كشف وتقدير المركبات الفعالة لمستخلص الكمون المائي وزيته المستخلص ودراسة تأثيرها على إطالة فترة حفظ اللحم المفروم وفعاليتها على البكتيريا المرضية المعزولة منه

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- **تاريخ استلام البحث 2021/11/16 وقبوله 2021/12/30**
- **البحث مستل من رسالة دبلوم للباحث الأول .**

الخلاصة

أجُرِيتَ الدراسة الحالية في مختبرات قسم علوم الأغذية في كلية الزراعة جامعة تكريت وعدد من مختبرات محافظة بغداد ، تضمنت عزل وتشخيص نوعين من البكتريا المرضية من اللحم المفروم الذي تم الحصول عليه من الأسواق المحلية لمدينة تكريت من خلال زراعتها على الأوساط الزرعية Nutrient , Cetrimide Agar , MacCkonkey Agar وAgar Salmonella Shigella Agar تم تشخيصها مبدئيا اعتماداً على الصفات المجهرية والمزرعية الخاصة بها وتم تأكيد التشخيص لمستوى النوع باستخدام جهاز الفايتك Vitek 2 compact system حيث تم عزل و تشخيص نوعين من البكتريا السالبة لصبغة كرام وهما *E.coli* و*Salmonella enterica sub sp diarizonae*، بعد ذلك تم تحضير المستخلص المائي والزيت المستخلص ،كما تم تشخيص وتقدير المركبات الفعالة بالنسبة للزيت المستخلص باستعمال كروموتوغرافيا الغاز المتصل بجهاز مطياف الكتلة نوع Shimazu Qp 2010 plus GC/MS حيث تم تشخيص عدد من المركبات وعددها 29 واهم هذه المركبات الكومينول ويوجينول وبورنيل وغيرها ، بعدها تم دراسة الفعالية التثبيطية لهذه المستخلصات على أنواع البكتيريا التي تم تشخيصها مسبقا وبالتراكيز 2% و4% باستخدام طريقة الانتشار في الحفر Well diffusion assay method حيث أظهرت النتائج زيادة الفعالية المضادة للمستخلصات بزيادة التركيز المستخدم وان الزيت المستخلص كان أكثر فعالية في تثبيط نمو الميكروبات مقارنة بالمستخلص المائي فقد سجل اعلى قطر تثبيط 18 و17ملم عند التركيز 4% تجاه بكتريا *Salmonella enterica sub sp diarizonae* و*E.coli* على التوالي للزيت المستخلص ،كما تمت دراسة تأثير المستخلص المائي والزيت المستخلص للكمون في لوغاريتم أعداد البكتريا المختبرة والمشخصة مسبقا حيث سجلت اعداد قليلة في بداية فترة الحفظ بينما حصلت زيادة كبيرة في لوغاريتم أعداد البكتيريا قيد الدراسة عند اليوم السابع و لجميع التراكيز والأنواع البكتيرية المختبرة، اما فيما يخص الصفات الحسية للحم البقري المفروم والمضاف له الزيت العطري للكمون لوحظ ارتفاع بقيمة النكهة والطراوة والعصيرية والقبول العام بزيادة الكمية المضافة كما لوحظ انخفاض هذه الصفات بزيادة فترات الحفظ.

الكلمات المفتاحية : الكمون،الزيت الأساسي، المستخلص المائي،المواد الفعالة، الفعالية التثبيطية

Detection and estimation of active compounds for cumin aqueous extract and essential oil extracted and studying its effect on prolonged minced meat preservation and their effect on pathogenic bacteria isolated from it

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- **Date of research received 16 /11/2021 and accepted 30/12/2021.**
- **Part of the diploma dissertation for the first authors.**

Abstract

This study was conducted in the laboratories of Food Science dept. in the Faculty of Agriculture, Tikrit University and a number of laboratories in Baghdad, as it included the isolation and diagnosis of two types of pathogenic bacteria from minced meat samples obtained from the markets of Tikrit through cultivation on the agricultural media MacCkonkey Agar, SS. Agar, Nutrient Agar and Cetrimide Agar, where it was diagnosed based on its microscopic and agricultural characteristics, where two types of Gramnegative bacteria were included, *E. coli* and *Salmonella enterica sub sp diarizonae*and diagnosed to the species level using the Vitek 2 system . The aqueous and oil extract of the study samples were prepared, from which concentrations were prepared 2%, 4 %, after which the process of diagnosing and estimating the effective compounds in relation to the oil extract of cumin was performed using the gas chromatography technique connected to the mass spectrometer device type Shimazu Qp 2010 plus GC / MS, where a number of 29 compounds were diagnosed, the most important these are cominol, eugenol, borneil and others. The inhibitory activity of these extracts was studied on bacterial species by Well diffusion assay method, with concentrations of 2% , 4% , where the results showed , the highest inhibition diameter at concentration is 4% was 18 mm and 17 mm towards the growth of *Salmonella enterica sub sp diarizonae* and *E.coli,* respectively, for the oil extracted,and the effect of the aqueous extract and the oil extracted of cumin , was studied in the logarithm of the preparation of the test bacteria from study models and stored at a temperature of 5 ° C where no bacterial growth appeared the emergence of a few numbers at the beginning of the preservation period, while there was a significant increase in the logarithm of the number of bacteria at the seventh day for all concentrations and tested bacterial species,. focusing on the sensory characteristics of minced beef and added to it each of the aromatic oil of cumin, we notice an increase in the value of flavor, juiciness, freshness, general acceptance by an increase in the amount of addition, but these characteristics are reduced by increasing storage times.

