The Evaluation Of Antibacterial Activity Of Gum Arabic Against Methicillin-Resistant Staphylococcus Aureus (MRSA) And Effectiveness On The Sensory Properties Of Beef Meat

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KEYWORDS

Methicillin-resistant Staphylococcus aureus, MRSA, Gum Arabic, beef meat, Sensory evaluation

ABSTRACT

The effectiveness of Gum Arabic as antibacterial agent against methicillin-resistant *Staphylococcus aureus* isolated from imported (frozen) and local (fresh) beef sold in Baghdad city was investigated. Gum Arabic (GA) was used at different concentrations (10%, 20%, 30%, and 40%), Gum Arabic powder was dissolved the in sterile distal water and stirred with a magnetic stirrer at room temperature for 4 hrs. at 55 °C until it was completely dissolved, bacterial isolates was confirmation by cultural, biochemical and vitk system 2. All suspected isolates of (MRSA) subjected to identification tests showed positive reactions as (MRSA), the number and isolation percentage (%) of S. *aureus* and (MRSA) isolated from (50) frozen meat samples pointed as 11/50 (22. %) and 4 / 50 (8.00%) respectively and the number and isolation percentage of *S. aureus* and (MRSA) were isolated from (50) fresh meat samples revealed that 16/50 (32.00%) and 3 / 50 (6.00%) respectively. The results showed that the antibacterial activity of Gum Arabic against (MRSA) needed high concentration as effective concentration (40%). Antibacterial activity of Gum Arabic against (MRSA) isolated from frozen meat gives inhibition zone (10 mm). The evaluation of antibacterial activity conducted through immersed the contaminated meat samples in the Gum Arabic solutions at both of ambient and refrigeration temperatures for four hours as contact time to studied the reduction in the logarithmic count of (MRSA), the initial numbers of bacterial counts before dipping treatment ranged between 5-6 $\log_{10}$ CFU/g. while the reduction after dipping and storage at refrigeration temperature (4ºC), reduced were 2 $\log_{10}$ CFU/g in addition the results showed that there was no difference observed in the organoleptic properties (Color, Flavor, tenderness, juiciness and color of cooked meat treated with Gum Arabic (40%) at different storage temperature compering to non-treated meat. **Conclusion** Gum Arabic can be used as antibacterial substance against MRSA and for keeping the bacteriological and properties of beef meat.

1. Introduction

Most of the Middle East peoples used meat as the main meals as one their habits (Geiker et al ., 2021) Meat characterization by higher quality of protein, essential, non-essential amino acids, important minerals and vitamins (Baik et al., 2023). Staphylococcus aureus is causing food poisoning due to its capacity to produce enterotoxins that can be contaminate the different kind's food and food products as meat, meat products through the slaughter or during different stages of processing of meat also the bovine milk and raw milk products regarded as important causes of mastitis (Ahmed, 2017; Al-Rasheed, et al., 2022). Staphylococcus aureus is opportunistic pathogen that leads in a wide spectrum of infections (Ibrahim, et al., 2018).

Resistance of pathogenic bacteria recently are increase through to the abuse use of antibiotics, for preventing the emergence pathogenic effects and reduced the spread of drug resistance microorganisms, natural substances can be used to control and inhibited the drug resistance bacteria (Keyvani-Ghamsari et al ., 2023). Methicillin-resistant Staphylococcus aureus (MRSA) is recently listed as the most prevalent resistant pathogen around the world, in Europe, United States, North Africa, many countries in the Middle East, and East Asia (Mohammed et al ., 2023). Gum Arabic (GA) also known as Acacia gum, is composed from both of poly-saccharides and glycoprotein this substance obtained from the branches of Acacia Senegal and Acacia seyal tress (Suleiman and Brima, 2021; Jiang, et al., 2023). GA has numerous applications due its high solubility and low-viscosity compared to other polysaccharides with excellent emulsifying ability and lack of toxicity (Daoub et al., 2018). The film-forming capacity, biocompatibility, and biodegradability can also support its application as film-forming matrix therefore recently used in the development of food packaging and /or coatings materials for food preservation (Ali and Alsayeqh, 2023).There are growing demands and interests for

the used natural plant extracts for protection the foods from oxidative and microbial deterioration, many researchers pointed out that used GA-based films and as edible coating with excellent functional properties by introducing the natural bioactive ingredients (Lin, et al., 2021 ;Alnadari et al., 2022;). Recent information revealed that there are multiple pharmacological and medical effects, of (GA) such as antibacterial effects, antibiotic, anti-inflammatory and nephroprotective activities (Jaafar, 2019).

