





Bacteriological evaluation of some meat products with some trials to improve their quality Alaa Eldin M. A. Morshdy, Abdallah Fikry A. Mahmoud and Ghada Eid Ahmed Hassona

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Abstract

Meat products are an excellent source of many nutrients; particularly protein, vitamins and minerals. Despite being one of the most important items in the human diet, they are considered as one of the main vehicles of pathogens. These pathogens can result in many cases of food poisoning. Therefore, the present study was conducted to evaluate the bacteriological quality of some meat products (minced meat, sausage, burger and luncheon) in Zagazig City, Sharkia Governorate, Egypt through determination of psychrotrophic count, pseudomonas count, enterococci count and isolation of Salmonella spp. In addition to, study the anti-microbial activities of some essential oils (clove oil, oregano oil and garlic oil) on minced meat quality and shelf life. Results revealed that the mean count of psychrotrophic bacteria was 5.4 ± 0.097 , $5.1 \pm$ 0.066, 3.5 ± 0.117 and 2.7 ± 0.114 log10CFU/g, pseudomonaswas 5.2 ± 0.075 , 4.5 ± 0.112 , 3.1 ± 0.006 0.108 and $2.9 \pm 0.066 \log_{10}$ CFU/g and enterococci was 4.7 ± 0.104 , 3.8 ± 0.079 , 3.4 ± 0.114 and $2.8 \pm 0.074 \log_{10}$ CFU/g in the examined minced meat, burger, sausage, and luncheon, respectively. Salmonella spp failed to be detected in all examined meat products. The three essential oils (clove, oregano and garlic) used in this were effective in improving the bacteriological quality of meat. Garlic oil was the most effective one followed by oregano then clove oil.

Key words: Meat products, Pseudomonas, Psychrotrophic, Essential oils

1. Introduction

Meat products are an excellent source of many nutrients; particularly protein, unsaturated fats like omega-3 fatty acids as well as several vitamins such as vitamins E, B1, B2, B3 and B6. Also, meat products contain several minerals including magnesium, iron and zinc.

In Egypt, meat products are favored by numerous peoples because they are easy to purchase, quickly to cook, tasty to eat and low in cost. Despite being one of the most important items in the human diet and an industry with considerable potential for growth, meat and meat products are considered one of the main vehicles of pathogens to humans (**Rhoades** *et al.* 2009), causing foodborne illne. There are various sources which can contaminate such products duringthe different stages on production process including food handlers, cross contamination and unhygienic preparation, processing and storage resulting in bacteriological contamination and therefore, meat spoilage and food borne illnesses.









Psychrotrophic bacteria develop on meat products at chill temperatures. They belong to microbial genera of both gram positive, such as lactic acid bacteria, and gram-negative bacteria, such as *Pseudomonas spp.* and Enterobacteriaceae(Ercoliniet al., 2009). *Pseudomonas* species are the major causative spoilage bacteria in meat, primarily due to their metabolic versatility and ability to produce extracellular proteases and lipases cause oxidation, color change, off- flavor, slimy form and animal tissues degradation (Doulgeraki et al. 2012). *Enterococcus* species are associated with the gastrointestinal tracts of animals and are responsible for morbidity and mortality in predisposed humans. They are used as indicators of animal fecal contamination of meat products. They are ubiquitous bacteria widely distributed in a variety of habitats; they also, comprise a high proportion of saprophyte bacteria (Domiget al. 2003).

Salmonella is a genus of rod-shaped Gram-negative bacteria of the family Enterobacteriaceae. According to the World Health Organization, Salmonella is one of the most relevant pathogens in meat and meat products; its presence poses a risk to consumers (WHO, 2005).