Key Wards: cumin, Essential oil, aqueous extract, active substances, the inhibitory activity.

Introduction

Aromatic herbs are defined as seeds, leaves, stems, fruits or flowers of plants were are added to foods in order to obtain a distinctive taste and aroma (E.A.SCD/ K, 2010) Initially it was used in foods and food industries to obtain distinct flavors but nowadays they are known It contains many minerals, vitamins and essential oils that are used in preserving food,It is also characterized as cheapness, and there are no strict limitations in its use in compression with other manufactured additives purity and stability within the conditions of product preservation and circulation (Gheisariand Ranjbar,2012) Thereare a significant increasing useing natural materials, as materials extracted from natural sources like natural plant extracts have very great benefits, as they are safe so that they do not cause health problems for consumers (Benzie and Wachtel-Galor , 2011).

They are universally classified as Generally Recognized As Safe (GRAS) food additives, which are available and authorized to be added to meat and have no negative effects in compression to chemicals and antibiotics that are used as food additives that have recently been shown to have

carcinogenic effects such as nitrate and nitrite compounds, and therefore that plant products have an antimicrobial effect can be used as food additives to prevent the growth of food-spoil microorganisms (Lanciotti et al., 2004) . The importance of aromatic herbs are due to the aromatic oils that are concentrated in the active parts of the plant, according to the different plant variety, environmental conditions , drying, the degree of purity of impurities varies the proportion of oil and the type of oils in one plant (Zoubiri, 2011) Essential oils are complex compounds in terms of chemical composition it includes many components, which are chemically derived from terpenes and hydrophobic fluids (Gohri, 2011). Essential oils produced by plants are vital because they possess antioxidant and antimicrobial properties (Kirbaslar et al., 2009).

The mechanism of inhibition of aromatic herbs for microbes includes different forms of effect, such as effectiveness and activity of microbial enzymes ,effect of microbial cell protein, cell wall or membranes, mechanism of nutrient transport, the nature of active ingredient in essential oils as well as the target microbe type(Montivile and mattews , 2005; Aliabadi et al., 2012; Dixit,2013).

The effectiveness of essential oil depends mainly on the amount and concentration of the active substance, as well as the surrounding chemical and physical factors, bearing in mind that at the beginning of the adaptive phase of microbial growth the effect is more effective, not in the logarithmic phase due to high microbial load (Adeleye et al., 2003; Benavides et al., 2012),It also works as an antioxidant as it seizes newborn oxygen, and works to disrupt the action of mineral ions that aid in the oxidation process .These compoundswere *Caryophyllene Oxide*،*1.8-Cineole*، *α-phellandrene*،*α&β-Pinene*،*Carene*، *α-Terpinene*، *Germacrene*، *Sabinene*، *Camphene*، *β-Myrcene*، *Thymol*، *α-Terbineol*، *β-Cymene*،*[α-Cubebene*(Maestri et al., 2006).There are many different compounds present in cumin, including Alcohols, phenols, Terpenes, and Aldehydes. More accurately, Cuminaldehyde, eugenol, B-benin and some other minor compounds have been known. Cumin oils have been used as active antimicrobials against pathogens such as *Klebsiella pneumoniae*(Gohri, 2011)Cumin also contains a wide range of phenolic acids, such as gallic acid, cinnamic, rosmarinic, and vanillic acid.Cumin seeds contain many compounds of flavonoids including Luteolin, catechin, comedic, quercitin, and apigenin (Rebey et al., 2012).

Materials and methods

1. The Samples Collection

Minced beef was purchased from Tikrit city markets and transported to the Tikrit University Faculty of Agriculture laboratory using sterile polyethylene bags containing ice blocks and transfer to lab. ,As for the cumin plant, good qualities were bought from the local markets of the city of Baghdad and dust was removed from them, then they were ground with the German-made Blender electric mill, directly before extraction, The seeds of the cumin were dried and kept in sealed bags until use (Said et al., 2002).

2. Isolation and Diagnosis of Bacteria

The model was implanted on the appropriate Culture media, which included (MacConkey agar Nutrient, Cetrimide agar, and S. S agar,) supplied by the US Oxoid company . After the appearance of isolates, they were purified in media for each species, The isolation were initially diagnosed by cultured and morphologyical test using compound microscopic based on Burke Classifier in (Holt et al., 1994, Carr et al., 2000) and biochemical tests were achived using Vitek 2 system.

3. Prepare the aqueous extract of cumin

50 g of dry powder of the plant were weighed and mixed with 500 ml of distilled water and left for 24 hours at room temperature after which the mixture was filtered using several layers of medical gauze to get rid of plankton and impurities and then final filtering using a 3000 rpm centrifuge for 15 minutes Then filtered with Whatman No 0.1 filter papers,The filtrate was placed in a Petri dish at 40 ° C and then left to obtain the dry powder that was kept in the refrigerator in sterile bottles tightly closed until use,(The dry weight of the plant sample was 10 g dissolved in 10 ml of distilled water to obtain thestock solution 100%) the concentrations used in the experiment were prepared, which are 2% and 4% (El-Shennawy, 2009).

