

**Original Paper****Effect of different concentrations of lemon oil on some food poisoning bacteria and histamine residue in fish fillet**Aya, T. Elbalsy^{1*}, Hemmat, M. Ibrahim¹, Mohamed A. Hassan¹, Shimaa, M. Nada²¹Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, Egypt²Food Hygiene Department, Animal Health Research Institute (Shebin Elkom), ARC, Egypt**ARTICLE INFO****Keywords**

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ABSTRACT

The current study aimed to investigate the sensory impact, antibacterial effect, and histamine degradation effect of lemon essential oil (LEO) (1 and 2% conc.) on fish fillet experimentally inoculated with *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) during cold storage. Referring to the obtained results, treatment with LEO extended the sensory characters acceptability when compared with control group. LEO 2% showed higher sensory scores up to the 9th day of the experiment, while spoilage mildly appeared at the end of the experiment in 1% LEO treated samples, indicating that the LEO preservative effect is a concentration dependent. Regarding to the antibacterial effect, there is a significant reduction in *S. aureus* count at 9th days of cold storage with reduction percent of 68.9 and 78.3%; furthermore, *E. coli* showed significant reduction (48.1 and 75.3%) in the treated samples with 1 and 2% LEO, respectively. Moreover, lemon oil showed significant retardation in the histamine levels in the treated samples in comparison with the untreated control sample, where the progression levels were significantly lower than the untreated control sample (112.5%) to be 36.2 and 21.1% in the treated samples with lemon oil 1 and 2%. Conclusively, lemon oil showed a strong antibacterial effect with a potential adverse effect on the formation of histamine in chilled fish fillet making it as a promising natural food-additive for bacterial and chemical quality enhancement.

1. INTRODUCTION

Microbial food safety is of a major health concern in our life. Food spoilage leads to reduction of shelf life, food poisoning, and economic losses. For example, *Staphylococcus aureus* that considered a main cause of food poisoning, toxic shock syndrome, endocarditis, and osteomyelitis worldwide (Pinchuk *et al.*, 2010). Among these organisms, *E. coli* has become recognized as a serious foodborne pathogen and has been associated with numerous food poisoning outbreaks around the world (Bintsis, 2017); which is characterized by hemolytic uremic syndrome (HUS) and gastroenteritis, especially in severe cases of shiga toxin producing *E. coli* (STEC) infections (Yang *et al.*, 2017).

In decades, chemical food-additives have been widely used; but recently it's strictly recommended its limitation because of their toxic and residual effects. So, a powerful interest in using natural additives of herbal origins, especially their extracts and essential oils, for food preservation due to their wide spectrum of bio-active constituents revealing antibacterial, antifungal and antioxidant activities (Khorshidian *et al.*, 2018).

Throughout history, the medicinal plants have provided a variety of usage as food flavoring spices and preservation that has given researchable interests to replace chemical

additives (Martínez-Graciá *et al.*, 2015). Aromatic plants and their extracts have been evaluated for their effectiveness for food safety and preservation applications. Most of their preservative properties were attributed to their essential oil's (EOs) active principles and other secondary plant metabolite components. EOs have well-recognized properties such as antimicrobial as well as antioxidant properties (Laranjo *et al.*, 2019). *Citrus* fruits belong to the family *Rutaceae* that includes about 140 genera; for instance, *Citrus limon* (Lemon) is among important species of genus *Citrus* (Kamal *et al.*, 2011). Lemon EO composes many valuable products as mixtures of terpenes, sesquiterpenes, aldehydes, alcohols, esters and sterols which have the antimicrobial, antioxidant and flavoring characters (Ambrosio *et al.*, 2021).

Due to its significant health concern, many previous studies had reported the strong antibacterial effect of different lemon by-products such as essential oil, peel extracts, powders and juice on different major foodborne pathogens as *Escherichia coli* and *Staphylococcus aureus* (Li *et al.*, 2019), and biogenic amines residues (El-Khabaz *et al.*, 2017) revealing enhanced shelf-life and quality of the examined food item; so, lemon EO was considered as a promising food-additives of dual flavoring and preservative application.

