

Anti- *Staphylococcus aureus* activity of some essential oils and their impacts on physicochemical properties of chilled minced meat

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ABSTRACT

Raw minced beef is one of the most used meat products and exposing to microbial contamination as well. So, improving its preservation techniques is highly recommended, especially with raising of microbial multidrug resistance worldwide. The recent study aimed to evaluate impacts of olive and garlic essential oils on the physicochemical and *S. aureus* multiplication in chilled minced beef along nine days of refrigeration. The minimum inhibitory concentration (MIC) of oils was determined using disc diffusion technique. Results revealed higher inhibitory effect of garlic oil than olive oil on *S. aureus*, combination of olive and garlic (GOC) treated samples showed significant synergistic effect appeared as wider zone of inhibition and higher enhancement effects on the physicochemical and bacterial quality of the treated chilled minced meat. Although, the treated samples were sensory acceptable at the 9th day, when the control group showed spoilage characteristics; they, all, showed signs of spoilage in various degrees at the 12th day of storage. In addition, results of pH, TBA and TVN were 6.3, 0.82 and 18.7; 6.2, 0.79 and 18.3; 6.0, 0.75 and 17.5 for the olive oil (1.5%), garlic oil (1.0%) and GOC treated groups at the 9th day of storage, respectively. On the other hand, significant retardation in the *S. aureus* growth was observed in all the treated samples revealing that the used oils were a promising and recommended meat additive of significant preservation characteristic on the meat safety and quality.

Introduction

Around the world and in Egypt, ground beef is a common ingredient used in a variety of recipes. However, because of process contaminations, improper handling, and preservation, which encourage the growth of both spoilage and pathogenic bacteria, this meat can easily become contaminated with microorganisms, leading to a loss of quality and potential health issues (food poisoning) (USDA, 2016).

The increasing frequency of foodborne illness outbreaks is a severe issue, despite improved cleanliness and sophisticated food production practices, commercially manufactured meals may be more vulnerable to microbial contamination. Recently, virulent pathogenic bacteria have been found in acidified food products as well (WHO, 2022). Consumers prefer foods with natural additives than those containing chemical preservatives. Consequently, there is a growing need for safer foods and natural biopreservatives (Teshome *et al.*, 2022).

Plants, herbs, spices, and certain foods contain substances that possess antimicrobial activity. Among them, garlic and olive oils are among the most reported foods with bactericidal activity.

Olive oil is a common ingredient in many recipes and commercially processed meals like tuna, tomato, and mayonnaise; in addition to be eaten directly on toast and in fresh salads. According to Medina *et al.* (2007), polyphenols found in olive leaves, olive oil waste fluids, and olive fruits have antibacterial activity against a variety of microbes. There have been few studies on the antimicrobial activity of polyphenols found in olive oil, but those that have been found to be active include hydroxytyrosol, tyrosol, the dialdehydic form of decarboxymethyl ligstroside, and oleuropein aglycons; which, have the strongest bactericidal activity (Nazaro *et al.*, 2019).

On the other hand, many cultures have utilized garlic (*Allium sativum*) and its derivatives to preserve food and treat infectious diseases (Kumar, 2020). Garlic is high in organosulfur compounds and selenium,

both of which have well-established and noticeable antioxidant action. Garlic also possesses broad spectrum antimicrobial properties due to its antiviral, antifungal, antibacterial, and antiprotozoal properties. Garlic's primary antibacterial properties are ascribed to its allicin, or diallyl disulfide and diallyl trisulfide, organosulfur compounds. Nevertheless, intact garlic bulbs have alliin (*S*-allyl-L-cysteine sulphoxide), which is the precursor to allicin and has broad spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria (Mukhtar and Ghori, 2012; Muhammad *et al.*, 2014).