4. Cumin oil extraction by water distillation method

Cumin seeds (dry form) were ground with an electric mill before extraction to avoid loss of the volatile oil and by using the Clevenger device where the 200 g dry matter and the solvent (distilled water) were heated in a glass beaker and then condensed its vapor, collecting the extracted oil over the solvent layer on top The funnel and left for an hour for the oil to completely separate from the water, then the Volatile oil was collected in glass bottles and refrigerated until use (Mohamed et al., 2004).

5. Diagnosis and measurement of the active compounds present in cumin oil

Gas chromatography technique connected to the Shimadzu Qp 2010 plus GC / Ms mass spectrometer was used for the purpose of knowing its quality, proportions and molecular weight, the capillary column dimensions were 1MS 0.25µm،30mm ,0.25mm ,Inert.Cap ،capillary column,Injection volume 5 µl, The gas carrier is helium, and the rate flow constant is 1ml/min.

In gas chromatography (GC), separation occurs when the sample mixture is introduced (injected) into a mobile phase, the mobile phase is an inert gas. The mobile phase carries the sample mixture through what is referred to as a stationary phase. The stationary phase is usually a chemical that can selectively attract components in a sample mixture. The stationary phase is usually contained in a tube of some sort called a column.

6. Sensory tests

The sensory measurement of the transactions was done. Suraj (2011) method was followed to perform the sensory measurement of the transactions by five degrees of measurement, as follows: the flavor 5 - very strong, 1 - not completely present, juiciness 5 - very juicy, 1 - dry, freshness 5-soft Very, 1-rejected and general acceptance 5-Very acceptable, 1-rejected.

7. The effect of adding aqueous and oily extract of cumin to prolonging the shelf life of minced beef: 2 kg of beef was taken from the local market and placed in sterile polyethylene bags, then placed in sterile plastic containers with ice until they reached the laboratory. Samples washed with distilled water after removal of the external lipid and bone and then sterilized with autoclave to the microbial load become zero, then the meat sample was divided into two equal groups and each group was divided into 10 equal groups 100 g. different concentration of extracts 2% and 4% of the aqueous extract and the oily extract of the cumin put in each bag with two control bags with each group does not contain extracts but only distilled water (group Control),The first group was injected with *Salmonella . diarizona* and the second group was injected with *E. coli*, mixed well and kept for 1 days at 5°C. Ten grams of each sample were taken in a sterile and disinfected atmosphere at the beginning of the experiment and the process was repeated on the 3, 5 and 7 days. A series of dilutions were shown to the sample by adding 90 ml of peptone water and left to mix well for two minutes. Agar.SS and Maconkey and incubated for 24 hours at 37°C **(**Abdel-Malik, 2012). The purpose of this experiment is to studythe effect of the aqueous extract and the essential oil extracted for cumin in the logarithm of the number of test bacteria from the study samples and stored at a temperature of 5 ° C and at concentrations of 2% and 4% for a period of 1, 2, 5 and 7 days.

8. Inhibitory activity of different concentrations of essential oil by Well diffusion assay method: Antibacterial activity was determined by the Well diffusion assay ,each microorganism was inoculated by streaking the swab over surface of Mueller-Hinton Agar plates, were compared with MacFarland's solution 0.5 , and then holes were made with a diameter of 8 mm on the surface of the cultured medium using sterile cork piercing and after 15 minutes 200 micrograms of the prepared extracts 2% and 4% of the aqueous extract and the oily extract of the cumin for each hole, with a control hole containing sterile distilled water, after which the dishes are placed For two hours in the refrigerator, in order to spread the extract in the medium ,The inhibitory activity was determined for the extracts by measuring the diameters of the zone inhibition formed around the holes estimated in milliliters ,After incubating for 24 hours at 37°C (Kuete et al., 2008).

Results and discussion

1. Isolation and Diagnosis of Bacteria

Two types of pathogenic bacteria were diagnosed from minced beef samples by culturing them in the culture media MacConkey Agar, Nutrient Agar, S. S Agar and Cetrimide Agar were initially diagnosed based on their microscopic, morphological, and cultured properties. Microscopic examination of the isolated species showed that they are gram negative and bacilli forms, so all isolates initially be within the enteric family (Collee et al.1996).

Then, bacterial isolatesl were diagnosed to genes and species level using the Vitek2 system through a number of biochemical tests of isolated bacterial species of beef samples and included both the *E. coli* and*Salmonella enterica sub sp diarizonae*.