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Therefore, the current study was conducted to evaluate the antibacterial effect of lemon EO (1 and 2%) on experimentally inoculated food poisoning bacteria (*E. coli* and *S. aureus* counts), and histamine residue in chilled fish fillet.

2. MATERIAL AND METHODS

2.1. Fish fillet sampling:

Fresh *Tilapia niloticus* fillets (750 g) was cut with a sterile scalpel and put under the UV light in the cabinet for 20 minutes to reduce the number of the commensal microorganisms (Lopez-Malo and Palou, 2004).

2.2. Lemon Essential oil (Alfonzo et al., 2016):

Ready to use prepared lemon oil (LO) (100% conc./w.) was purchased from Al-Badawi Company for oil extraction and natural preparations, Mansoura governorate, Egypt.

2.3. Inoculation of *Staphylococcus aureus* and *Escherichia coli*:

Staphylococcus aureus (3.2 log₁₀ CFU/g) and *Escherichia coli* (3.6 log₁₀ CFU/g) (field isolate from fish samples) were obtained from Animal Health Research Institute. Strains were kept frozen in glycerol brain heart infusion broth to be used. Bacterial strains were enriched separately in BHI broth at 37±1°C for 24h aerobically before inoculation. Preparation and inoculation of the used strains were adjusted according to Saad et al. (2020).

2.4. Experimental Design:

The effect of Lemon Essential Oil (LEO) on the bacterial counts (log₁₀ CFU/g) of *Staphylococcus aureus* and *Escherichia coli*; and histamine levels (mg%) were studied. Accordingly, fish fillets (*Tilapia niloticus*), after inoculation and LEO immersing, were classified into 7 principal groups as follow:

The 1st group represented control untreated sample (200 g weight); which was used as control samples for bacterial count and histamine level estimation.

The 2nd group: 100 g weighted fillet sample was inoculated by 3.2 log₁₀ CFU/g of *S. aureus*, then immersed in lemon oil (1%) for 15 min and stored in refrigerator at 4±1°C for zero-time, 3, 6 and 9 days.

The 3rd group: 100 g weighted fillet sample was inoculated by 3.2 log₁₀ CFU/g of *S. aureus*, then immersed in lemon oil (2%) for 15 min and stored in refrigerator at 4±1°C for zero-time, 3, 6 and 9 days.

The 4th group: 100 g weighted fillet sample was inoculated by 3.6 log₁₀ CFU/g of *E. coli*, then immersed in lemon oil (1%) for 15 min and stored in refrigerator at 4±1°C for zero-time, 3, 6 and 9 days.

The 5th group: 100 g weighted fillet sample was inoculated by 3.6 log₁₀ CFU/g of *E. coli*, then immersed in lemon oil (2%) for 15 min and stored in refrigerator at 4±1°C for zero-time, 3, 6 and 9 days.

The 6th group: 50 g weighted fillet sample was immersed in lemon oil (1%) for 15 min, stored in refrigerator at 4°C for zero-time, 3, 6 and 9 days for histamine levels determination.

The 7th group: 50 g weighted fillet sample was immersed in lemon oil (2%) for 15 min and stored in refrigerator at 4°C for zero-time, 3, 6 and 9 days for histamine levels determination.

The experiment was repeated for three times and the mean ± SE were calculated.

After grouping and preparation, each sample was subjected to the following examinations:

Sensory evaluation (color, odor, texture and overall) following Mörlein (2019) in scores (1 to 5), where ≤1 represented the worst while 5- represented the excellent mark.