Regarding health concerns, *S. aureus* is a significant foodborne pathogen. It can affect both humans and animals. It can cause a broad range of illnesses, from minor skin infections to more serious conditions like pneumonia and septicemia. Meat contaminated with *S. aureus* can occur at several points along the food supply chain, from farms to markets (Hamad *et al.*, 2023). One of particular relevance to the food processing industry is the ability of some *S. aureus* strains to produce heat stable enterotoxins (SEs) that cause *Staphylococcus* food poisoning (SFP), results from ingestion of one or more performed SEs on food that has been contaminated with *S. aureus* and ranks as one of the most prevalent causes of gastroenteritis worldwide which is that is manifested clinically as emesis with or without diarrhea (Tong *et al.*, 2015).

Owing to the widespread consumption of ground beef which commonly use it after mixing with various spices and subsequent storage which may reach to several days, this study designed to evaluate the antibacterial effect of olive and garlic oils on *Staphylococcus aureus* inoculated in chilled minced meat along nine days of storage.

Materials and methods

Collection of minced meat samples

A total of 6400 g of fresh minced beef were purchased from one

butcher, who applies high hygienic levels, located in Benha city, Qalubiya governorate, Egypt. The minced beef sample was put in sterile plastic bag and hygienically transported in an ice box to the laboratory of Animal Health Research Institute – Benha lab. for conducting the experiment.

Collection of essential oils

Commercially prepared ready-to-use essential oils of olive (*Olea europaea*), and garlic (*Allium sativum*) were collected from the plant extraction unit, national research center (NRC), Egypt. Hydro-distillation is used to extract the essential oils (EOs) from their sources with a purity of 99%. EOs were kept in dry sealed dark glass vials at 4°C until use (Barros et al., 2022).

In vitro determination the minimum inhibitory concentration (MIC) of the used essential oils

Preparation of *S. aureus* strain (de Assis et al., 2011; ISO 6888-1, 2023)

One ml from the *S. aureus* stock culture was pre-enriched on tryptic soy broth for 24 h at 37°C; from which, tenfold serial dilution was made on a sterile normal saline (0.9%) followed by plating on Baird Parker agar and incubated at 35°C for 48 h for obtaining the 10⁶ CFU/ml concentration for the further applications.

Determination of MIC technique

In vitro determination the MIC of the used essential oils was performed by Agar disk-diffusion technique according to CLSI (2012). Original oil was diluted by Tween-80 as an oil emulsifier into three concentrations (0.5%, 1.0% and 1.5%). Each of these concentrations was examined against foodborne *S. aureus* strain (10⁶ CFU/ml) to obtain the minimum concentration that reveal a significant inhibitory effect, represented by clear zone of inhibition. According to the obtained results, samples of minced beef were treated by olive oil, garlic oil, and in combination to assess their effect on the physicochemical and bacteriological quality of the treated minced beef samples in chilling storage.

Experimental design

Preparation of minced beef sample

Before treatment, minced beef was arranged in thin layer, and exposed to UV light to inhibit most of commensal bacteria according to Soro et al. (2023), followed by inoculation of *Staphylococcus aureus* (about 10⁶ CFU/g). After which, minced beef sample was grouped into four primary groups (1600 g/each group). Each group was sub-grouped into two subgroups (1450 g for chemical and sensory evaluation, 150 g for *S. aureus* counting); then each subgroup was furtherly divided into four portions. Groups were represented as follow:

- Group 1: untreated minced beef + *S. aureus* (10⁶ CFU/g)
- Group 2: treated minced beef with olive oil (1.5%) + *S. aureus* (10⁶ CFU/g).
- Group 3: treated minced beef with garlic oil (1.0%) + *S. aureus* (10⁶ CFU/g).
- Group 4: treated minced beef with olive-garlic oil combination (1.5:1%) + *S. aureus* (10⁶ CFU/g).