Table (1-1) Isolation and Diagnosis of Bacteria by *Vitek 2 system*

Well	Test	Mnemonic
2	Ala-Phe-Pro-ARYLAMIDASE	APPA
3	ADONITOL	ADO
4	L-Pyrrolydonyl-ARYLAMIDASE	PyrA
5	L-ARABITOL	lARL
7	D-CELLOBIOSE	dCEL
9	BETA-GALACTOSIDASE	BGAL
10	H2S PRODUCTION	H2S
11	BETA-N-ACETYL-GLUCOSAMINIDASE	BNAG
12	Glutamyl Arylamidase pNA	AGLTp
13	D-GLUCOSE	dGLU
14	GAMMA-GLUTAMYL-TRANSFERASE	GGT
15	FERMENTATION/ GLUCOSE	OFF
17	BETA-GLUCOSIDASE	BGLU
18	D-MALTOSE	dMAL
19	D-MANNITOL	dMAN
20	D-MANNOSE	dMNE
21	BETA-XYLOSIDASE	BXYL
22	BETA-Alanine arylamidase pNA	BAlap
23	L-Proline ARYLAMIDASE	ProA
26	LIPASE	LIP
27	PALATINOSE	PLE
29	Tyrosine ARYLAMIDASE	TyrA
31	UREASE	URE
32	D-SORBITOL	dSOR
33	SACCHAROSE/SUCROSE	SAC
34	D-TAGATOSE	dTAG
35	D-TREHALOSE	dTRE
36	CITRATE (SODIUM)	CIT
37	MALONATE	MNT
39	5-KETO-D-GLUCONATE	5KG

2. Diagnosis and measurement of effective compounds in cumin oil

Table, Figure (2-1), Table and Figure (2-1) show the separated active compounds and their molecular weights using GC-Ms technique, which numbered 29 compounds for cumin oil , The most important of these compounds are cominol, eugenol, borneil and others, as these compounds have an anti-bacterial activity against the gram negative (Lee et al., 2007)

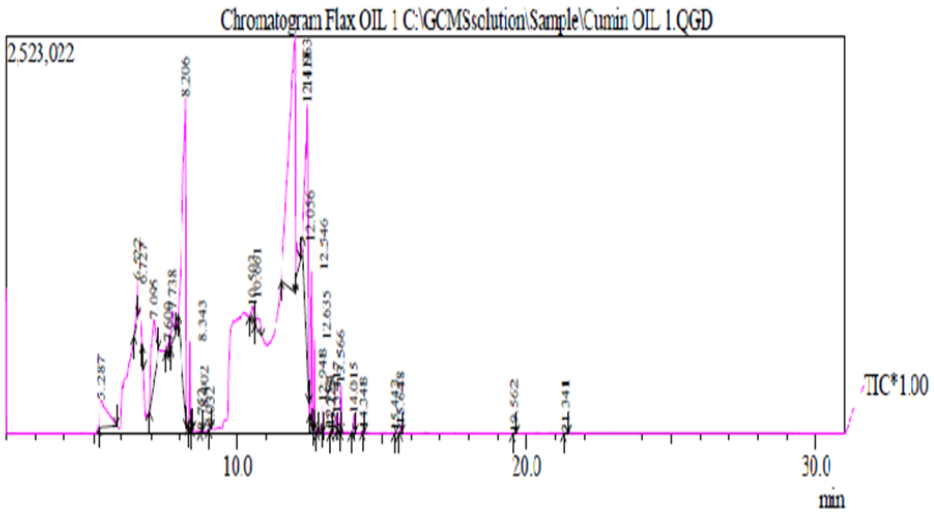
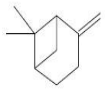
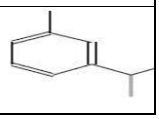
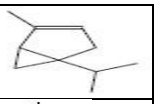
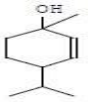
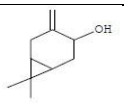
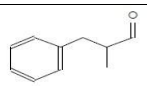
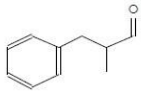
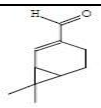
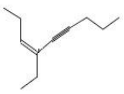
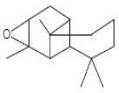
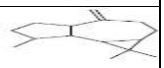
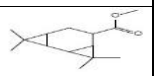
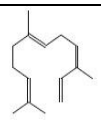
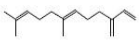
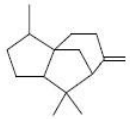
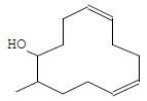
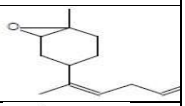
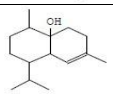
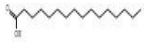
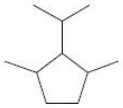


Figure (2-1): GC-Ms chromotagram for the cumin essential oil.

Table (2-1) Type of chemical compounds separated by GC-Ms technique and their proportions in cumin oil

No.	COMP. NAME	AREA	M.wt	Structure
1	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (.+/-.)- $$ 6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane # $$	3046002	136	
2	beta.-Pinene $$ Bicyclo[3.1.1]heptane, 6,6-dimethyl-2methylene- $$ 2(10)-Pinene $$ Nopinen $$ Nopinene $$ Pseudopinen $$ Pseudop	1010550	136	
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- $$ 2(10)-Pinene, (1S,5S)-(-)- $$ (-)-.beta.-Pinene $$ (-) 2(10)-Pinene $$ L-.beta	66358	136	
4	Benzene, 1-methyl-2-(1-methylethyl)- $$ o-Cymene $$ o-Cymol $$ o-Isopropyltoluene $$ 1-Isopropyl-2 methylbenzene $$ 1-Methyl-2-i	4297219	134	
5	Benzene, 1-methyl-3-(1-methylethyl)- $$ m-Cymene $$ .beta.-Cymene $$ m-Cymol $$ m-Isopropyltoluene $$ m-Methylisopropylbenzen	169669	134	