Bacteriological profile

Preparation of samples (ISO 6887-2, 2017): tenfold serial dilutions were prepared on sterile peptone water (0.1%); from which the following parameters were examined by pour-plate method:

Enumeration of Staphylococcus aureus (log₁₀ CFU/g) following ISO 6888- 1 (2003) on Baird Parker agar supplemented with egg yolk tellurite, and then incubated at 35±2°C for 24-48h.

Enumeration of Escherichia coli (log₁₀ CFU/g) following ISO 16649-2 (2001) on TBX agar, and then incubated at 44±1°C for 24h.

Determination of Histamine levels (mg%) using ELISA lateral flow test kit (Cat. No.: S9024/S9048), manufactured by ProGnosis Biotech S.A. following the manufacturer instruction (Pais et al., 2022).

2.5. Statistical analysis:

After triplicate examination of the designed treatment experiment, the obtained data were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

2.6. Calculation of reduction percent:

$$\frac{(B - A)}{A} \times 100$$

A

A = control reading

B = reading after treatment

3. RESULTS

The current study scoped the physical, bacteriological, and biogenic amine residue in lemon oil treated chilled fish fillet samples in comparison with control untreated samples. Table (1) indicated that treatment with LEO extended the acceptable sensory characters significantly when compared with a control group which showed spoilage signs on the 9th day of chilling storage. LEO 2% showed higher sensory quality up to the 9th day of the experiment (2.9: acceptable), while spoilage mildly appeared at the end of the experiment in the other treated groups indicating that the LEO preservative effect is a concentration dependent.

Regarding to the bacterial counts of the experimentally inoculated *S. aureus* and *E. coli* as were recorded. The results in Table (2) showed significant reduction in *S. aureus* count after nine days of cold storage with reduction percent of 68.9 and 78.3%, respectively in the treated samples with 1 and 2% lemon oil when compared with the untreated control group. In addition, *E. coli* showed a significant reduction in the treated samples with 1 and 2% lemon oil, 48.1 and 75.3% as was recorded in Table (3); indicating the more reduction effect of LEO on *S. aureus* (Gram- positive) than *E. coli* (Gram-negative).

Moreover, lemon oil showed significant retardation in the histamine levels in the treated samples as was recorded in Table (4), where the progression levels were significantly lower than the untreated control sample (112.5%) to be 36.2 and 21.1% in the treated samples with lemon oil 1 and 2%.

Table 1 Sensory profile of untreated and treated fish fillet samples with lemon EO in cold storage (4±10C).

Groups	Tested parameter	Control	LEO (1%)	LEO (2%)
Zero day	Color	4.8±0.26	4.7±0.36	4.6±0.21
	Odour	4.6±0.41	4.6±0.41	4.6±0.28
	Texture	4.7±0.36	4.6±0.26	4.5±0.32
	Overall	4.7±0.1a	4.6±0.03a	4.6±0.03 a
3rd day	Color	3.3±0.31	4.2±0.25	4.4±0.1
	Odour	3.4±0.28	4.2±0.21	4.5±0.2
	Texture	3.2±0.2	4.0±0.30	4.2±0.3
6th day	Overall	3.3±0.1c	4.1±0.1b	4.4±0.1a
	Color	2.1±0.12	3.6±0.31	3.9±0.1
	Odour	2.0±0.23	3.6±0.24	3.8±0.2
	Texture	1.6±0.18	3.3±0.3	3.5±0.3
9th day	Overall	1.9±0.2b	3.5±0.1a	3.7±0.1a
	Color	S	2.6±0.20	3.0±0.1
	Odour	S	2.5±0.33	3.0±0.3
	Texture	S	2.1±0.21	2.8±0.3
	Overall	S	2.4±0.15b	2.9±0.1a

The values represent Mean ± SE of three experiments. Means within the same row (abcd) followed by different superscript letters are highly significantly different ($P \leq 0.05$). Zero time: 30min after inoculation. 4.0-5.0 very good 3.1-3.9 good 2.1-3.0 Acceptable 1.1-2.0 Unacceptable 0.0-1.0 spoiled

Table 2 The antibacterial effects and Reduction percentage of lemon oil on *S. aureus* counts (log10 CFU/g) inoculated into fish fillet.