Many naturally occurring extracts like essential oils from edible and medicinal plants, herbs and spices have been shown to possess antimicrobial functions and could serve as a source for antimicrobial agents against food spoilage organisms and pathogens (**Dhanzeet al., 2013**). For instance, clove, oregano and garlic show antimicrobial and antioxidant properties. Clove oil (*Syzygiumaromaticum L.*) has been used for many purposes since ancient times in various food applications. Clove oil has a widespectrum of actions not only antibacterial, antiviral, antifungal and antiprotozoal, but also have beneficial effects on the cardiovascular and immune system (**Maheshwariet al., 2012**). Essential oil of oregano(*Origanumvulgare L.*) is known for its relatively strong antimicrobial properties (**Teixeira et al., 2013**). It contains mainly carvacrol and considerably less thymol as well as small amounts of other constituents which may have antioxidant activity (**Michalczyket al., 2015**). Garlic oil (*Allium sativum L.*) is used as a preservative and additive to prevent lipid oxidation(**Salejdaet al., 2011**); it also characterized by high biological activity.

Keeping the above view, the present study was planned to evaluate the bacteriological quality of minced meat, sausage, burger and luncheonin Zagazige City, Sharkia Governorate in addition to, study the anti-microbial activities of clove, oregano and garlic on minced meat.

2. Materials and method

A. Bacteriological examination

Collection of samples:

A total of 80 random samples of minced meat, burger, sausage and luncheon (20 of each), were randomly collected from different markets in Zagazig City, Sharkia governorate, Egypt. All samples were transferred under complete a septic conditions to Food Control lab for, bacteriological examinations.







Preparation of samples:

According to APHA (2001).

Determination of total psychrotrophiccount:

On standard plate count agar (Oxiod CM325) according to APHA (2002)

Determination of Pseudomonas count:

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

Determination of Enterococci count:

On a Bile Esculin Ager, Himedia (M340) according to **ISO** (2000)

Isolation and identification of Salmonellaspp

According to **ISO** (2002), Pre- enrichment on a non- selective liquid medium, Selective enrichment Rappaport Vassiliadis with soya then Selective plating and identification on Xylose Lysine Desoxycholate ager (XLD agar).

B. Evaluation of the effect of some essential oils on the quality of minced meat

The experimental part aimed to testing the effect of selected oils on the shelf life of minced meat concerning the previous bacteriological andchemical parameters. 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. Clove treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 m1 of Clove oil to obtain final concentration 1% then separated to five clean Ziploc pages.
- 3. Oregano treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 m1 of oregano oil to obtain final concentration 1%, then separated to five clean Ziploc pages.
- 4. Garlic treated group: 500 grams of minced meat mixed and gently massaged by hand for the homogenous distribution with 5 ml of garlic 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 fridge at $4\pm1^{\circ}$ C. Bacteriological examination was conducted.

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 examination of meat products

Results illustrated in **Table** (1) revealed that the mean count of Psychrotrophic bacteria in the examined minced meat, burger, sausage, and luncheon samples was 5.4 ± 0.097 , 5.1 ± 0.066 , 3.5 ± 0.117 and $2.7 \pm 0.114 \text{Log}_{10}\text{CFU/g}$, respectively. While, the mean count of Pseudomonas was 5.2 ± 0.075 , 4.5 ± 0.112 , 3.1 ± 0.108 and 2.9 ± 0.066 Log₁₀CFU/g in the examined minced meat, burger, sausage and luncheon samples, respectively **Table** (1). Meanwhile, the mean count of enterococci in the examined minced meat, burger, sausage and luncheon samples were 4.7 ± 0.104 , 3.8 ± 0.079 , 3.4 ± 0.114 and $2.8 \pm 0.074 \text{Log}_{10}\text{CFU/g}$, respectively **Table** (1). Salmonella spp failed to be detected in all examined meat products.

Table (1) Statistical analytical results of Psychrotrophic, Pseudomonas and Enterococci count (n=20) \log_{10} CFU/g