6	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- $$ 3-Thujene $$ .alpha.-Thujene $$ Origanene $$ 5 Isopropyl-2-methylbicyclo[3.1	1152312	136	
7	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)- $$ .gamma.-Terpinen $$ .gamma.-Terpinene $$ p Mentha-1,4-diene $$ Crithmene $$ M	15874683	136	
8	Cyclohexene, 1-methyl-4-(1-methylethylidene)- $$ p-Mentha-1,4(8)-diene $$ Terpinolen $$ Terpinolene $$ UN 2541 $$ .alpha.-Terpino	950054	136	
9	1,6-Octadien-3-ol, 3,7-dimethyl- $$ .beta.-Linalool $$ Linalol $$ Linalool $$ Linalyl alcohol $$ 2,6-Dimethyl 2,7-octadien-6-ol $$ allo-O	163524	154	
10	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis- $$ 4-Isopropyl-1-methyl-2-cyclohexen-1-ol # $$	20684	154	
11	(E)-3(10)-Caren-4-ol $$ 7,7-Dimethyl-4 methylenebicyclo[4.1.0]heptan-3-ol # $$	17995	152	
12	Pulegone $$ Cyclohexanone, 5-methyl-2-(1-methylethylidene)-, (R)- $$ p-Menth-4(8)-en-3-one, (R) (+)- $$ (+)-(R)-Pulegone $$ (+)-Pul	443163	152	
13	Propanal, 2-methyl-3-phenyl-	802075	148	
14	Propanal, 2-methyl-3-phenyl-	23021673	148	
15	2,4-Decadien-1-ol, (E,Z)- $$ (2E,4E)-2,4-Decadien-1-ol # $$	1016109	154	
16	2-Caren-10-al	10147173	150	
17	1,4-Cyclohexadiene-1-methanol, 4-(1-methylethyl)- $$ p-Mentha-1,4-dien-7-ol $$ 1,4-p-Menthadien-7-ol $$ (4-Isopropyl-1,4-cyclohexad	2464826	152	
18	3-Nonen-5-yne, 4-ethyl-, (Z)- $$ (3Z)-4-Ethyl-3-nonen 5-yne # $$	818906	150	
19	Longipinene epoxide	48400	220	
20	Aromadendrene $$ 1,1,7-Trimethyl-4 methylenedecahydro-1H-cyclopropa[e]azulene # $$	249214	204	
21	Tricyclo[5.1.0.0(2,4)]octane-5-carboxylic acid, 3,3,8,8 tetramethyl-, methyl ester	22162	222	

22	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)- $$ N (Z,E)-.alpha.-Farnesene $$ (3Z,6E)-3,7,11-Trimethyl-1,3,6,10-dodecatetraene # $$	237703	204	
23	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)- $$ .beta.-Farnesene $$ 7,11-Dimethyl-3-methylene-1,6,10-dodecatriene $$ (6E)-7	524830	204	
24	Cedrene $$ Cedr-8(15)-ene # $$	345134	204	
25	Cyclododeca-5,9-dien-1-ol, 2-methyl-, (Z,Z)-	28995	194	
26	cis-Z-.alpha.-Bisabolene epoxide	14587	220	
27	Cubenol $$ 1-Isopropyl-4,7-dimethyl-1,3,4,5,6,8a hexahydro-4a(2H)-naphthalenol # $$	123982	222	
28	n-Hexadecanoic acid $$ Hexadecanoic acid $$ nHexadecoic acid $$ Palmitic acid $$ Pentadecanecarboxylic acid $$ 1-Pentadecanecarbo	21709	256	
29	Cyclopentane, 2-isopropyl-1,3-dimethyl- $$ 2 Isopropyl-1,3-dimethylcyclopentane # $$	12016	140	

3. Sensory tests

Table (3-1) shows an increase in the value of all measured sensory characteristics, which included flavor, juiciness, freshness, and general acceptance of minced beef and added to the cumin essential oil for different time times by increasing the amount of addition compared to the control treatment on the other hand, the value of those characteristics decreased with increasing storage times.

Table (3-1) shows the sensory characteristics of the beef with added concentrations of 2% and 4% of the essential oil of cumin.

Time / The sensory characteristics	1 day			2 day			5 day			7 day		
	control	essential oil extracts 2%	essential oil extracts %4	Control	essential oil extracts 2%	essential oil extracts 4%	control	essential oil extracts 2%	essential oil extracts 4%	control	essential oil extracts 2%	essential oil extracts 4%
Flavor	2	2.5	4.25	1.75	2.25	4	1.25	1.75	3.5	1	1.25	2.75
Juiciness	2	2.5	4.25	1.75	2.25	4	1.25	1.75	3.5	1	1.25	2.75
Freshness	2	2.5	4.25	1.75	2.25	4	1.25	1.75	3.5	1	1.25	2.75
general acceptance	2	2.5	4.25	1.75	2.25	4	1.25	1.75	3.5	1	1.25	2.75

The reason for improving the flavor of the beef flavor is the effectiveness of the phenolic compounds of the essential oil in inhibiting the undesirable flavors in the meat by inhibiting the oxidation of fats, in addition to the fact that cumin is a kind of flavors of meat or food products as they mainly contain Borneol as well as many Terpenes Al-Jubouri (2002), and the increase in the juicy attribute may be due to the increased susceptibility to the bonding and holding of water, in addition to that the essential oil provides protection for meat proteins from some changes in their nature during storage times because they contain multiple phenolic compounds that limit the oxidation process and then protect the components of the cell membranes, which results in This results in a lack of clear liquid and maintaining the nutritional value of meat and itsproducts (Shi. J ,2003; Botsoglou, 2003).