The treated minced beefs were kept in refrigerated at 4±1°C until sensory, chemical and bacteriological examination at day zero (2 h post treatment), then periodically every 3 days up to appearance of organoleptic spoilage during cold storage. Samples were subjected to the following examinations:

Sensory evaluation of examined meat samples

Meat samples were assessed for their sensory characters following

Mörlein (2019) in scores (1 to 5), where 1- represented the worst while 5- represented the excellent mark.

Bacteriological examination

Samples were prepared according to ISO 6887-2 (2017), followed by counting of *Staphylococcus aureus* according to ISO 6888- 1 (2023).

Chemical examinations

pH, total volatile nitrogen (TVN), and thiobarbituric acid (TBA) values were conducted according to EOS 63-11 (2006), EOS: 63-9 (2006), and EOS 63-10 (2006), respectively. The experiment was performed in triplicate.

Statistical analysis

Data was analyzed using SPSS version 20 (SPSS Inc., Chicago, IL). ANOVA analyses, followed by Duncan post-hoc value were used to ascertain the significance of differences between mean values of the examined groups. The level $p \leq 0.05$ was considered as significance.

Results

Table 1 and Fig. 1 showed the obtained findings of MIC of the used olive oil, garlic oil of 0.5, 1 and 1.5% conc., and the garlic-olive combination (1%: 1.5%). Although, it appeared that the antibacterial effect of the used oils is concentration dependent, and all of the used concentrations had an inhibitory effect on *S. aureus* growth, garlic-olive combination had the widest and clearest zone of inhibition (2.1 cm in diameter).

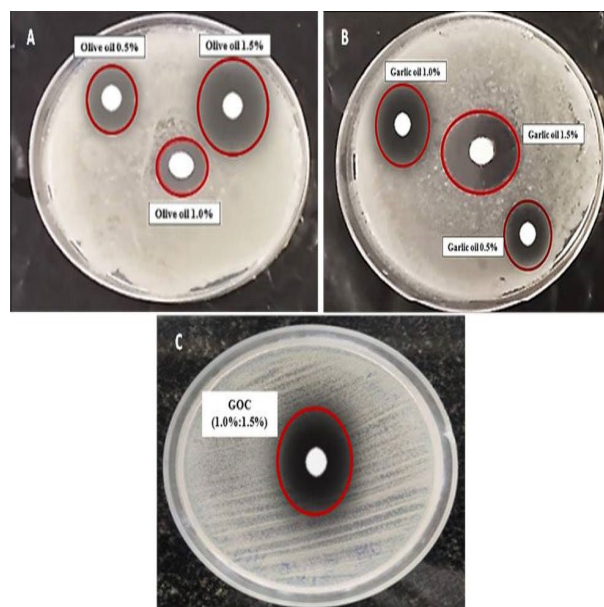


Fig. 1. Zone of inhibition of the used olive oil (A), garlic oil (B), and garlic-olive combination "GOC" (C)

Table 1. Zone of inhibition of the used oils (mm) against *S. aureus*.

Oil concentration	Zone of inhibition (diameter cm)
Olive oil (0.5%)	0.8
Olive oil (1.0%)	1.3
Olive oil (1.5%)	1.5
Garlic oil (0.5%)	1
Garlic oil (1.0%)	1.5
Garlic oil (1.5%)	1.8
GOC (1%:1.5%)	2.1

GOC: Garlic-Olive Combination

Table 2. Sensory profile of untreated and treated minced beef samples in cold storage (4±1°C).