Storage time	Control	Lemon oil 1%		Lemon oil 2%	
	Average count	Average count	R%	Average count	R%
Zero time	3.22± 0.28 ^a	3.22± 0.28 ^a	-----	3.22± 0.28 ^a	-----
3rd day	4.4± 0.27 ^a	2.4± 0.24 ^b	25.5	2.0±0.18 ^c	37.8
6th day	5.8± 0.33 ^a	1.8± 0.19 ^b	44.1	1.49± 0.02 ^c	53.7
9th day	6.6± 0.42 ^a	1.0± 0.12 ^b	68.9	0.70± 0.06 ^c	78.3

R % = Reduction percent. Results are expressed as mean ± S.E.M. a, b & c: There is no significant difference ($P > 0.05$) between any two means, within the same row (of each group) have the same superscript letter.

Table 3 The antibacterial effects and Reduction percentage of lemon oil on *E. coli* counts (log10 CFU/g) inoculated into fish fillet.

Storage time	Control	Lemon oil 1%		Lemon oil 2%	
	Average count	Average count	R%	Average count	R%
Zero time	3.64± 0.31 ^a	3.64± 0.31 ^a	-----	3.64± 0.31 ^a	-----
3rd day	4.3± 0.42 ^a	2.75± 0.22 ^b	24.5	2.31± 0.11 ^c	36.5
6th day	5.2± 0.41 ^a	2.10± 0.20 ^b	42.3	1.41±0.06 ^c	61.3
9th day	6.4± 0.47 ^a	1.89± 0.15 ^b	48.1	0.90±0.01 ^c	75.3

Table 4 The effect and Reduction percent of lemon oil on histamine level (mg%) in fish fillet.

Storage time	Control		Lemon oil 1%		Lemon oil 2%	
	Histamine level	P%	Histamine level	P%	Histamine level	P%
Zero time	15.2± 1.1 ^a	----	15.2± 1.1 ^a	-----	15.2± 1.1 ^a	-----
3rd day	22.4± 2.1 ^a	47.4	17.4± 1.2 ^b	14.5	16.1± 1.1 ^c	5.9
6th day	29.1± 2.8 ^a	91.4	19.1± 1.5 ^b	25.6	17.6±1.6 ^c	15.8
9th day	32.3± 4.1 ^a	>100	20.7± 2.1 ^b	36.2	18.4±1.9 ^c	21.1

P % = Progression percent. Results are expressed as mean ± S.E.M. a, b & c: There is no significant difference ($P > 0.05$) between any two means, within the same row (of each group) have the same superscript letter.

4. DISCUSSION

Fish is an excellent nutritive value food having high-quality lipo-proteins and a huge variety of vitamins and minerals (Pal *et al.*, 2018). Adversely, because of its high moisture and easily utilized nutrient content, fish is susceptible to spoilage rapidly even during chilling conditioning with short-shelf life (Li *et al.*, 2012); associated with initial loss of freshness affecting the sensory characters of fish; so, sensory evaluation plays a significant role in food acceptability where the eating quality of any food is a human response (Mlian *et al.*, 2017). Fish can be an important source of foodborne pathogens like different emerging pathogens of serious threat to a public health concern (Igbinsosa *et al.*, 2012).

Different investigations announced that use of chemical substances and additionally manufactured food added substances can prompt degenerative sicknesses, and even malignant neoplasms. This initiated the need to search for elective techniques to broaden timeframe of realistic usability and cover different antimicrobial properties. As of late, food conservation strategies have shown impressive interest in using natural plant's byproducts because of their capacity to control the development of pathogenic bacteria with their antioxidant activity (Aminzare *et al.*, 2016).