	Psychrotrophic		Pseudomonas		Enterococci	
Samples	Range	Mean± S.E	Range	Mean± S.E	Range	Mean± S.E
Minced meat	4.6-6.4	$5.4^{a} \pm 0.097$	4.6-5.9	$5.2^{a} \pm 0.075$	3.4-5.2	$4.7^{a} \pm 0.104$
Burger	4.2-5.4	$5.1^{\text{b}} \pm 0.066$	3.5-5.2	$4.5^{b} \pm 0.112$	3.2-4.7	$3.8^{b} \pm 0.079$
Sausage	2.4-4.3	$3.5^{c} \pm 0.117$	2.5-3.7	$3.1^{c} \pm 0.108$	2.7-4.3	$3.4^{c} \pm 0.114$
Luncheon	2.1-3.6	$2.7^{d} \pm 0.114$	2-3.5	$2.9^{c} \pm 0.066$	2.3-3.3	2.8 ^d ±0.074

n: number of the examined samples, CFU/g: Colony Forming Unit per gram

B. Effect of clove, oregano and garlic oil 1% on the bacteriological quality of minced meat:

As shown in **Figure (1)**, Psychrotrophic counts of control untreated samples gradually increased along storage period; the initial Psychrotrophic count was $5.05\pm0.293 \log_{10}$ CFU/g. By the third day the count increased to $6.49\pm0.329 \log_{10}$ CFU/g, while, at the 5^{th} day of storage it reached to $7.48\pm0.309 \log_{10}$ CFU/g, by the 7^{th} day, the count highly increased to $8.1\pm0.391\log_{10}$ CFU/g. The mean Psychrotrophic counts in treated samples by clove, oregano and garlic oil1% at the third day of storage were 5.68 ± 0.147 , 5.53 ± 0.068 and $5.11\pm0.318 \log_{10}$ CFU/g, respectively, meanwhile at the 5^{th} day of cold storage, the countwas 6.83 ± 0.229 , 6.69 ± 0.177 and $6.26\pm0.289\log_{10}$ CFU/g, respectively, by the 7^{th} day of storage, Psychrotrophiccountwas 7.57 ± 0.373 , 7.48 ± 0.368 and $7.46\pm0.320\log_{10}$ CFU/g, respectively.

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).







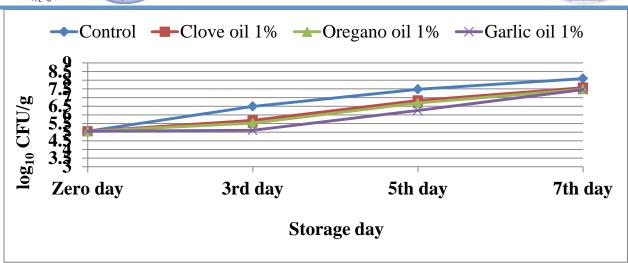


Figure (1): Effect of Clove, Oregano and Garlic oil 1% on Psychrotrophic count ($log_{10}cfu/g$) of chilled minced beef meat samples at $4\pm1^{\circ}$ C at zero, 3^{rd} , 5^{th} , and 7^{th} day.

The initial Pseudomonas count was $4.66\pm0.029~\log_{10}~CFU/g$, while it reached to 6.26 ± 0.375 , 7.53 ± 0.399 and $7.99\pm0.341~\log_{10}~CFU/g$ at the 3^{rd} , 5^{th} and 7^{th} day of storage, respectively. Regarding to treated samples, Pseudomonas count after treatment by clove, oregano and garlic oil 1%, at the third day of storage, was 5.25 ± 0.124 , 5.09 ± 0.291 and $4.49\pm0.313~\log_{10}~CFU/g$, respectively. Meanwhile at the 5^{th} day, it was 6.18 ± 0.057 , 5.72 ± 0.117 and $5.13\pm0.386~\log_{10}~CFU/g$. By the 7^{th} day of storage, Pseudomonas count in treated samples by clove, oregano and garlic oil 1% reduced to 6.64 ± 0.278 , 6.40 ± 0.087 and $5.73\pm0.119~\log_{10}~CFU/g$, respectively (**Figure 2**).