The objective of the current study was to evaluate the sensory properties of beef meat after subjecting to dipping or washing application and its effectiveness against (MRSA) isolated from two kinds of meat imported (frozen) and local (fresh) sold in Baghdad markets.

2. Methodology

Samples collection

All meat samples (100 samples) were obtained from the local butchers and super marks in Baghdad city, during the period extended from March 2022 to January 2023.

Frozen sample:

Frozen meat samples (500) g vacuumed- sealed bag, the meat samples thawed in the refrigerator for 4 - 6 hours before subjecting to the confirmed tests .Frozen minced meat that kept in the original vacuum packaged and frozen bulk samples were obtained from supermarkets in Baghdad city, the meat cuts about (2 x 2 x 5 cm) thickens, width and length respectively these samples used for sensory properties , after holding the samples were thawed in aseptic conditions ,minced meat homogenized vigorously for 2-5 minutes inside sterile plastic bags by lab stomacher. One ml of the meat well mixed in the sterile 0.1% peptone water was transfer to universal bottle contain 9 ml of sterile peptone water for series of dilutions from (101 up 106) to detection and enumeration of staph aureus and MRSA before and after to subjecting to the different treatments.

Fresh sample:

Fresh beef meat (500) g. samples, were rapidly transported in a cooling icebox to the lab of Vet Medicine/ University of Baghdad laboratory, prepared for both of bacteriological and sensory analysis directly.

Processing of samples:

Identification of S. aureus were performed by pre-enrichment broth culture prepared as 25 g portion of meat homogenized with 225 mL of sterile buffered peptone water, incubated at 37 °C for 18–24 h. after pre-enrichment in buffered peptone water, 1 mL of the culture was uniformly mixed with 5 mL of sterile nutrient broth and incubated for 24 h at 37 °C. a loopful of culture broth was streaked onto Mannitol Salt Agar and Baird–parker agar with egg yolk in triplicate, incubated at 37 °C for 24 h. Three to five presumptive of S. aureus yellow color colonies from selective agar plate were picked, sub cultured to obtain a pure culture for further biochemical tests as Gram staining, catalase, coagulase Oxidase, DNase tests and VITEK-2 Compact System according to company's instructions (ISO 6888-1:1999/AMD 1:2003).

Preparation of Gum Arabic solution as natural antibacterial solution

Commercially available Gum Arabic as powder (Sudan / AL-Nuser) were used for preparation of stock solutions, to achieved the final concentration of 10,20,30 and 40% (w/v) by dissolved 10 ,20 ,30 ,40 gm in 100 ml of sterile distal water and stirred at room temperature by magnetic stirrer for 4 hrs. at 55 °C until it was completely dissolved dissolution, the solutions were sterilized by Millipore filters (0.45 µm),Meat cubes divided into two groups, the first group was kept without dipping in the Gum Arabic solution, kept in the sterile distal water as (control), second and third groups were dipped in the GA containing the best concentrations 40% for (4 hrs). as contact time at both temperature ambient and refiguration temperature. (Khudhir .,2021a).

Inoculum preparation:

MRSA isolates were inoculated in BHI broth and incubated for 6-8 hours; the bacterial suspension was adjusted with sterile phosphate buffer saline (PBS) to match the density of McFarland standard 0.5 (Umerska et al .,2017).