As consumers tend to adopt towards eating food containing no chemicals; EO come to fulfill part of the increasing demand for products of "green image" being obtained from natural sources (Holley and Patel, 2005). Essential oils may be added to food product by different techniques as dipping or spraying (Khalafalla *et al.*, 2015).

Referring to the sensory evaluation of the treated samples in Table (1), which indicated a promising enhancement in the sensory impact of the treated samples comparing with the control untreated sample. Moreover, referring to the obtained results indicating the antibacterial effect of lemon oil on *E. coli* and *S. aureus* in chilled fish fillet as were recorded in Tables (2 and 3); lemon oil showed significant antibacterial effect on the examined bacterial counts; furthermore, it showed higher reduction effect on *S. aureus* (positive Gram bacteria) than *E. coli* (negative Gram bacteria); which came in agreement with the previously recorded by Hyldgaard *et al.* (2012) who determined that the antibacterial activity of essential oils not only depend on chemical characteristics of essential oils, but also on the type of bacteria. Essential oils more effective against Gram positive bacteria than Gram negative bacteria, which could be attributed to the better effective permeability barrier of Gram- negative bacteria to restrict the penetration of amphipathic compounds than Gram positive microbes (Fisher and Phillips, 2009). The most common mode of

antibacterial action of essential oils is through damage of bacterial cell membrane by their effect on morphology, structure, function, modification in the transport of nutrients, membrane disruption and extensive leakages from the bacterial cells leading to cell death (Schelz *et al.*, 2010 and Nazzaro *et al.*, 2014).

The main component of lemon EO is α -limonene, in the range of 51% to 68% (Mancuso *et al.*, 2019 and Djenane, 2015), together with other monoterpenes. Lemon EO has been tested for its preservation effect on different forms and different meat matrices included fish fillet; Giarratana *et al.* (2016) tested lemon EO on some specific spoilage bacteria isolated from fish during 15 days of refrigerated storage plus evaluation of sensory quality of treated samples, the results revealed that the addition of lemon oil improved the treated fish sample's microbial quality and sensory scores in a concentration-dependent manner; in addition, other studies done by Alfonzo *et al.* (2016) who examined the preservative effects of lemon essential oil (0.3 and 1.0%) on salted sardine's microbial quality, where it revealed great inhibition to the microbial growth of Enterobacteriaceae, staphylococci and Lactic Acid Bacteria in the treated samples, and Kunová *et al.* (2021) recorded significant inhibition of coliform bacteria in lemon oil treated samples.

The antibacterial effect of the lemon essential oil is increased by increase their concentration, and their immediate reduction of bacterial population might be more effective against food borne pathogens and spoilage bacteria when applied directly on foods ready to be used (Salem *et al.*, 2017).

The results declared in Table (4) showed the degradation effect of lemon EO (1% and 2%) on the histamine level in the examined fish samples, where the progression levels were significantly lower than the untreated control sample (112.5%) to be 36.2 and 21.1% in the treated samples with lemon oil 1 and 2%; and these findings came in agree with the recorded results by Alfonzo *et al.* (2016) in which, the addition of EOs at two different concentrations (0.3 and 1.0%) determined lower accumulation of histamine in sardine than the control sample; El-Khabaz *et al.* (2017) who declared reduction of histamine levels in the treated fish fillets with lemon EO (1%) by 15.8% - 29.3% after 2 and 4 days of cold storage.

Fishery foods present a high risk of histamine intoxication and many regulations have been worldwide legislated to limit this risk (FDA, 1998 and EC, 2007). Ingestion of food containing small amounts of histamine has little effect on humans, but large amounts histamine can be toxic. The characteristic symptoms of histamine poisoning are rash, urticaria, edema and localized inflammation (Taylor and Mathew, 2021).

5. CONCLUSION

Afterall, it can be concluded that the lemon oil has a strong antibacterial effect with a potential adverse effect on the formation of histamine residue in chilled fish fillet making it as a promising natural food-additive for bacteriological and chemical quality enhancement.

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