HYGIENIC STATUS OF SOME MEAT PRODUCTS WITH SOME TRIALS TO IMPROVE THE QUALITY AND EXTEND SHELF LIFE

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Abstract

Meat products are good sources of all essential amino acids, B-complex vitamins and minerals. Despite this high biological value, meat products act as good substrates for microbial growth and have been implicated in many foodborne disease outbreaks. Therefore, A total of 80 random samples of minced meat, sausage, burger and luncheon (20 of each), were randomly collected from different markets at Zagazig City, Sharkia governorate, Egypt for bacteriological examinations. Results revealed contamination of the examined products by Pseudomonas, Enterobacteriaceae and Hydrogen sulfide producing bacteria. Furthermore, the antimicrobial activity of some natural oils (cumin, thyme and rosemary oil 1%) were investigated; results revealed that they act as good preservatives to meat. Rosemary oil was the most effective oil followed by thyme then cumin oil.

Key words: Meat products, spoilage, Food poisoning, Essential oils

1. Introduction

Meat and its products are considered as an excellent source of protein, fat, B-vitamins, Iron, Zinc and Vitamin A as well as the essential amino-acids. Meat products are favored by numerous people groups, but if these products are not processed, packaged, distributed and stored correctly; it will spoil rapidly and represent a risk to consumers' health. Meat products can be contaminated with microorganisms from meat handlers during processes of manufacturing, packing and marketing in addition to improper cooking, refrigeration and storage which may lead to meat borne illness. P*seudomonas spp*. are proteolytic microorganisms, they can separate protein, delivering an assortment of smell and flavor deteriorations as well as slime appearance on meat surfaces. The Enterobacteriaceae is a family of Gram-negative, non-spore-forming bacteria and is a standout amongst the most critical gatherings of microscopic organisms known to man which are the most dominating species in all food contamination cases related with some meat products.Hydrogen sulfide-producing bacteria include a wide assortment of bacterial species that are universal in the earth and can develop in high protein products such as meat and meat products. They can prompt generation of hydrogen sulfide, which is dangerous to wellbeing. These include H_2S producing microorganisms include *Pseudomonas spp., Citrobacter spp., Aeromonas spp., Salmonella spp. and Escherichia coli***(Atterbury*et al*., 2007)**.

Essential oils are aromatic and oily liquids that are extracted from different parts of plant materials, (seeds, leaves and fruits)**(Gutierrez *et al.*, 2008)**. These substances had various antimicrobial activities as they act mainly on the cytoplasmic membrane of the bacterial cells in addition to hydroxyl group related to the deactivation of many enzymes. Furthermore, this group can cause loss of cell component, changes on fatty acids and phospholipids, and antagonize energy metabolism and genetic materials synthesis **(Di Pascua *et al.*, 2005)**. Among these essential oils, rosemary (*Rosmarinusofficinalis L.*) oil which is broadly utilized to preserve meat and meat products. Thyme (*Thymus vulgaris L*.) is an aromatic herbbelongs toLabiateae family, utilized in foods for many purposes. The most important compounds of thyme are the phenols thymol (44-60%) and carvacrol (2.2-4.2%), as well as the monoterpene hydrocarbons ρ-cymene (18.5-23.5%) and γ-terpinene (16.1-18.9%) **(Di Pasqua*et al.*, 2005)**; these active have antimicrobial action against a wide range of gram negative or positive bacteria **(Ozcan*et al*., 2003)**. Cumin (*Cuminumcyminum* L.) is a flavor generally utilized as a germicide specialist, and it additionally has an amazing antimicrobial action on various types of microscopic organisms, pathogenic and non-pathogenic growths for people **(De *et al*., 2003).**Subsequently, the present investigation was planned to evaluate to what degree meat products in Sharkia Province are bacteriologicallycontaminated in addition to determine the antimicrobial activity of cumin, thyme and rosemary oil 1%.

2. Materials and method

Collection of samples

A total of 80 random samples of minced meat, sausage, burger and luncheon (20 of each), were randomly collected from different markets in Zagazig City, Sharkia governorate,Egypt. All collected samples were immediately transferred, aseptically handled and moved promptly to Food Control lab for, bacteriological and chemical examination.