As for the reason for the decrease in juices of minced meat during storage times, it is due to the increase in the loss of the clear liquid and the evaporation of moisture during storage and the lost weight during cooking (Miles et al. 1993). Also, the improvement in freshness may be due to the increase in the percentage of moisture and hence the freshness of the beef, as well as an increase in the solubility of muscle fiber proteins and provide protection and stability to the protein in the meat by the effect of adding essential oil, which is reflected in the improvement in the degree of that quality, King, A. J and others (1990),As for the decrease in the degree of freshness, it can be explained as a result of the loss of clear liquid and then the decrease in beef juice, and the improvement in the degree of general acceptance is due to the increase in flavor, juiciness and freshness that was reflected in the degree of general acceptance of the meat, which improved its sensory degrees.

4. The effect of adding aqueous extracts and essential oil extracts of cumin to extend the shelf life of minced beef

Tables (4-1) and (4-2) show the effect of the aqueous extract and the essential oil extracted for cumin in the logarithm of the number of test bacteria from the study samples and stored at a temperature of 5 ° C and at concentrations of 2% and 4% and for a period of 1, 2, 5 ,, 7 days, and it was observed through Table (4-1) that no bacterial growths appeared at the concentration of 4% of cumin oil and the appearance of a few numbers at the concentration of 2% compared to the control sample, The table also shows a small increase in the number of bacterial colonies tested during day 2 and 5 of the cold storage compared to the control sample, while a significant increase was observed in the logarithm of the numbers of test bacteria types after the seventh day, which amounted to more than 35 * 10^{-5}colony formation unit (CFU) / ml $^{-1}$ for all concentrations and tested bacterial species.

This is due to the decrease or fading of the role of the oil extracts used when storing for longer periods, and the results were encouraging the use of the essential oil extracted from cumin in increasing the period of keeping minced meats for periods longer than 5 days compared to the control sample.

Table (4-1) shows the effect of oily plants extracts with 2% and 4% concentrations of cumin in the logarithm of the number of test bacteria injected into the meat for specific time periods.

concentration of extract / Type of bacteria		essential oil extracts of cumin 4%	essential oil extracts of cumin 2%	Control
Salmonella enterica sub sp diarizonae	The logarithm of the number of bacteria	(1)day		
		No growth	$1*10^1$	More than 35
E. coli		No growth	$1*10^1$	More than 35
Salmonella enterica sub sp diarizonae		(2)day		
		$1*10^1$	$1*10^1$	More than 35
E. coli		$1*10^1$	$2*10^1$	More than 35
Salmonella enterica sub sp diarizonae		(5)day		
		$2*10^2$	$4*10^2$	More than 35
E. coli		$5*10^3$	$7*10^3$	More than 35
Salmonella enterica sub sp diarizonae		(7)day		
		More than $35*10^5$	More than $35*10^5$	More than 35
E. coli		More than $35*10^5$	More than $35*10^5$	More than 35

Table (4-2) showed the effect of aqueous extracts of cumin, at 2% and 4% concentrations on species of test bacteria and for periods of 1 , 2, 5, 7 days on the temperature of 5 ° C,As the results showed that very little growth occurred for both*Salmonella enterica sub sp diarizonae* and *E. coli* at 4% for the extract, while at 2% there was higher growth, due to the difference in concentration and thus a difference in the concentration of active substances, while On the second day, there was a dense growth of bacteria at a concentration of 2%. As for the concentration of 4%, bacterial growth increased more than the first day by a small percentage. Upon reaching the seventh day, dense growths appeared in relation to the concentration of 4% due to the decrease in the role of water extracts used when advancing storage periods.

The main reason for the ability of extracts to inhibit the bacteria is to contain them on a wide range of secondary metabolism compounds, for example flavonoids are attributed to their effectiveness towards bacteria because of their ability to form a complex compound with dissolved cell proteins.

Alkaloids interfere with cell DNA while tannins and phenolic compounds inhibit the action of enzymes and transporting proteins (Al-bayati 2008).