Groups	Tested parameter	G1	G2	G3	G4
Zero day	Color	4.9±0.3	4.9±0.3	4.9±0.3	4.9±0.3
	Odor	4.7±0.3	4.7±0.3	4.7±0.3	4.7±0.3
	Texture	4.8±0.4	4.8±0.4	4.8±0.4	4.8±0.4
	Overall	4.8±0.2 ^a	4.8±0.2 ^a	4.8±0.2 ^a	4.8±0.2 ^a
3 rd day	Color	3.0±0.2	4.5±0.4	4.5±0.1	4.7±0.5
	odor	3.3±0.2	4.5±0.2	4.3±0.2	4.5±0.4
	Texture	3.5±0.3	4.7±0.3	4.7±0.3	4.8±0.3
	Overall	3.3±0.2 ^b	4.6±0.3 ^a	4.5±0.3 ^a	4.6±0.4 ^a
6 th day	Color	2.1±0.4	3.8±0.1	4.0±0.1	4.4±0.2
	odor	2.2±0.3	3.6±0.2	3.8±0.2	4.0±0.5
	Texture	2.0±0.3	3.5±0.3	4.1±0.3	4.2±0.3
	Overall	2.1±0.2 ^c	3.6±0.3 ^b	4.3±0.2 ^a	4.2±0.1 ^a
9 th day	Color	<1	3.0±0.2	3.5±0.1	3.9±0.3
	odor	<1	3.2±0.2	3.4±0.3	3.5±0.2
	Texture	<1	2.8±0.3	3.0±0.3	3.4±0.2
	Overall	Spoiled	3.0±0.2 ^c	3.3±0.2 ^b	3.6±0.3 ^a
12 th day	Color	<1	<1	<1	<1
	odor	<1	<1	<1	<1
	Texture	<1	<1	<1	<1
	Overall	Spoiled	Spoiled	Spoiled	Spoiled

Values are presented as Mean ± SE of three trials.

Means within the same row followed by different superscript letters (^{abcd}) are significantly different ($P \leq 0.05$).

Zero time: 2h 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.

Referring to the recorded results in Table 2, the used additives showed a significant ($p \leq 0.05$) improvement in the sensory quality of the treated samples in comparing with the control untreated group. Although, the overall sensory scores of the examined groups were 3.0, 3.3 and 3.9 for the G2, G3 and G4, respectively, revealed them acceptable at the ninth days of storage, they showed spoilage characteristics at the 12th day of storage; whereas, control untreated group showed spoilage after the 6th day of storage.

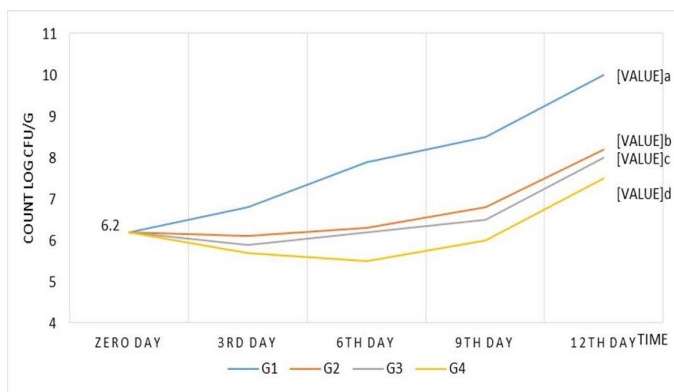


Fig. 2. Average values of *S. aureus* count (CFU/g) in minced beef groups at cold storage (4±1°C).

Means within followed by different superscript letters (abcd) are significantly different ($p \leq 0.05$).

Chemical analyses of the treated groups (Table 3) showed significant ($p \leq 0.05$) enhancement in the pH, TVN and TBA parameters of the treated samples in relation to the control untreated sample; where they still fit for human consumption up to the 9th day of refrigeration, while the control group exceeded the permissible limits before the 9th day of refrigeration; whereas the treated samples spoiled at the 12th day of storage.

Regarding with the inhibitory effect on *S. aureus* growth, addition of olive oil (1.5%) and garlic oil (1%), and their combination showed significant ($p \leq 0.05$) inhibitory effects on *S. aureus* appeared as a retardation

in the bacterial growth in the treated groups in relation to the control untreated groups (Fig. 1). The original inoculum counted 6.2 log CFU/g in all the examined groups, and recorded 10, 8.2, 8.0 and 7.5 log CFU/g in the control, olive oil (1.5%), garlic oil (1.0%) and GOC treated samples at the 12th day of storage, respectively.