***Preparation of samples*:**

According to technique recommended by**APHA(2001)**.

Determination of Pseudomonas count:

On Pseudomonas Agar Base (CM 559; Oxoid) supplemented with cetrimide, fucidin, and cephaloridine (CFC) supplements **(Roberts and Greenwood, 2003).**

Determination of Enterobacteriaceae count:

On violet red bile glucose agar (VRBG) agar(**ISO, 2004)**

Determination of Hydrogen Sulphide producing bacteria count:

On iron agar Lyngby (CM 964; Oxoid, Basingstoke, Hampshire, UK) according to **Gram et al. (1987)**.

Evaluation of the effect of some essential oils on the quality of minced meat:

The selected oils arecumin oil 1% (*Cuminumcyminum* L.), thyme oil 1% (*Thymus vulgaris L.*) and C. rosemary 1%(*Rosmarinusofficinalis L.*). These oils were obtained from the squeezing and extraction of natural oils in the National Research Center, Dokki, Giza.

Design of the experiment:

In the laboratory, minced meat was divided into four equal groups:

1. Control group: 500 grams of minced meat, separated to five clean Ziploc bags.
2. Cumin treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 m1 of cumin oil to obtain final concentration 1% then separated to five clean Ziploc pages.
3. Thyme treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 m1 of thyme oil to obtain final concentration 1%, then separated to five clean Ziploc pages.
4. Rosemary treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 ml of rosemary oil to obtain final concentration 1% then separated to five clean Ziploc pages.All the groups were sampled immediately after treatment (zero time) and every 48 hours. All groups were kept in afridge at 4° C.

Statistical analysis.

One way analysis of variance (ANOVA) was done by using the statistical package for social sciences (SPSS-14; Chicago, IL, USA). Statistical significance was evaluated using tukey-kramer honestly significant difference tests with $p < 0.05$.

3.Results

A. Bacteriological evaluation of meat products

Results in **Table (1)** declared that the mean count of pseudomonas in the examined minced meat, sausage,burger and luncheonsamples was4.8 ± 0.092, 3.7 ± 0.1129, 3.5± 0.1978 and 2.7 ± 0.1288Log_{10}CFU/g, respectively.While, the mean count of Enterobacteriaceae was4.2 ± 0.1294**,** 3.6 ± 0.1059, 3.2 ± 0.1510and 2.7 ± 0.1628Log_{10}CFU/g in the examined minced meat, sausage,burger and luncheon samples, respectively (**Table 1)**.

Table (1) Bacteriological examination of some meat products (n=20 of each) log_{10} CFU/g

Samples	Pseudomonas		Enterobacteriaceae		H_2S producing bacteria	
	Range	Mean± S.E	Range	Mean± S.E	Range	Mean± S.E
Minced meat	3.8-5.3	4.8^a± 0.0921	2.9-5.1	4.2^a± 0.1294	3.4-4.9	4.3a± 0.0921
Sausage	2.7-4.2	3.7^b± 0.1129	2-4.3	3.6^b± 0.1059	2.3-3.7	3.1b± 0.1306
Burger	2.4-4.5	3.5^b± 0.1978	2.4-3.7	3.2b± 0.1510	2.7-3.9	3.3b± 0.0985
Luncheon	2-3.7	2.7^c± 0.1288	1.8-3.6	2.7c±0.1628	1.3-3.2	2.5c± 0.0935

n: number of the examined samples, CFU/g: Colony Forming Unit per gram S.E: Standard error of mean, (a, b, c and d): Means within the same column bearing different superscript letters are significantly different (P< 0.05).

It was found that, the mean count of hydrogen sulphide producing bacteria was 4.3 ± 0.0921, 3.1± 0.1306, 3.3 ± 0.0985 and2.5 ± 0.0935Log_{10}CFU/g in the examined minced meat, sausage,burger and luncheon samples, respectively.

