

Efficacy of Olive Leaves Extract on the Survival Pattern of *Salmonella Typhimurium* and *Staphylococcus Aureus* in Minced Meat

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Abstract

The application of plant extract as bio-preservatives, for meat products, was recommended by many researchers; therefore an aqueous extract of olive leaves (*Olea europaea* L.) was examined for its effect on the survival pattern of *Salmonella typhimurium* and *Staphylococcus aureus* which were experimentally inoculated in minced meat with an initial load of approximately 10^6 cfu/g. All samples of meat were stored at $7 \pm 1^\circ\text{C}$ for 24 hours. The results indicated that olive leaves extract showed a significant effect on the survival pattern of *Staphylococcus aureus* and a lower effect against *Salmonella typhimurium*. Sensory examination revealed that the addition of 1% OLE to minced meat had significantly better overall acceptability. From this study we can conclude that olive leaves extract contain compounds that have significant antimicrobial properties, which can be applied to use in the meat industry.

Keywords: Olive leaves; Phenolic compounds; Antibacterial activity; Sensory quality. *Salmonella typhimurium*, *Staphylococcus aureus*

Introduction

Safety of food is an important priority as 1 in 6 Americans (48 million people) get sick, 128,000 are hospitalized and 3,000 die of food borne diseases each year according to (CDC, 2011) Antimicrobials are used in food to control natural spoilage processes, and to prevent/control growth of micro-organisms, including pathogenic micro-organisms. Due to the increase concerns on

chemical preservatives among consumer, natural antimicrobials are receiving a good deal of attention. Although chemical preservatives are approved for human consumption by government agencies but many of these chemical preservatives are threaten our health. So, the food researchers have given more attention towards the useful use of natural products as antimicrobials. (Mathew et al, 2007; Abou-taleb and Kawai,

2008; Fisher and Phillips, 2008 and Sofos, 2008).

Natural antimicrobials are promising solve for the increasing concerns antibiotic resistance and could yield better results than synthetic antimicrobials (Ngwoke, 2011).

Olive Leaf Extract (OLE) has been used clinically for many years and have promising therapeutic action against many common and chronic conditions. (Healthy Christian Living, 2014)

Antimicrobial and antioxidant activity of olive Leaf Extract (OLE) is directly related with its polyphenols namely oleuropein, tyrosol, hydroxytyrosol, rutin, verbacoside, apigenin- 7-glucoside and luteolin-7-glucoside (Korukluoglu et al, 2010). There are some studies revealing olive leaf extract inhibits many gram-negative and -positive bacteria, and can inhibit the sporulation of *Bacillus cereus* and growth of *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Vibrio parahaemolyticus* and *Staphylococcus aureus*, that all known as food pathogens (Markin et al, 2003 and Pereira et al, 2007). Unlike synthetic antibiotics, it destroys only the bad bacteria and protects the good. Olive leaf extract offers the benefits of being a broad-spectrum antimicrobial compound while at the same time exhibiting no harmful effects on beneficial microbes. Because it will not induce any side effects which could be

associated with antibiotics, and will not lead to secondary infections related to a preponderance of harmful bacteria (Hansen et al, 2013). The application of plant extract as bio-preservatives, for meat products, was recommended by many researchers (Erdohan, 2011). Many plant extracts were evaluated to decontaminate and maintain the quality of meat steaks (Tayel et al, 2012) and ground beef (Tayel and El-Tras, 2012). The present study was designed to evaluate the effect of olive leaves extract on the survival pattern of *Salmonella typhimurium* and *Staphylococcus aureus* in minced meat.

Materials and methods

Preparation of OLE

Olive leaves were collected from *Olea europaea* L. trees. They were collected in winter season and dried in air. The extract was prepared according to (Devatkal et al, 2012).

Inoculation of meat

Reference *Staphylococcus aureus* and *Salmonella typhimurium* strains were obtained from The Animal Health Research Institute- Food Hygiene Department, Agriculture Center Research, and Egypt.

Antibacterial activity of OLE

Meat sample was obtained from the local supermarket at Port- Said City and transferred directly to the laboratory under complete aseptic conditions without delay. The meat samples were cut into pieces, and minced using meat grinder. Minced

meat (1kg) examined bacteriologically for the presence of *Staphylococcus aureus* and *Salmonella typhimurium* according to (FDA, 2001) and (FDA, 2007) respectively. The minced meat sample was divided into 3 equal samples each weight nearly of 330g, the first portion was inoculated by the reference strain of *Staphylococcus aureus* and the second with *Salmonella typhimurium*, and the third portion was used for the sensory evaluation. Preliminary work was conducted to determine the concentration of inoculums of the reference strain volume which are needed to yield 10^6 cfu/g in minced meat according to QSOP 18, (2005).

