4.2 TBA:**

Effect of alcoholic extract from *F. carica*: (25, 50, 100, 200 mg/ml) on thiobarbituric acid reactive substances (TBA) values in fresh beef stored at  $4 \pm 1$  °C for 15 day are shown in Figure 1

\*Vertical bars show the standard error  $\pm$  of the three replicates samples.



**Figure 1:** TBA values of treatment meat samples with different concentrations of alcoholic extract of *F. carica*.

TBA gradually increased with the storage time in all samples. However, the treatment of meat with alcoholic extract of *F. carica* caused a significant reduction ( $p < 0.05$ ) in TBA value as compared to the control samples.

Values in control samples increased from 0.25 on day 5 to 1.75 mg/kg on day 15. When compared to control samples, treatment meat samples with 25, 50, 100, 200 mg/ml had lower TBA values (0.28, 0.25, 0.24 mg/kg) on day 5, and (1.72, 1.05, 0.92, 0.53 mg/kg) on day 15 (Figure 1). Generally, TBA values are accepted in all meat samples, and they refer to their quality (32).

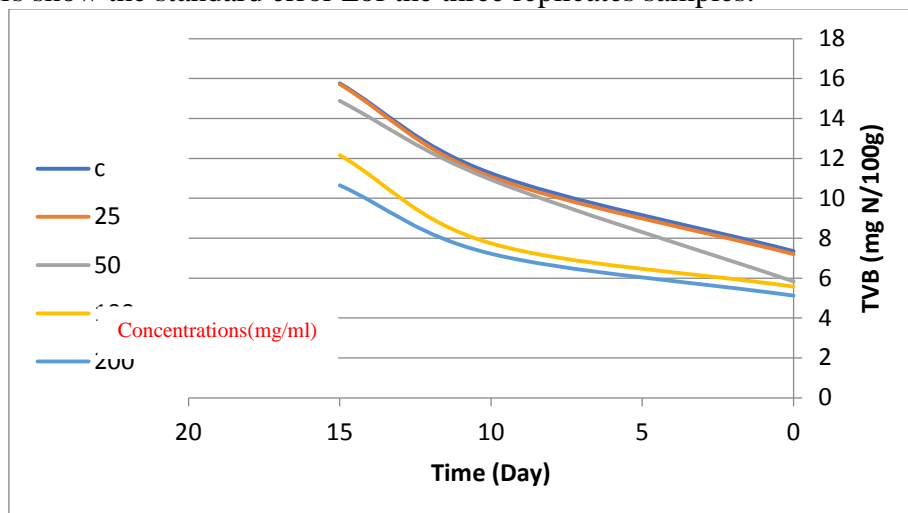
During frozen storage, TBA values increased in all treatments because of the lipid oxidation in meat. (33).

Phenolic compounds in *F. carica* cause an inhibition of the chain reactions during lipid oxidation. Again, these phenolic compounds might be involved in the inhibition of lipid oxidation, by inhibiting free radical formation through chelation of transition metal ions, specifically Fe and Cu (34).

#### 4.3 TVN (Total Volatile Nitrogen):

The results showed that treating meat samples with alcoholic extract of *F. carica* had significant effects on TVN values compared to control samples on the storage period (Figure 2).

Vertical bars show the standard error  $\pm$  of the three replicates samples.



**Figure 2:** TVN values of treatment meat samples with different concentrations of alcoholic extract of *F. carica*.

The TVN in control samples increased significantly ( $p < 0.05$ ) from 7.35 mg/day (5 to 15.76 mg N/ 100 g meat). In contrast, the TVN values of treatment meat samples with 100 and 200 mg/ml (5.57, 5.12) mg N/ 100 g meat, respectively, on day 5 were 12.16 and 10.65 mg, N/100 g meat on day 15 (Figure 2).

TVN values increased in all meat samples during the storage period. This may be due to the degradation of protein in meat and the activity of microbial and proteolysis enzymes accumulation of the free nitrogen groups that increased in meat during storage, resulting in a high concentration of TVN [35].

Based on the results, chemical analysis of TBA, TVN, and moisture content values showed that meat samples with alcoholic extract from the leaves of *F. carica* especially concentrations of 100 and 200 mg/ml, showed good natural antioxidant properties. Another study reported that phenolic compounds within *F. carica* have anti-oxidative activity against lipid oxidation [36].

The results of this study agree with those of Abdel-Aziz *et al.*[21], who reported that leaf extract of *F. carica* contained bioactive compounds (flavonoids, steroids, tannins, saponins, and alkaloids), specifically corresponding to the antioxidant and antimicrobial exercises. The disposition of activity is based on harm to the enzymatic forms included in vitality generation amid wrapping up or pulverization of the penetrability square of the cell film by shifting the physiological state of the cells or influencing the blend of the auxiliary components. The secondary metabolites of plants have a promising point of view as a source of viable antifungal specialists, such as compounds extracted from plants. These components, counting hydroquinones, naphthoquinones, alkaloids, and flavonoids, have shown antimicrobial activities.

A separate study also stated in their review that riboflavin, carotenoids and triterpenes in *F. carica* extract are responsible for its antioxidant property by preventing oxygen reactive, which prevents cellular component damage such as DNA, proteins, and lipids from being damaged [37].

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