B.Effect of cumin, thyme and rosemary oil 1% on bacteriological quality of minced meat:

Pseudomonas counts of control untreated samples gradually increased along storage period; the initial Pseudomonas count value was 4.19±0.1925Log_{10}CFU/g**(Figure 1)**, while it increased to 4.93± 0.0712,5.69± 0.1867 and 7.08± 0.3298Log_{10}CFU /g at the 3rd, 5th and 7thday of storage, respectively**(Figure 1)**. Pseudomonas counts in treated samples by cumin, thyme and rosemary oil1% at zero daywas 4.08±0.1672, 3.98±0.0454 and 3.83±0.1514 Log_{10}CFU/g, respectively.Rosemary oil1% was the most effective in Pseudomonas reduction followed by thyme oil 1% >cumin oil 1%.By the third day of storage, treatment by thyme and rosemary oil 1% proved significant reduction ($p<0.05$) inpseudomonas count;it was 4.28±0.0352 and 3.04±0.2783Log_{10}CFU/g. Meanwhile at the 5th day, the three treatment trials proved significant reductions in Pseudomonas counts ($p<0.05$). It ranged from 4.94 to 5.48, 4.38 to 5.42 and 4.20 to 4.90 Log_{10}CFU/g after treatment by cumin, thyme and rosemary oil 1%, respectively.By the 7th day of storage, after treatment by cumin, thyme and rosemary oil 1%,Pseudomonas counts reduced to 6.13±0.2427, 5.87±0.1154 and 5.19±0.2131Log_{10}CFU/g, respectively **(Figure 1)**.

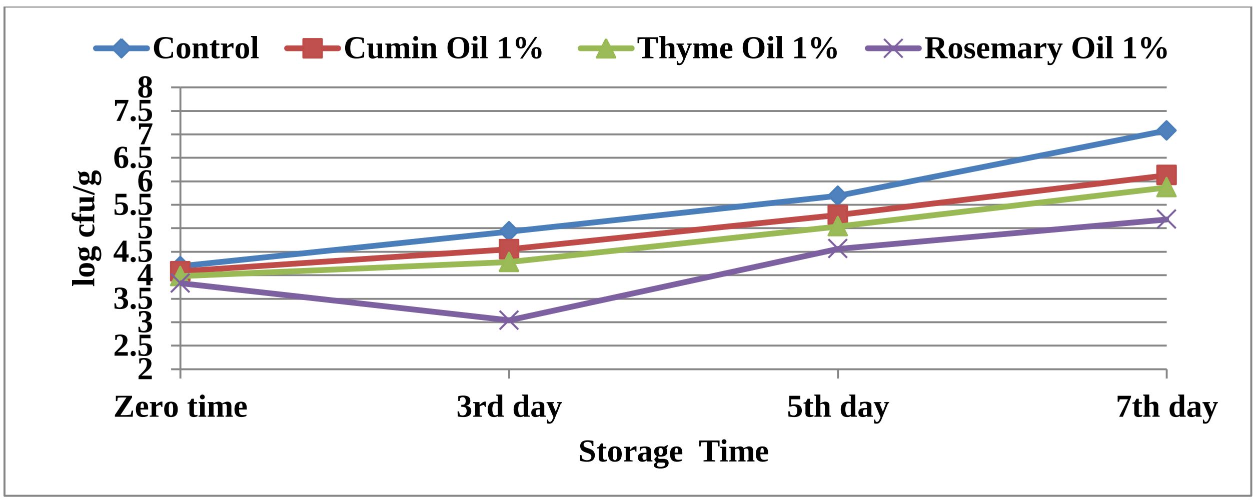

Figure (1): Effect of Cumin, Thyme and Rosemary oil 1% on Pseudomonas count (log CFU/g) of chilled minced beef meat samples at 4±1° C at zero, 3rd, 5th, and 7th day.