Antibacterial efficacy of gum Arabic as aqueous solution against MRSA:

Antibacterial solution of Gum Arabic was evaluated against MRSA isolated from meat, sterile plates of Mueller Hinton agar were uniformly swabbed with 100 µl of bacterial suspension containing 5x108 colony-forming unit/mL, inoculums of overnight cultures were prepared. Three to five collected colonies were picked by sterile wire loop and suspended into sterile peptone water. The turbidity of suspension to be inoculated was adjusted in line chart with 0.5 Mc Farland (5x 10-5) standard solution. Tested bacteria were swabbed over the Mueller-Hinton agar surface. seven-millimeter diameter well was made on the seeded agar surface using a sterile cork borer and 100 µL of the appropriate of each concentration of gum Arabic (40%) was placed inside the wells. Levofloxacin 250 mg/ml was served as positive controls and distal water was used as negative controls. The assays were conducted in triplicate. The Petri plates were incubated for 24 h at 37 ºC and the inhibitory activity was measured by calculating by the diameter (mm) of a clear zone around the well using ruler (Manilal et al., 2020).

Cooking procedure of meat after subjecting to antibacterial solutions for evaluation the sensory properties :The effect of Gum Arabic solution at (40% v/w) for keeping the sensory properties of meat , was assessed at the initial time and after the dipping of samples for (4hrs) as contact time at (4 ºC), beef meat sample was aseptically portioned into smaller pieces each peace about (25) g , were cooked at 190 ºC by electric oven for 40 mins, the internal temperature of meat was checked by a food thermometer and cooked meat with the internal temperature of 75±2ºC (Weerasinghe, et al., 3013).

Sensory evaluation of cooked meat samples

Panel consisted of ten panelists —five female and five males with aged ranged from 24 to 62 these assessors chosen from the staff of the Department of Veterinary pubic Heath at the University of Baghdad. Each one was separately asked to evaluated the cooked samples, for color, flavor, juiciness and overall acceptability, according to a nine-point hedonic test. Panelists were asked and answer write on the sample code of each sample 9-point hedonic scale1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither dislike or like, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely. All tests were conducted under good lights specially for examination of visual color to avoided any differences in the samples (Meilgaard, et al., 2006).

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Least significant differences (LSD) post hoc test was performed to assess significant differences among means whereas dependent and independent t tests were used in this current study.

3. Result and Discussion

Laboratory identification of MRSA by Cultural and Biochemical characteristics:

Gram staining of suspected colonies of *S. aurous* (MRSA) showed typical gram-positive cocci under light microscope. The biochemical tests, as oxidase negative results, positive for Dry spot (Staphytect Plus), latex agglutination, DNAse, catalase and coagulase testes while gave negative for oxidase test, meat samples were cultured directly on chromagar™ (MeReSa Agar Base), mannitol salt agar, Baird–parker agar, Nutrient agar, Mueller-Hinton agar and blood agar, the suspected colonies were appeared as shown in Table 1, also all suspected isolated showed positive results by the VITEK-2 Compact System.

Table (1) Cultural characteristics of *Methicillin resistance staphylococcus aureus (*MRSA*)* isolated from fresh and frozen meat

Cultural and biochemical characteristics (MRSA)		
Cultural characteristics	**Media**	**Cultural characteristics**
	MeReSa Agar Base	green to greenish blue colonies
	Mannitol salt agar	yellow (gold) colonies
	Baird–parker agar	dark gray to black colonies
	Nutrient agar	White colonies
	Mueller-Hinton agar	golden colonies
	Blood agar	beta hemolysis (incomplete hemolysis)

The *Methicillin resistance Staphylococcus aureus* MRSA appeared as yellow (golden) due to the fermenting the mannitol salt leads to change the phenol red to the golden color while can resist high salts concentration in Mannitol salt agar which used as selective medium, these characterization of the typical morphological of *S. aureus* (**Aziz and Lafta., 2021, Ahmed & Yousif, 2021).**The used of chromogenic medium with supplements aid to inhibit all *Methicillin susceptible Staphylococcus aureus* MSSA isolates and allowed the growth and multiplication of MRSA isolates, which are developed the bluish green color due to action of the chromogenic mixture (chromogenic substance, inhibitor mixture, carbonaceous, nitrogenous and vitamin B complex) incorporate in the medium , chromogenic agar promotes the detection of MRSA from the primary isolation plates during 24h after direct plating, without needing for extra biochemical tests (**Abd Zaid and Kandala, 2021**).