Discussion

Minced meat is the product of the transformation of the whole muscle of slaughtered food animal to meat product by means of grinding. Because of its high water content, fat, protein and nutritional value, meat is essential indispensable in a balanced diet. However, these same reasons make it a very favorable media for the development of microorganisms.

The agri-food industry is regarded as one of those that has a direct impact on human health. The food sector needs to be able to supply meals that will probably guarantee the consumer's nutritional satisfaction. Many ancient cultures have been using herbal byproducts for long times to enhance the flavor and aroma of their meals. Additionally, because of their antibacterial qualities, they have been utilized as a food preservative (Hintz *et al.*, 2015). According to data on foodborne illnesses worldwide, two thirds of foodborne disease outbreaks are caused by bacterial food poisoning (Abebe *et al.*, 2020); *S. aureus* accounts for two thirds of these cases (Bukhari *et al.*, 2021).

The need to create novel and inventive antimicrobial drugs is urgent because of the rise in microbial resistance to antibiotics. Plants have long been studied as possible sources of novel drugs since they contain a variety of bioactive chemicals with potential medicinal applications. There is a long history of using dietary plants to cure microbial diseases due to their low toxicity (Djeussi *et al.*, 2013).

Antimicrobial combinations may be resulted in synergistic, antagonistic, indifferent or additive action, the *in vitro* tests which may be performed included disc diffusion sensitivity testing and determination of minimum inhibitory concentration (MIC) (Makie and McCartney, 1996).

Referring to the obtained results in Table 1 and Fig. 1, all of the used oils had a noticed inhibitory effect against *S. aureus* growth with various ranges depending on their concentration, where the GOC combination had the highest inhibitory effect appeared as wider zone of inhibition; which came in line with the recorded results of Cottarel and Wierzbowski (2007) and Abd EL-Tawab *et al.* (2017) who concluded that Combination therapies have a real and broad potential to address significant synergism competing MDR microorganisms.

Table 3. Effect of different concentration of olive and garlic oils on TVN and TBA of minced meat during refrigerator storage (Means \pm SE).

T	pH				TBA				TVN			
	Zero day	3 rd day	6 th day	12 th day	Zero day	3 rd day	6 th day	12 th day	Zero day	3 rd day	6 th day	12 th day
G1	5.80 \pm 0.5 ^{dA}	6.1 \pm 0.3 ^{CA}	6.5 \pm 0.3 ^{BA}	S.	0.57 \pm 0.5 ^{dA}	0.74 \pm 0.08 ^{CA}	0.86 \pm 0.1 ^{BA}	S.	15.5 \pm 1.5 ^{dA}	17.7 \pm 2.1 ^{CA}	19.9 \pm 1.2 ^{BA}	S.
G2	5.77 \pm 0.5 ^{CA}	5.81 \pm 0.1 ^{dB}	5.96 \pm 0.6 ^{BB}	S.	0.57 \pm 0.5 ^{CA}	0.63 \pm 0.04 ^{dB}	0.75 \pm 0.05 ^{BB}	0.82 \pm 0.04 ^{BB}	15.5 \pm 1.5 ^{CA}	16.1 \pm 2.5 ^{BB}	17.5 \pm 2.3 ^{BB}	18.7 \pm 2.4 ^{BB}
G3	5.76 \pm 0.5 ^{CA}	5.64 \pm 0.6 ^{CC}	5.86 \pm 0.5 ^{CC}	S.	0.57 \pm 0.5 ^{CA}	0.60 \pm 0.04 ^{CC}	0.72 \pm 0.05 ^{CC}	0.79 \pm 0.06 ^{CC}	15.5 \pm 1.5 ^{CA}	15.8 \pm 2.0 ^{CC}	17.0 \pm 2.0 ^{CC}	18.3 \pm 1.6 ^{CC}
G4	5.75 \pm 0.5 ^{CA}	5.68 \pm 0.2 ^{DD}	5.8 \pm 0.5 ^{DD}	S.	0.57 \pm 0.5 ^{CA}	0.60 \pm 0.06 ^{CC}	0.68 \pm 0.07 ^{DD}	0.75 \pm 0.06 ^{DD}	15.5 \pm 1.5 ^{CA}	15.8 \pm 2.0 ^{DD}	16.7 \pm 1.0 ^{DD}	17.5 \pm 1.6 ^{DD}