Enterobacteriaceae counts after treatment by cumin, thyme and rosemary oil1% at zero daywas3.99±0.2220, 3.96±0.1456 and 3.74±0.1006 Log_{10}CFU/g, respectively**(Figure 2)**.By the third day of storage, treatment by thyme and rosemary oil 1% proved significant reduction ($p<0.05$) in Enterobacteriaceae counts ranged from 3.63 to 4.6 3 and 3.11 to 4.45 Log_{10}CFU/g. However, treatment by cumin oil 1% was the lowest in Enterobacteriaceae reduction. Meanwhile at the 5th day, after treatment by rosemary oil 1%, Enterobacteriaceae count reduced to 4.30±0.0706Log_{10}CFU/g**(Figure 2)**.While, after treatment by cumin and thyme oil 1%, Enterobacteriaceae count was5±0.0815 and 4.84±0.1831 Log_{10}CFU/g, respectively. By the 7th day of storage, treatment by cumin, thyme and rosemary oil 1% reduced Enterobacteriaceae counts to 5.83±0.1676, 5.74±0.1102 and 5.41±0.1702 Log_{10}CFU/g, respectively. **(Figure 2)**.

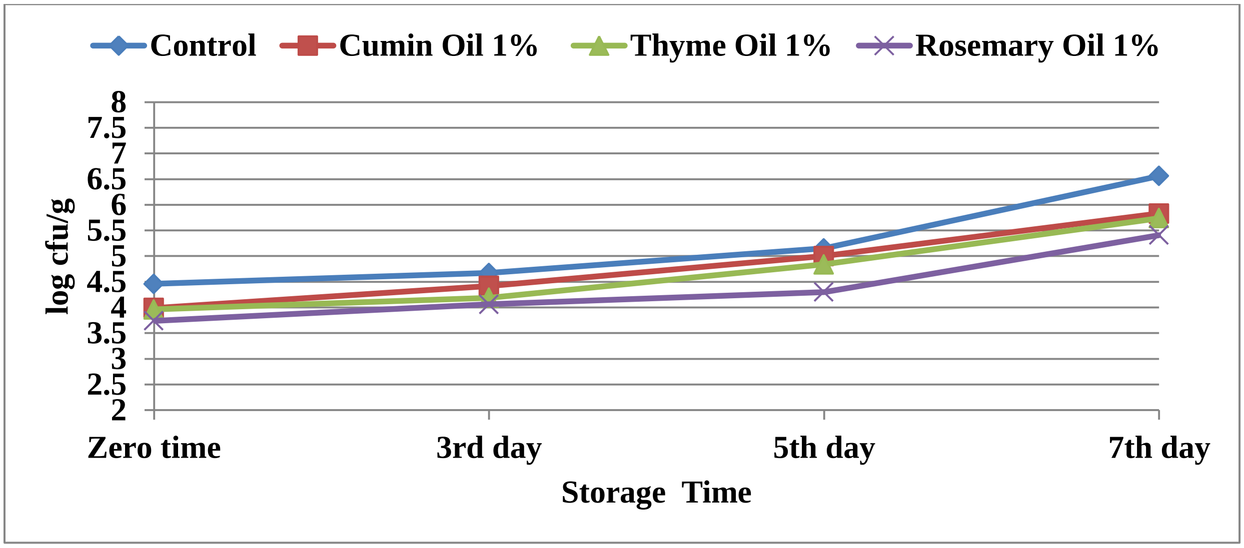

Figure (2): Effect of Cumin, Thyme and Rosemary oil 1% on Enterobacteriaceae count (log CFU/g)of chilled minced beef meat samples at 4±1° C at zero, 3rd, 5th, and 7th day

The counts of H_2S producing bacteria of control untreated samples gradually increased along storage period; it was3.63±0.0439 Log_{10}CFU/g**(Figure 3)**. By the third day, the count increased to 5.03±0.1927 Log_{10}CFU/g. While, at the 5th day of storage the counts of H_2S producing bacteriawas 5.60±0.1791Log_{10}CFU/g**(Figure 3)**. By the 7th day, the count highly increased to 6.60±0.1576 Log_{10}CFU /g. Concerning to treated samples,the counts of H_2S producing bacteria after treatment by cumin, thyme and rosemary oil1% at zero daywas3.56±0.0444, 3.35±0.1027and 3.15±0.1184 Log_{10}CFU/g, respectively.Rosemary oil1% was the most effective in reduction of H_2S producing bacteriafollowed by thyme oil 1% >cumin oil 1%.Meanwhile at the third day, after treatment by rosemary oil 1%, the counts of H_2S producing bacteria decreased to 4.79±0.1050Log_{10}CFU/g. While, after treatment by cumin and thyme oil 1%, the counts of H_2S producing bacteriawas 4.71±0.1501 and 4.19±0.1667 Log_{10}CFU/g, respectively.By the 5th day of storage, the counts were4.96±0.2610, 4.83±0.1322 and 4.47±0.1424 Log_{10}CFU/g. However at the 7thafter treatment by cumin, thyme and rosemary oil 1%, the counts of H_2S producing bacteriadecreased to 6.42±0.1952, 6.10±0.2262 and 5.49±0.1704 Log_{10}CFU/g, respectively**(Figure 3)**.