Table (4-2) shows the effect of aqueous extracts with 2% and 4% concentrations of cumin in the logarithm of the number of test bacteria injected into the meat for specific time periods.

concentration of extract / Type of bacteria		aqueous extracts of cumin 4 %	aqueous extracts of cumin 2%	Control
Salmonella enterica sub sp diarizonae	The logarithm of the number of bacteria	(1)day		
		$1*10^3$	$10*10^4$	More than 35
E. coli		$1*10^3$	$12*10^4$	More than 35
Salmonella enterica sub sp diarizonae		(2)day		
		$2*10^3$	More than $35*10^5$	More than 35
E. coli		$1*10^3$	More than $35*10^5$	More than 35
Salmonella enterica sub sp diarizonae		(5)day		
		$4*10^4$	More than $35*10^5$	More than 35
E. coli		$7*10^4$	More than $35*10^5$	More than 35
Salmonella enterica sub sp diarizonae		(7)day		
		More than $35*10^5$	More than $35*10^5$	More than 35
E. coli		More than $35*10^5$	More than $35*10^5$	More than 35

5. Determination of the inhibitory efficacy of aqueous extracts and the oil extracted from cumin towards the isolated bacteria under study by means of spread by drilling: The results of Table (5-1) showed the inhibitory efficacy of the aqueous extract and the extracted essential oil of cumin against the tested bacterial species. The ratio of the concentrations used was 2% and 4%, as the results showed an increase in the anti-aqueous extract and the essential oil's effectiveness by increasing the concentration used when it reached the highest inhibition diameter at The concentration was 4%, 18 mm and 17 mm towards the growth of *Salmonella enterica sub sp diarizonae* and *E. coli* for the oil extracted from cumin, respectively, while the aqueous extract of

the cumin recorded the highest inhibition diameter 14,15 at the concentration 4% towards the growth of *E. coli* and *Salmonella enterica sub sp diarizonae*, respectively.

Table (5-1) Diameters of the inhibition regions (mm), growth of bacteria treated with different concentrations of aqueous and oily extracts of cumin.

Type of bacteria / concentration of extract	*Salmonella enterica sub sp diarizonae*	*E. coli*
	Inhibition Zone (mm)	
Aqueousextracts4%	**14**	**15**
Aqueousextracts2%	**12**	**12**
essential oil extracts 4%	**18**	**17**
essential oil extracts 2 %	**13**	**12**

The inhibitory efficacy of extracts and extracted oil is due to the presence of phenolic compounds and tannins in oily extracts which have a high effect on inhibiting the growth of microorganisms, especially bacteria De and others (1998) explained the efficacy of inhibitory cumin fruit extracts to the alcohol and aldehyde compounds that were extracted from experiments that have proven inhibitory and killer efficacy for many pathogenic and spoilage microorganisms, and at a concentration of 2% the inhibition regions were less due to the lack of concentration of active substances. With regard to aqueous extracts, the results showed that inhibition was achieved, but with different diameters with regard to the used concentrations 2% and 4%, as the inhibition diameters in relation to the concentration were 4% higher, and this was due to the increased concentration of inhibitors in it.

Conclusions

The results in this study showed the plant extracts have led to an increase in the shelf life of minced beef, and that they are materials added to meat not only as food additives that give a distinctive flavor and smell, but also as materials to help preserve by inhibiting the growth of bacteria to contaminate food and thus extending the shelf life of meat and suitability for human consumption.

References

1. **Adeleyea . I. A., Ogunniyia . A. A. And Omonigbehinb . E. A. (2003)**:Antimicrobial Activity Of Some Local Herbs On Common Skin Pathogens . Bioscience Research Communications . 15 : 231236.
2. **Al-Bayati, M.K.A,(2001)**. A taxonomic study of the species Mentha, Micromeria, Thymbra, Thymus, and Ziziphora belonging to the Labiatae family in Iraq. PhD thesis. College of Science - University of Baghdad.
3. **Al-Jubouri, I.T,(2002).** Black Seed Overview and Overview through the Department of Herbal Medicine Specialized Symposium on Black Seed Research. May 10 - College of Pharmacy – University.
4. **Abdel-Malik, A. M**،(2013). The effect of spices on increasing the period of keeping poultry meat. Assiut Journal of Environmental Studies - Issue thirty-eight. Faculty of Veterinary Medicine, Assiut University.
5. **Aliabadi, M. Darsanaki, R, Rokhi, M. Nourbakhsh, M. Raeisi, G.(2012).** Antimicrobial activity of olive leaf aqueous extract Scholars Research Library Annals of Biological Research, 3 (8):4189-4191.
6. **Benavides . S., Villalobos-Carvajal . R. And Reyes . J. E. (2012)**. Physical,Mechanical And Antibacterial Properties Of Alginate Film: Effect Of The Crosslinking Degree And Oregano Essential Oil Concentration .Journal Of Food Engineering . 110: 232-239.
7. **Benzie, I.F.F. and Wachtel-Galor, S.(2011).** Herbal Medicine: Biomolecular and clinical aspects, 2 nd ed., CRC press, 488p.
8. **Botsoglou, A. G. and Papageorgiou, G.(2003)**. The effects of dietary oregano essential oil and alpha- Tocopherol acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. Meat Sci., 65: 1193- 1200.
9. **Colle ,J.G; Fraser,A.G; Marmino,B.P. and Simmons, A. (1996)** .Practical medical microbiology .14th ed. Churchill Livingstone Inc., New York.
10. **Carr, F. J.; Chill, D. and Maida, N. (2002).** The lactic acid bacteria. A literature survey. Critical Reviews in Microbiology, 28 (4): 281-370.
11. **De, G . H. and Rauen, U . (1998) .** Tissue injury by reactive oxygen species and the protective effect of flavonoids . J. Fundam. Clin. Pharmacol. 12 , 249-255 .
12. **Dixit , p.(2013)** Acomparative screening of antibacterial activity of Inisomeles indica with Mentha piperita against human pathogenic microorganism. indian journal of fundamental and applied life sciences,3(1):85-88pp.issn:2231-6345