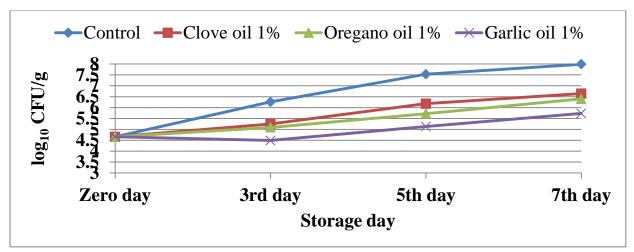


Figure (2): Effect of Clove, Oregano and Garlic oil 1% on Pseudomonas count (log_{10} CFU/g) of chilled minced beef meat samples at $4\pm1^{\circ}$ Cat zero, 3^{rd} , 5^{th} , and 7^{th} day







TheEnterococci count of control untreated samples gradually increased along the storage period; its initial count was $4.02\pm0.047~\log_{10}$ CFU/g, by the third day of cold storage, the count increased to $6.50\pm0.106~\log_{10}$ CFU/g. Concerning to treated samples,the counts of enterococci after treatment by clove, oregano and garlic oil 1% at 3rd day of cold storage ranged from 5.40 to 6.23, 4.95 to 5.32 and 4.32 to 5.45 \log_{10} CFU/g, respectively. Meanwhile at the 5th day of storage, enterococci count in control samples was $7.21\pm0.428~\log_{10}$ CFU/g, while, treatment by clove, oregano and garlic oil 1% reduced the enterococci count to 6.46 ± 0.096 , 6.08 ± 0.449 and $5.86\pm0.345~\log_{10}$ CFU/g, respectively. By the 7th day of storage, enterococci count was 7.57 ± 0.341 , 7.56 ± 0.315 and $6.61\pm0.368~\log_{10}$ CFU/g, respectively (**Figure 3**).

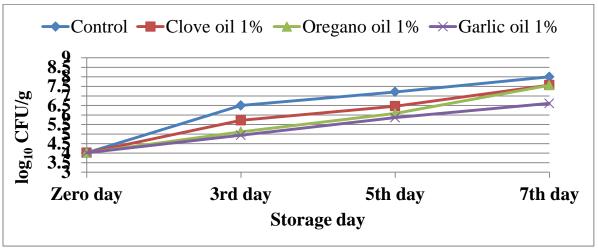


Figure (3): Effect of Clove, Oregano and Garlic oil 1% on Enterococci count (log_{10} CFU/g)of chilled minced beef meat samples at $4\pm1^{\circ}$ Cat zero, 3^{rd} , 5^{th} , and 7^{th} day

4. Discussion

Psychrotrophic bacteria are capable of surviving in extremely cold environment. They provide an estimation of the shelf life of meat. Minced meat samples had the highest count of Psychrotrophic bacteria compared with other samples. Significance differences were detected between the examined samples (P < 0.05). The variation in counts may be attributed to improper handling and poor sanitation level during the processing steps and storage. Nearly similar results were detected by Selvanet al. (2007) for sausage, Mousaet al. (2014) for luncheonMashaket al. (2015). Meanwhile, lower results were recorded by Selvanet al. (2007) for minced meat (3.33 log₁₀ CFU/g); Pao and Ettinger (2009) andMousaet al. (2014) (3.3and 2.7 log₁₀ CFU/g) for burger and Mousaet al. (2014) for sausage (2.9 log₁₀ CFU/g). But higher results were reported by Ercoliniet al. (2009) for minced meat (6.04 to 7.4 log₁₀ CFU/g) and Shaltoutet al. (2017) for sausage (4.3 log₁₀ CFU/g).

Pseudomonas spp., are used as general indicators of processing hygiene, storage conditions and spoilage in meat industries. It is an important meat spoilage indicator as









nitrogenous compounds, including primary, secondary, tertiary amines and others, are released. The obtained results in this study agreed with **Siriken** (2004) for minced meat; **El-Said** (2010) and **El-Shopary** (2010) for sausage; and **Gaafaret** *al.* (2012) for burger. But disagreed with **El-Shopary** (2010), **El-Said** (2010) and **Gaafaret** *al.* (2012) who reported lower counts of Pseudomonas (4.7, 3.4 and 4.1 log₁₀ CFU/g) in the examined minced meat samples, while, **El-Said** (2010) and **El-Shopary** (2010) reported lower counts of Pseudomonas (2.9 and 3.3log₁₀ CFU/g) in the examined burger samples. Higher counts of Pseudomonas for the examined luncheon, sausage and minced meat samples 4, 4.5 and 6.1 log₁₀ CFU/g were recorded by **El-Shopary** (2010), **Gaafaret** *al.* (2012) and **Erdemet** *al.* (2014), respectively.