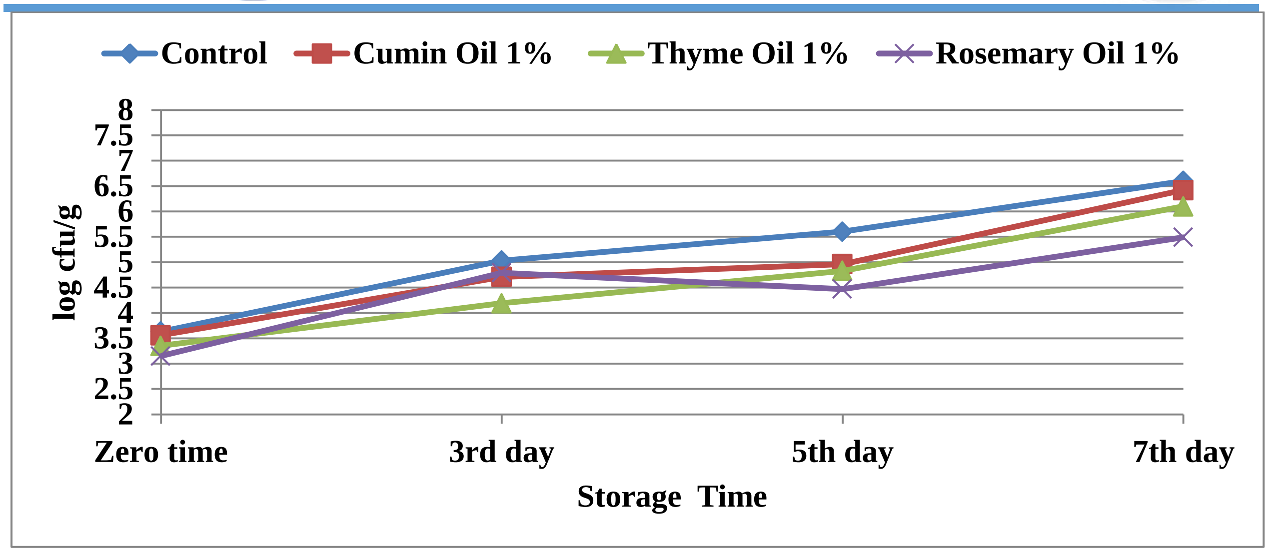

Figure (3): Effect of Cumin, Thyme and Rosemary oil 1% on Hydrogen Sulphide (H_2S) producing bacteria count (log CFU/g) of chilled minced beef meat samples at 4±1° C at zero, 3rd, 5th, and 7th day

4. Discussion

Meat spoilage and foodborne diseases pose a serious threat to the public health causing huge economic losses. *Pseudomonas spp*., are used as general pointers of hygiene in meat industries because they have the ability to release spoilage indicator as nitrogenous compounds, including primary, secondary and tertiary amines.

It was found that, minced meat samples had the highest count of *Pseudomonas*. This result was in line with **El-Said (2010)** and, **El-Shopary (2010)**. On the other hand, higher result (6.25 log_{10}CFU/g) was detected by **Erdem*et al*. (2014)**. However, lower results (2.85 and 3.11 log_{10}CFU/g)were detected by **Zhanga*et al*. (2011)** and **Gaafar*et al*. (2012)**, respectively.