Each inoculated meat samples was subdivided into 4 portions and mixed with 0%, 1%, 2% and 3% OLE (w/v) and stored at 7°C and examined after 2 hours and 24 hours for *Staphylococcus aureus* and *Salmonella typhimurium*

according to (FDA, 2001) and (FDA, 2007) respectively.

Sensory evaluation

The Sensory characteristics of minced meat samples were evaluated in the 3rd portion which was subdivided into 4 portions and each one was mixed with the corresponding dilutions 0%, 1%, 2% and 3% OLE (w/v). These concentrations were chosen to be the same as the concentrations that were used in the microbiological examination. The sensory evaluation was conducted by the 5 points hedonic scale: 1, very poor; 2, poor; 3, common; 4, good; 5, very good (Szczesniak, 1987).

Statistical analysis

All measurements were repeated three times. The results are expressed as mean values and standard deviations. The data were statistically analyzed by ANOVA and Duncan's multiple range tests. Statistical significance was accepted at a level of $P < 0.05$ (SAS Institute, 1988).

Results

Table 1. Effect of olive leaves extract on populations of *Staphylococcus aureus* and *Salmonella typhimurium*

OLE %	<i>Staphylococcus aureus</i>		<i>Salmonella typhimurium</i>	
	2 hours	24 hours	2 hours	24 hours
0%	7.4×10^6 ± 0.010	8.2×10^7 ± 0.150	6.5×10^6 ± 0.076	7.1×10^7 ± 0.100
1%	1.5×10^6 ± 0.07	4.1×10^5 ± 0.150	4.8×10^6 ± 0.100	3.1×10^6 ± 0.100
2%	7.4×10^5 ± 0.100	9.8×10^4 ± 0.170	2.9×10^6 ± 0.115	9.8×10^5 ± 0.057
3%	1.2×10^5 ± 0.057	$3.3 \times 10^4 \pm 0.100$	$1.2 \times 10^6 \pm 0.100$	$5.7 \times 10^5 \pm 0.07$

Table 2. Effect of different concentration of olive leaves extract on sensory attributes

OLE %	Flavour	Colour	General acceptance
0%	4.40 ± 0.57 ^a	3.00 ± 0.00 ^a	4.66 ± 0.57 ^a
1%	4.00 ± 0.33 ^b	3.00 ± 0.66 ^a	4.33 ± 0.33 ^a
2%	3.60 ± 0.57 ^c	2.53 ± 0.33 ^b	3.00 ± 0.00 ^b
3%	3.00 ± 0.57 ^d	2.00 ± 0.33 ^c	2.33 ± 0.33 ^c

Means bearing similar letters within each column are not differ significantly ($p < 0.01$) otherwise, they differ significantly ($p < 0.01$)

Discussion

From the results given in (Table 1) we observed that the population of *Staphylococcus aureus* was reduced by 2 log cfu/g after 24 hours of storage at 7°C with 1% of OLE concentration, with 2% concentration the reduction in *Staphylococcus aureus* was 1 log and 3 log after 2 hours and 24 hours respectively. This means that the reduction was affected by the concentration % of OLE and the time of storage. The findings observed with *Salmonella typhimurium* were, after 24 hours of storage the reduction was 1, 2 and 2 log reductions with 1%, 2% and 3% OLE concentration respectively.

The current results indicating that, the count of *Staphylococcus aureus* and *Salmonella typhimurium* were decreased while OLE concentration and time of storage increased (Table 1). These findings were paralleled with the findings of *Tayel and El-Tras, (2012) and Ahmed et al (2014)*. In vitro studies performed by *(Tranter et al, 1993; Tassou and Nychas, 1994; Tassou, 1995; Markin, 2003; Pereira et al,*