Isolation of (MRSA) form meat:

The result of cultural examination of frozen meat samples collected from different markets in Baghdad city revealed that four MRSA isolates, from different brand at different locations were positive for all cultural, biochemical, serological and vitke system 2 tests while, only three of MRSA isolates were isolated from (50) fresh meat samples as shown in Table 2 .

Table (2): Number and percentage (%) of *Methicillin-resistant Staphylococcus aureus* (MRSA) isolated from imported (frozen) and local (fresh) meat collected from different locations.

Source of meat sample	**Total number and percentage of (MRSA)**		
	No. of samples	**No. and percentage of *S. aureus***	**No. and percentage of MRSA**
Imported meat	50	11/50 (22.00)	4 / 50 (8.00%)
Local meat	50	16/50 (32.00%)	3 / 50 (6.00%)
Total	100	27/100 (27.00%)	7 / 100 (7.00%)
P-value		0.26	0.69

Contaminations of food by *S. aureus* bacteria in Iraq can occurs due the poor hygiene practices by infected workers ,bad transport conditions specially in the poor rural area ,through the different stages of food preparation from production, distribution and finally during the storage in the markets and butches shops specially when used improper refrigeration temperatures that enhance the bacterial growth and multiplication all these factors finally leads to productions of enterotoxins that causes human hazard . Sheet *et al* ., (2023) reported that (MRSA) isolated from different sources as meat samples and other tools and utensils used in the local butcher shops in Al- Mosul city the results indicated that meat had the higher prevalence of bacterial isolated (75%) compered to machines (38.9%), butchers tables (36.7%) and worker hands (38.5%). Aziz and Lafta., (2021) recorded that staphylococcus recorded as (27%) of the isolates that isolated from healthy and infected sheep. The results of the hygienic profile of the examined frozen meat samples, recorded more isolation percentage due to poor knowledge personal hygiene as frequent thawing and refreezing of frozen meat resulting

decrease in the meat quality and became unfit for human consumption, local authorities should educate the people that consumption this kind of meat, that must be insure to the safety about the danger of diseases that may be transmitted through the imported frozen meats and consumption the meat under suit cooked methods, the occurrence of bacterial isolations in the present study from frozen meats may indicated that imported bovine meats regarded as dangerous foods vehicle for (MRSA) infection. The incidence of (MRSA) recently recorded with increasing the infection in the calves, sheep, horses and poultry (Guo *et al*., 2022).

Antibacterial efficiency of Gum Arabic:

In this study, Gum Arabic (GA) was used to find out its effectiveness as a natural antibacterial against (MRSA) that isolated from frozen (imported) and fresh (local) meat collected from different markets and different places in Baghdad city, at different concentrations (10 %, 20%, 30 %, 40 %). the results recorded that the effectiveness of Gum Arabic solution at (40 %) with highest zone of inhibition (10 mm) as shown in the Tables (3 and 4) figures (1).

Table (3) Antibacterial activity of Gum Arabic against (MRSA) isolated from frozen meat.

MRSA isolated from frozen meat	**Zone of inhibition (mm) means**					
	Gum Arabic concentrations %				Control positive (levofloxacin 250mg/l)	Control negative (distal water)
	(10%)	(20%)	(30%)	(40%)		
Mean ±SE	0.00±0.00 e	3.75±0.47 d	4.75±0.47 c	10.00±0.40 b	47.00±0.00 a	0.00±0.00 e
LSD	0.95					

Table (4) Antibacterial activity of Gum Arabic against (MRSA) isolated from fresh meat.