Concerning to sausage samples, Pseudomonas countrecorded in this study was nearly similar to what had been reported by **El-Said (2010)**, **Gaafar*et al*. (2012)** and **Sofy*et al.* (2017)** who found the mean count of Pseudomonas in the examined sausage samples was 3.5, 3.5 and 3.88 log_{10}CFU/g, respectively. But lower result (3.3 log_{10}CFU/g) was recorded by **El-Shopary (2010).**Contamination of the examined burger samples was in accordance with **El-Shopary (2010)**, **Gaafar*et al.* (2012)** and**Sofy*et al.* (2017)**,but disagreed with **El-Said (2010)** who detected higher count. The examined luncheon samples had the lowest count of pseudomonas compared with the other samples. In contrast to this result, higher counts of (4 and 3.6 Log_{10} CFU/g) in luncheonsamples were detected by **El-Shopary (2010)** and **Sofy*et al.* (2017)**.Contamination with *Pseudomonas spp*. attributes to inappropriate handling by food handlers during the different stages of production as well as inadequate storage temperature.

Enterobacteriaceae are considered as indicator bacteria to evaluate the hygienic condition of meat. Minced meat samples had a high count of Enterobacteriaceae compared with other samples. This result agreed with **El-Sheikh (2014)**, however, lower results (3.4 Log_{10} CFU/g) was registered by **Gaafar*et al*. (2012).** On the other side**,** higher result (4.92 Log_{10} CFU/g) was detected by **Kilonzo-Nthenge*et al.* (2013).** Concerning to sausage samples, the mean count of Enterobacteriaceae was consistent with **Gaafar*et al.* (2012)** and **Sofy*et al.* (2017)**.Meanwhile, **Mousa*et al.* (2014)** reported lower result (2.9 Log_{10} CFU/g). However, higher counts were detected by **El-Sheikh (2014)**(4.9CFU/g)in sausage samples**.** Regarding to burger samples**,** Enterobacteriaceaecount corroborated previous works conducted by **Guillier*et al.* (2013)** and **Sofy*et al.* (2017)**. On the other side, higher results of 5.4 Log_{10} CFU/g were registered by **Gaafar*et al*. (2012)**. Meanwhile, lower counts (2.7 and 2.13 Log_{10} CFU/g) were detected by **Mousa*et al*. (2014)** and **Shaltout*et al.* (2017)**.In the examined luncheon samples, theEnterobacteriaceaecount was lower than all examined samples. Nearly similar result was reported by **Samaha*et al.* (2016)**, but,this result disagreed with **Zaghloul*et al.* (2014)** and **Sofy*et al*. (2017)** who reported higher counts of Enterobacteriaceae (3.6 and 4.55 Log_{10} CFU/g).Contamination with family Enterobacteriaceae is an indicative of unhygienic environment like sewage, improper waste disposal systemas well ascontamination of meat handler's hands, tools and handling surfaces.Members of the family Enterobacteriaceae are potent causes of foodborne diseases and might pose a health risk to consumers.

Different species of bacteria (*Pseudomonas spp., Citrobacter spp., Aeromonas spp., Salmonella spp. and Escherichia coli*) are able to convert sulfur-containing amino acids into hydrogen sulphide (H_2S) which is a colorless and irritant gas with a characteristic odor of rotten eggs. Minced meat samples had a high count of hydrogen sulphide producing bacteria, followed by burger then sausage samples, while, luncheon samples had the lowest count of hydrogen sulphide producing bacteria. Many other previous investigations reported contamination of meat products by hydrogen sulphide producing bacteria as **Vogel *et al.* (2005)** and**Nychas*et al.* (2008)**. Contamination of meat products by hydrogen sulphide producing bacteria could be attributed to inadequate practices, mainly poor hygienic conditions. Also, unsanitary preparation places, inadequate clean utensils, cross contamination from raw meat, poor personal hygiene and hygienic practices of meat handlers.