13. **E.A.S., East African Standard (2010):** CD/K/714, ICS 67.120 Guide For The Microbiological Quality Of Spices And Herbs Used In Processed Meat And Poultry Products . Tanzania.
14. **El-Shennawy, Fawzia Ahmed (2009)**. Effect of a mixture of Peganum harmala seed extract and Artemisia harba-alba wormwood leaves against Entamoeba histolytica in glass. Iraqi Journal of Science 50 (3): 290-295.
15. **Gohari, A. R. and Saeidnia, S. (2011).** A review on phytochemistry of Cuminum cyminum seeds and its standards from field to market. Pharmacognosy Journal, *3*(25), 1-5.
16. **Gheisari,H.R.and Ranjbar,V.R.(2012)**.Antioxidative antimicrobial effects of garlic in ground camel meat .Turk.J.vet.Anim.Sci.;36(1):13-20
17. **Holt, J. G.; Kring, N. R.; Sneath, P. H. A.; Staley, J. J. and Williams, S. T . (1994).** Group 17 gram-positive cocci. Bergey's Manual of Determinative Bacteriology. 9th ed. W. R. and Forlifer, L. E. pp. 527-543.
18. **King, A. J.; Dobbs, J. and Earl, L. A. (1990)**. Effect of selected sodium and potassium salts on the quality of cooked, Dark-meat Turky patties. Poultry Sci., 69:471-476.
19. **Kırbaşlar, F. G., Tavman, A., Dülger, B. And Türker, G (2009):** Antimicrobial Activity Of Turkish Citrus Peel Oils. Pak. J. Bot., V. 41(6), Pp. 3207-3212.
20. **Kuete V, Ngameni B, Simo CCF, Tankeu RK, Ngadjui BT, Meyer JJM, Lall N,Kuiate JR, 2008.** Antimicrobial activity of the crude extracts and compounds from Ficus chlamydocarpa and Ficus cordata (Moraceae). J Ethnopharmacol 120:17-24.
21. **Lanciotti, R.; Gianotti, A.; Patrignani, N.; Belleti, N.; Guerzoni, M.E. And Gardini, F. (2004):** Use Of Natural Aroma Compounds To Improve Shelf-Life Of Minimally Processed Fruits. Trends In Food Science & Technology, 15, 201-208.
22. **Lee, S. O., Choi, G. J., Jang, K. S., Lim, H. K., Cho, K. Y. And Kim, J. C.(2007):**Antifungal Activity Of Five Plant Essential Oils As Fumigant Against Postharvest And Soilborne Plant Pathogenic Fungi. Plant Pathol. J., V. 23(2), P. 97.
23. **Maestri . D. M., Nepote . V., Lamarque . A. L. And Zygadlo . J.A(2006):** Natural Products as Antioxidants. Research Signpost. 37/661: 105-135.
24. **Mohamed, N. A.;Murad, N.A. and Abd. Aziz M.K. (2004).** EDO_4 process design of hydrodistillation in ginger oil production. The 4th Annual seminar of national Science fellowship. Center of lipids Engineering and applie Research (CLEAR), University Teknologi Malaysia.Pp:527-530.
25. **Miles, R. D. 1993**. Manipulation of micro flora of the gastrointestinal tract: natural ways to prevent colonization by Pathogens. PP. 133-150 in: biotechnology in feed industry. Proc. Alltech's 9th Ann. Symp. T.P. Lyons, Ed., Alltech Technical Publications, Nicholasville. KY.
26. **Montville, T. J. and K. R. Mattews (2005).** Food Microbiology: an Introduction .ASM Press. New York.
27. **Peter . K. V(2004):**Handbook Of Herbs And Spices 2 . CRC Press .New York.

28. **Rebey, I. B.; Zakhama, N.; Karoui, I. J.; Marzouk, B. (2012).** Polyphenol composition and antioxidant activity of cumin (Cuminum cyminum L.) seed extract under drought. *Journal of food science*, *77*(6).
29. **Said . O., Khalil . K., Fulder . S. And Azaizeh . H. (2002):**Ethnopharmacological Survey Of Medicinal Herbs In The Golan Heights And The West Bank Region . Journal Of Ethnopharmacology. 83: 251– 265.
30. **Shi, J.; Jianmuls, Y; Joseph, Yu.; Pohorly, E. and Kakudas, Y. O. (2003).** Polyphenolics in grape seeds Bio chemistry and Functionality. J. Med. Food, 6: 291-299.
31. **Siraj, I., H., S.(2011).** Effect of using natural additives rich in catechins and lycopene on some traits of cold-stored buffalo meat. Master Thesis. College of Agriculture, University of Baghdad.
32. **Zoubiri . S. And Baaliouamer . A(2011):** Chemical Composition And Insecticidal Properties Of Some Aromatic Herbs Essential Oils From Algeria . Food Chemistry . 129: 179-182.