MRSA isolated from fresh meat	**Zone of inhibition (mm) means**					
	Gum Arabic concentrations %				Control positive (levofloxacin 250mg/l)	Control negative (distal water)
	(10%)	(20%)	(30%)	(40%)		
Mean ± SE	0.00±0.00 e	2.00±1.00 d	5.33±o.33 c	9.00±0.57 b	47.00±0.00 a	0.00±0.00 e
LSD	1.51					

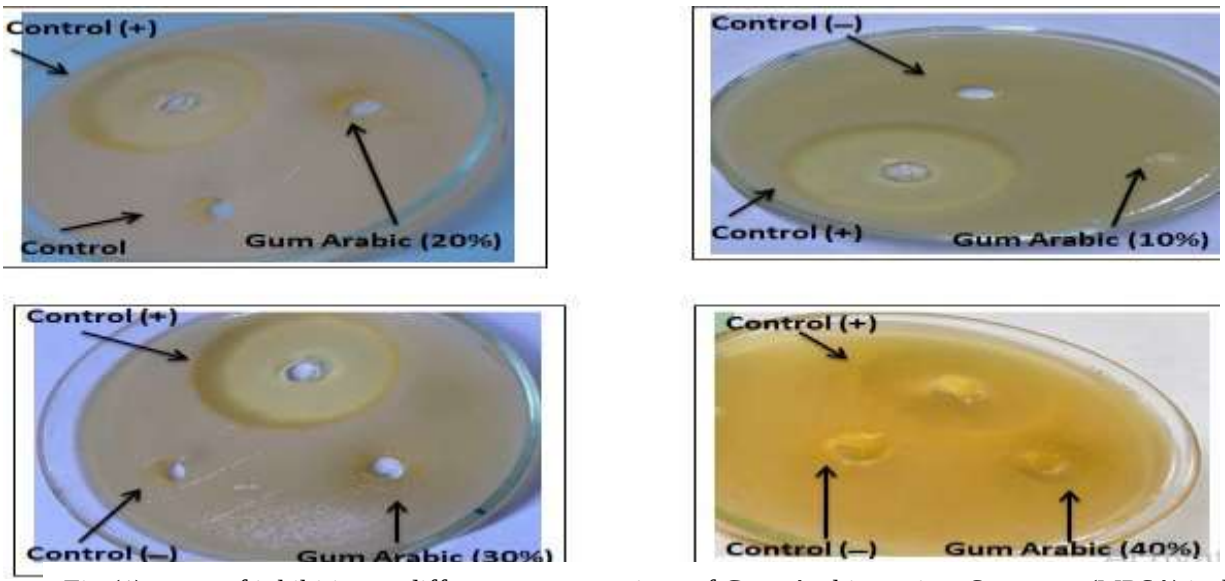

Fig (1): zone of inhibition at different concentrations of Gum Arabic against *S. aurous (MRSA)* isolated from frozen and fresh meat

Gum Arabic was used in the current study at different concentrations of 10, 20, 30, and 40%, higher concentration (40%) was showed the effectiveness against bacterial isolates. While in another study **Suhail and Khudhir, (2022**) who studied the effect of Gum Arabic (GA) at (5%) combined with ozonated water (0.5) ppm with contact time (30) minutes combined with other technology ozonated water (OW) at refrigeration (4^{o}C) temperature and evaluated the effectiveness on the antimicrobial activity and organoleptic characterizations of locally produced Cows and buffalo cheese, the results indicated that Gum Arabic at concentration (5%) and/or ozonated water was effective as antibacterial solution can improved the chemical quality properties of both locally produced cows and buffaloes' soft cheese. **Bnuyan, *et al*. (2015**) illustrate that the antimicrobial activity of Acacia Senegal and Acacia seyal two products used as prebiotics (Al Manna and Tayebat) were detected against both gram positive and negative bacteria and *C. albicans* aqueous extract was used, the highest zone of inhibition of Tayebat was (20) mm while Al Manna shown inhibition zone (10) mm aginst different microorganisms. secondary metabolites or alkaloids, flavonoids, saponin, tannin, glycosides, different volatile oil, hydrolysable and phenol substance were effective in the antimicrobial effect of Gum Arabic **(Idriss *et al*. 2023**)