Means within the same row followed by different superscript letters (^{abcd}) are significantly different ($P \leq 0.05$). Means within the same column followed by different superscript letters (^{abcd}) are significantly different ($P \leq 0.05$). S: Spoiled physically.

Referring to the obtained results in Table 1, the used extracts revealed significant enhancement in the sensory quality of the treated samples, represented as acceptable meat sensory quality up to nine days of storage. Although the significant recorded elongation in the acceptability criteria, olive-garlic oils combination showed higher sensory scores along the treatment period, which may be referred to the synergistic effect between the antimicrobial and the antioxidant effects of the used oils.

Olive oil is a liquid fat that is made from pressing whole olives, which are the fruit of the *Olea europaea* plant. Its beneficial antimicrobial and antioxidant properties are primarily attributed to the polyphenols in the olive oil, which appear to inhibit the growth of pathogenic bacteria while encouraging the growth of probiotic bacteria that are good for the gut

and combating free radicals and oxidative stress (Jimenez-Lopez et al., 2020).

Garlic (*Allium sativum*) has been used as a spice with medicinal and functional properties; which have been known since ancient times. Garlic extracts have been known for their antimicrobial and antioxidant effects because of their contents of organosulfur compounds (allicin, ajoene, and various aliphatic sulfides), which play to inhibiting reactive oxygen species, radical scavenging, and affecting microbial cell permeability revealing a reduction potential on the microbial growth, even against multidrug resistant bacteria (Bhatwalkar et al., 2021).

Garlic infused olive oil is now a known commercial product used in many countries for health and cooking concerns. Referring to the food technology, it has been used as food additive for its strong aroma, ripening effect and preservative effect (Paz, 2021).

Additionally, the used oils showed powerful antioxidant effect appeared as lower pH, TVN and TBA scores in relation to the untreated control group. Treated samples showed longer keeping quality represented by pH, TVN and TBA levels, which still within acceptable range up to nine days of storage. However, the treated samples with the olive-garlic oils combination had the best chemical indices (Table 2); which came in agreement with the recorded antibacterial effect of the used oils against *S. aureus*. Olive, garlic and oil combination showed significant retardation in the *S. aureus* multiplication in comparing with the untreated control group (Fig. 2).

The recorded significantly enhanced physicochemical and bacteriological quality of the treated samples may be attributed to the powerful antioxidant and antibacterial compounds present in the olive and garlic oils, as was mentioned by Medina et al. (2007) who attributed olive oil's antibacterial effect to its content of hydroxytyrosol, tyrosol, and the dialdehydic form of decarboxymethyl ligstroside and oleuropein aglycons, and other organosulfur compounds of garlic oil (Bhatwalkar et al., 2021).

The recorded results came in line with those recorded by Kota and Paladi (2013) who stated that the synergistic effect is generally a result of components of one spice aiding the other and improving the total efficacy. Zaqzouq et al. (2022) used garlic oil of (0.3, 0.5 and 1% v/w) against *S. aureus* in minced meat and recorded a significant reduction in *S. aureus* counts in all of the used concentrations.

Conclusion

Olive and garlic oils have shown to be a promising food preservative, that have an enhancement effect on the sensory, physicochemical, and microbiological quality of minced meat in the refrigerator storage, especially in olive-garlic oils combination.

Conflict of interest

The authors declare that they have no conflict of interest.

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