Plant extracts such as essential oils have indicated antimicrobial activity against both foodborne pathogenic and spoilage microorganisms **(Hashemi*et al.*, 2017).** Results in the present study indicated a comparison of effectiveness of cumin, thyme and rosemary oil 1%used separately on the count pseudomonas, Enterobacteriaceae and hydrogen Sulphide producing bacteriaof raw minced meat samples stored at 4 °C. These oils proved their effectiveness in meat preservation, reduction of bacterial count and extend the shelf life.These results were in accordance with **Conner (1993)** who reported that the essential oils of thyme and rosemary were shown to possess strong antibacterial activity against *Pseudomonas aeruginosa.* However **Elgayyar*et al.* (2001)** reported the antibacterial potential of rosemary essential oils against meat spoilage bacterial pathogens such as *Pseudomonas fluorescens*and *P. aeruginosa*, respectively.**Agaoglu*et al.* (2007)** revealed that the extracted essential oils of cumin showed an antimicrobial effect against *Pseudomonas aeruginosa*. While, **Karabagias*et al.* (2011)** reported the inhibitory effect of cumin and thyme on *Pseudomonas spp*.Concerning to the effect of the essential oils on Enterobacteriaceae count, nearly similar results were registered by **Imelouane*et al.* (2009)** who reported that thyme oil has been shown to effectively inhibit food-borne pathogens of familyEnterobacteriaceae. Meanwhile, **Djeddi*et al.* (2007)** reported that Enterobacteriaceae were susceptible to the activity of rosemary essential oil. **Rasool (2013)** stated that the extracted essential oils of cumin showed an antimicrobial effect against members of family Enterobacteriaceae. cumin, thyme and rosemary oil 1% were effective in reduction of hydrogen Sulphide producing bacteria. Similar results were obtained by **Viuda-Martos*et al*.(2010).** Also, **Solomakos*et al.* (2008)** illustrated that differentconcentrations (0.3, 0.6 or 0.9 %) of thyme essential oil successfully inhibited the bacterial growth intreated minced beef.It was found that rosemary oil 1% was the most effective oil in meat preservation followed by thyme oil then cumin oil.The reduction in the count of pseudomonas, Enterobacteriaceae and hydrogen sulphide producing bacteriaafter treatment of minced meat by cumin, thyme and rosemary oil 1%, attributed to the antimicrobial activities of the phenolic compounds of these oils against wide range of bacteria. Therefore, these oils especially rosemary oil act as good preservatives to improve the shelf life of meat products.

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الملخص العربي

الحاله الصحيه لبعض منتجات اللحوم مع بعض المحاولات لتحسين حالتها الصحيه واطاله مده حفظها

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تُعد اللحوم ومنتجاتها من أهم الأغذية التي يحتاج إليها الإنسان نظراًلقيمتها الغذائية العالية فهي تعتبر مصدرً ممتازً للعديد من العناصر الغذائية ؛ خاصة البروتين والدهون والفيتامينات والحديد والزنك بالإضافة إلي العديد من الحموض الأمينية الأساسية التي تلعب دورًا أساسيًا في بناء وإصلاح جميع أنسجة الجسم.لذا إشتملت هذه الدراسة على جزئين أساسيين تناول الجزء الأول منها فحص الحالة الصحية لبعض منتجات اللحوم المتداولة بأسواق مدينة الزقازيق حيث تم تجميع عدد ٨٠ عينة من منتجات اللحوم (اللحم المفروم، السجق، البرجر، اللانشون) بواقع ٢٠ عينة من كل نوع علي حده لفحصها بكتيريولوجيًا. بينما تناول الجزء الثانى بعض المحاولات لتحسين الحالة الصحية لبعض منتجات اللحوم (اللحم المفروم) بواسطة إستخدام بعض الزيوت الطبيعية مثل زيت الكمون وزيت الزعتر وزيت إكليل الجبل (الروزماري) بتركيز ١% من كل نوع. أظهرت النتائج تلوث العينات بالبكتريا مع زيادة في العد الكلي لكل من السودوموناس والبكتيريا المعوية وأيضا البكتريا المنتجة لغاز كبريتيد الهيدروجين. كما أظهرت أيضاً كفاءة الزيوت التي إستخدمت في تحسين الحالة الصحية للحم المفروم وزيادة فترة حفظه. خلصت الدراسة إلى أن استخدام بعض الإضافات مثل زيت الكمون ١% وزيت الزعتر ١% وزيت الروزماري ١% يؤدى إلى إطالة مدة الصلاحية وتقليل الحمل الميكروبى فى منتجات اللحوم ، لذا نوصى باستخدام تلك الإضافات فى التصنيع. وننصح بإستخدامهم في حفظ منتجات اللحوم.