Counting of *Methicillin-resistant Staphylococcus aureus* (MRSA) before and after subjecting to the Gum Arabic as antibacterial solutions

In current study, after confirmatory diagnosis for *Methicillin-resistant Staphylococcus aureus* (MRSA) isolates, subjected to antibacterial solution (Gum Arabic 40%) at two different temperatures, (ambient and refrigerator temperature). Meat samples were immersed or dipping in the 40% of gum Arabic as antibacterial washing solution, the best effective concentration, with contact time for 4 hours to determine the antibacterial effect of the treatment solution against MRSA, as shown in the Tables (5 and 6).

Table (5): Counting of *Methicillin-resistant Staphylococcus aureus* (MRSA) isolated from imported and fresh meat after Gum Arabic treatment (40 %) at refrigeration temperature for 4 hrs.

MRSA isolates isolated from frozen meat	**Count of (MRSA) CFU log /g before treatment (control)**	**Count of (MRSA) CFU log /g after treatment**
Mean ± SE	6.37±0.29 a	4.23±0.28b
MRSA isolates isolated from fresh meat	**Count of (MRSA) CFU log /g before treatment(control)**	**Count of (MRSA) CFU log /g after treatment**
Mean ± SE	5.46±0.28 a	3.38±0.27b

*Dependent test ($P<0.05$)

The results of present study agreed with (Binsi *et al* ., 2016) , who reported that application Gum Arabic vacuum packing and coating supported the preservation of the Indian mackerel to (11) days of chilled storage compared to conventional method of packing, also reported that edible coating with gum Arabic was increase the maintaining both of the textural integrity and elastic characterization due to improve the water holding capacity and prevent shrinkage the products by hindered the loss of water.

Means of reduction CFU.$\log_{10}$/g:

Reduction of bacterial counts according to the treatment was calculated as shown in Table (6) depending on the Bacterial reduction equation (Dickson *et al*., 1994).

Log Reduction= $\log_{10}$ (A) - $\log_{10}$ (B)

A= is the number of viable microorganisms before treatment.

B= is the number of viable microorganisms after treatment.

Table (6): Means of reduction CFU.$\log_{10}$/g of *Methicillin-resistant Staphylococcus aureus* (MRSA) isolated from local and imported meat after treatments with Gum Arabic (40%)

Treatments /Temperature	Mean ±SE
Reduction CFU.$\log_{10}$/g in imported (frozen) meat after subjecting to Gun Arabic treatment at refrigeration temperature.	2.14±0.02**b**
Reduction CFU.$\log_{10}$/g in local (fresh) meat after subjecting to action of gun Arabic treatment at ambient temperature.	2.08±0.01**b**
LSD	**0.72**

Results of the various treatments on the growth inhibition after treatment are displayed significant differences on the growth and inhibition of MRSA, as shown in Tables (6). The initial numbers of bacterial counts before dipping in the Gum Arabic (40%) ranged between 5-6 $\log^{10}$ CFU/g. The results showed that after dipping the meat pieces in the antibacterial solutions for 4 hours at ambient temperature and the refrigeration temperature (4ºC), the bacterial counts were reduced 2 $\log^{10}$ CFU/g (Table 6). With more efficacy at refrigeration temperature These results are in agreement with **Khudhir (2021b)** who reported that the antimicrobial activities of chitosan and/or Gum Arabic as aqueous solutions against many microorganisms in the local Iraqi cheese products showed significant antimicrobial activity at concentration (15%), without any inhibitory effect against *E. coli O157:H7*. Reducing the bacterial count of MRSA isolates by an amount 2 $\log_{10}$ CFU/g, indicated the effectiveness of this natural substance but on the higher concentration for preventing several bacterial infections. The antibacterial mechanisms behind these observations are still need more studying. Direct antimicrobial effect of (GA) was tested on three different *S. aureus* isolates which significantly inhibited the bacterial growth of *S. aureus* at concentration 40 mg/ml and 20 mg/ml the protection effects started at 240 min. (**Baien, *et al.*, 2020**), these results agreed with current study which showed that the inhibition the growth of MRSA after dipping meat sample in (40%) of gum Arabic for 4 hrs. Gum Arabic used in this study is Sudanese gum is more effective than other types, and this was proven by a study comparing the Omani gum and Sudanese gum, the result of Sudanese gum Arabic showed significant activity against all tested bacterial strains, compared with Omani *Gum acacia*, with higher antimicrobial activity against all bacterial strains (*S. aureus* and *Klebsiella pneumoniae*) , due to this substance contains several polar and non-polar bioactive materials (**Al Alawi, *et al.*, 2018**).