2007; Markin, 2008; Sudjana, et al, 2009 and Aliabadi et al, 2013) they recoded that lower concentrations of OLE were sufficient to kill *S. aureus* and most pathogenic bacteria. The antimicrobial effect could be also influenced by the original source, time of harvesting, and stage of development. In food applications, these natural antimicrobial compounds could be influenced by intrinsic factors such as composition (e.g., proteins, fat) as well as extrinsic factors (temperature, oxygen limitation) of the food affect the behavior of bacteria in food ecosystems and may act synergistically with preservatives such as antimicrobial agents. Indeed, food components, such as proteins and fat, are known to bind and/or solubilized Phenolic compounds, reducing their availability for antimicrobial activity. Furthermore, it has been reported by many authors that antimicrobial activity of spice is lower in food systems than in microbiological media *(Korukluoglu, 2010 and Saeed et al, 2013)*. In Studies performed by

(Aytul et al, 2010 and Gök and Bor, 2012) they discussed that using of 2 and 3 % OLE had the beneficial effect in controlling the microbial load of beef cubes and meatball during storage at 4°C. The strong antibacterial effect of olive leaves are due to the phenolic compounds including: caffeic acid, verbascoside, oleuropein, luteolin 7-O-glucoside, rutin, apigenin 7-O-glucoside and luteolin 4'-O-glucoside (Pereira et al, 2007; Aytul, 2010 and Tahir and Khan, 2012).

In the present study we observed that *Salmonella typhimurium* as a Gram-negative bacterium is more resistant to the antibacterial effect of olive leaves extract than *Staphylococcus aureus* (Gram-positive) bacteria (Table 1), this agreed with Srinivasan et al (2001) who stated that in general, plant extracts have much greater inhibition effect against Gram-positive than Gram-negative bacteria. It may be due to the external lipopolysaccharide wall that surrounds the peptidoglycan cell wall of the former Kuete et al (2007) and Lee and Lee (2010).

Sensory analysis

Result of analysis of variance revealed that adding of 1% OLE to minced meat had significantly better overall acceptability compared to untreated or other treated groups (Table 2). The same findings were observed by (Hayes et al, 2009, Hayes et al, 2011;

Tayel and El-Tras, 2012 and Baker, 2014).

Conclusion

From this study we can conclude that the addition of 1% OLE lead to reduce the *Staphylococcus aureus* and *Salmonella typhimurium* count after 24 hours of storage at 7°C and by 2 and 1 log cycle respectively in examined sample of minced meat. Thus the aqueous extracts of olive leaves could be considered as a suitable antibacterial agent with overall sensory acceptability at concentration of 1% against the tested organisms which are considered the most prevalent food pathogens responsible for meat borne illness.

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الملخص العربي

فاعلية مستخلص أوراق الزيتون على إزالة التلوث بميكروب السالمونيلا تيفيميوريم و المكور العنقودي الذهبي في اللحم المفري.

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قسم قسم صحة الأغذية معهد بحوث الصحة الحيوانية* و كلية الطب البيطري جامعة قناة السويس**
تم دراسة تأثير مستخلص أوراق لزيتون كمضاد للبكتريا الممرضة التي يمكن أن تنتقل و تسبب أمراض للإنسان عن طريق تناول اللحوم و قد تم اختيار نوعين من البكتريا الممرضة و هما السالمونيلا تيفيميوريم و و المكور العنقودي الذهبي كمثالين للبكتريا السالبة الجرام و الموجبة الجرام لدراسة تأثير المستخلص المائي لأوراق الزيتون على كل نوع على حده. تم تلويث اللحم المفري بعدد 10⁶ خلية/ جرام بكل من و المكور العنقودي الذهبي و السالمونيلا تيفيميوريم كل على حدة ثم تقسيم كل عينة لإضافة مستخلص أوراق الزيتون بتركيزات 0، 1، 2، 3% و حفظها بالثلاجة و فحصها بكتريولوجيا و إجراء عد لكل نوع من البكتريا المستخدمة في العينات موضع الدراسة بعد ساعتين و 24 ساعة. و قد لوحظ ان عدد البكتريا قد تأثر بزيادة تركيز مستخلص أوراق الزيتون و كذلك فترة الحفظ بالثلاجة. كما لوحظ أن ميكروب و المكور العنقودي الذهبي أكثر حساسية لمستخلص أوراق الزيتون عن السالمونيلا تيفيميوريم هذا و قد تم دراسة تأثير إضافة المستخلص على الخواص الحسية للحم المفري من حيث اللون والرائحة و التقبل العام و قد تبين أن تركيز 1% كان له تأثير جيد على المواصفات الحسية للحم المفري