Enterococci are common members of the microbiota in thegastrointestinaltract of mammals and other animals and can also be found in soil, water, and food. Minced meat samples were highly contaminated by enterococci, while luncheon samples had the lowest count. Contamination of meat products with enterococci is an indication on unhygienic conditions and fecal contamination. These findings were in line with Sadeghifardet al. (2015), Ike and Akortha (2017) who isolatedenterococci from the examined meat samples.

Salmonella spp. failed to be detected in all examined samples, these results coincided with **Selvanet** al. (2007) who did not isolate Salmonella spp. from the examined meat product samples. However, these results disagreed with **Mousaet** al. (2014) and **Roger** et al. (2015) who isolated Salmonella spp. from the examined meat product samples.

Treatment of minced meat samples by clove, oregano and garlic oil 1% was effective improving bacteriological quality and extension of shelf life compared with control untreated samples. Garlic oil was the most effective one followed by oregano then clove oil. Essential oils have different compounds with antibacterial activities such as; geraniol, menthol, cinnamylalcohol linalool, citronellol, carvacrol, cinnamaldehyde, eugenolthymol, estragole, carvone and chavicol(Ayala-Zavala et al., 2008). These results were in line with Gutierrez et al. (2009) who reported that essential oil of oregano is known for its relatively strong antimicrobial properties, while, Rattiet al. (2007) reported the antimicrobial activity of garlic. Michalczyket al. (2015) reported the antimicrobial activity of oregano and garlic, however, Kumudavallyet al. (2011) reported that ethanolic extract of clove exerted a strong inhibitory effect on meat spoilage organisms and pathogenic organisms.

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قسم مراقبة الأغذية ، كلية الطب البيطري جامعة الزقازيق مصر

تُعد منتجات اللحوم احد الأغذية الأكثر أهمية نظرًا لقيمتها الغذائية العالية حيث أنها مصدر غني لمختلف العناصر الغذائية التي يحتاج إليها جسم الإنسان وبخاصة البروتين والدهون والعديد من الفيتامينات والمعادن مثل الحديد والزنك بالإضافة الي الحموض الأمينية الأساسية التي يحتاجها الجسم في بناء وإصلاح جميع أنسجة الجسم التالفة. تضمنت هذه الدراسة جزئين رئسيين إحتوي الجزء الأول منها علي فحص الحالة الصحية (ظاهريًا- كيميائيًا- بكتيريولوجيا) لبعض من منتجات اللحوم التي يتم تداولها بأسواق مدينة الزقازيق تم تجميع عدد ٨٠ عينة من منتجات اللحوم (٢٠ عينة من اللحم المفروم، البرجر السجق، الملائشون) لفحصها بكتريولوجيا من حيث العد الكلي للبكتريا المحبة للبرودة و للسودوموناس المفروم، البرجر السجق، الملائشون) فحصها بكتريولوجيا من حيث العد الكلي للبكتريا المحبة للحم المفروم باستخدام والإنتيروكوكاي والسالمونيلا). بينما إحتوي الجزء الثاني علي بعض المحاولات لتحسين الحالة الصحية للحم المفروم باستخدام بعض الزيوت الطيارة مثل زيت القرنفل وزيت البردقوش وزيت الثوم بتركيز ١% من كل نوع علي حده و خلصت الدراسة البردقوش ١١% وزيت الثوم الكرق قي الأسواق ملوثة بكتيرياً وأن إستخدام بعض الإضافات مثل زيت القرنفل ١١% وزيت الدوم المتداولة في الأسواق ملوثة بكتيرياً وأن إستخدام بعض الإضافات مثل زيت القرنفل ١١% وزيت الدوم المختلفة كوسائل حفظ بديلة لتجنب خطر المواد توصى هذه الدراسة باستخدام تلك الزيوت في عمليات تصنيع منتجات اللحوم المختلفة كوسائل حفظ بديلة لتجنب خطر المواد الحافظة الكيميائية.