Organoleptic properties:

Organoleptic properties include color, Flavor, juiciness, tenderness and the effectiveness of gum Arabic on the pH of meat was evaluated. The examination was done by hedonic test consisting of Scale from 1-9 (9 = Like extremely /1 = Dislike extremely). As shown in Tables 8.

Table (7). pH value of fresh and frozen meat before subjecting to antibacterial treatment

Sources of samples	Mean of pH value	P-value*
Fresh meat	5.73±0.08	0.32NS
Frozen meat	5.66±0.12	

NS= no significant

Table (8): Evaluating the organoleptic properties of meat samples that subjecting to Gum Arabic (40%) at ambient temperature and refrigeration temperature (4ºC) for 4 hr.

Organoleptic Properties at ambient temperature	Control	Gum Arabic Group
color	8.6	8.6
Flavor	8.6	8.4

juiciness	8.8	8.8
tenderness	8.6	8.4
pH	5.8	5.7
Organoleptic index	8.00±0.65 a	7.90±0.65 a
LSD	2.02 NS	
Organoleptic Properties at refrigeration temperature (4ºC)	**Control**	**Gum Arabic Group**
color	8.6	8.6
Flavor	8.6	8.4
juiciness	7.4	7.8
tenderness	7.6	7.4
pH	5.9	6.0
Organoleptic index	7.54±0.56 a	7.52±0.57 a
LSD	1.77 NS	

*Dependent t test /NS= No significant

The food systems need to be evaluated with the aim of evaluation the sensory quality according to the consumer acceptance. In the present study, the sensory qualities of meat samples were evaluated in terms of color, Flavor, juiciness, tenderness with pH as shown in Tables (7,8) . The results of the organoleptic characteristics showed there were no significant differences after using Gum Arabic (40%) ,color, Flavor, juiciness and tenderness there are no significant differences between the gum Arabic group compared to the control group, and this indicates that gum Arabic do not affect the chemical or physical characteristics of meat, these findings in line with **Suhail and Khudhir, (2022)** who reported that the Gum Arabic as washing solution did not lead to any changes in appearance of the cheese products the keeping quality of food after subjecting with food products potentially leads to acceptance the products by the consumer

4. Conclusion and future scope

Gum Arabic used as antibacterial substance against MRSA with higher concentration and for keeping the sensory quality of beef meat with no significant effect on beef characterizations, as can improved the quality of cooked beef meat.

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Conflict Of Interest: The authors declare that there is no conflict of interest.

Authors Declaration: We hereby confirm that all the Tables in the manuscript are ours works.

Novelty Statement: This study points of the role of food (meat) as a harbor for very dangerous pathogenic bacteria (MRSA) and the risk for human health, natural food grade Gum Arabic is non expensive available as antibacterial substance and used as alternative material for keeping bacteriological quality and serving the organoleptic properties of meat sold in local Iraqi markets.

Authors' Contribution: Research is extracted from a doctoral Desertion of the Ph. D Student Qais Abdulrahman, University of Baghdad, Baghdad, Iraq. Proof Dr Zina Saab Khudhir (the adviser) was designed all the experiments. Qais Abdulrahman performed all experiments, collected the samples and data, and wrote the draft of the research under Dr zina supervision contributed to checked the analyses

of the data to the finalize the manuscript for journal submission. All authors approved the final version of the current manuscript for publishing in the respected Journal of Advances in animal and veterinary sciences

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