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Assessment of the potential impacts of garlic and/or sage essential oils on quality enhancement of chilled tilapia fish kofta

Shymaa Y.A. Ibrahim[1], Shaimaa A. Abd El-kader[2], Walaa M. Gomaa[3], Walid S. Arab[1], Rasha Elsabagh[1*]

[1]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Qaluobia 13736, Egypt.*
[2]*Bacteriology Department, Animal Health Research Institute (AHRI), Zagazig branch. Agriculture Research Center (ARC), Egypt.*
[3]*Food Hygiene Department, Animal Health Research Institute (AHRI), Shebin El Kom branch. Agriculture Research Center (ARC), Egypt.*

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***Correspondence:**

Corresponding author: Rasha Elsabagh
E-mail address: Rasha.alsbagh@fvtm.bu.edu.eg

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ABSTRACT

Fish and fish products are highly nutritional food that spoiled rapidly. Moreover, consumer needs for natural preservatives instead of synthetic ones have increased. So, the aim of this study was to evaluate the natural preservation effects of sage (*Salvia officinalis*) and garlic (*Allium sativum*) essential oils on the shelf life, sensory attributes, chemical quality, and bacteriological quality of chilled fish balls (Kofta). It was found that shelf life and sensory attributes of those group treated with garlic 2%, sage 2%, and mixtures (garlic: sage) has increased till the 15th day of chilled storage compared to control group (untreated) that rejected on the 6th day of storage. Examined EOs showed marked antioxidant efficacy that delayed chemical deterioration of tilapia fish kofta. Moreover, antibacterial impacts of EOS were detected by reducing total bacterial counts, Psychrotrophic count, Pseudomonas count, Aeromonas count, and *Staphylococcus* count. It was concluded to that using EOs especially garlic and/or sage is a promising solution to overcome risk of chemical preservatives.

Introduction

Fish and fish products are one of the highly nutritional standard foods. They are rich in protein of high quality, omega-3 fatty acids, vitamins and minerals (FAO, 2022). Nile tilapia, *Oreochromis niloticus* is one of the most widely consumed fish species. It contains a highly nutritional value (Dawood *et al*., 2020). Fish, on the other hand, has a shorter shelf life and is very prone to deterioration even during refrigerated storage due to its high moisture and nutritional content as well as a predisposition for a higher pH. This rotting is to blame for the early loss of freshness that affects the sensory qualities of fish Li *et al*. (2012). Moreover, it is implicated in many out breaks due to different food-borne pathogens.

Food safety as a newly emerging global issues impacts world trade and human health, and this increases the customer's willing towards natural preservation of fish products to decrease risks of synthetic preservatives.

New approaches in the level of fish industry have been focused on reducing chemical preservatives and replace them by natural bioactive compounds, especially for highly susceptible spoilage food, such as fish and fish products (Presenza *et al*. 2023). These bioactive compounds previously applied in fish and fish products in the form of plant extracts (Elsabagh *et al*., 2023), or Essential oils (EOs) (Cai *et al*., 2015).

Natural EOs proved its role to enhance fish quality, extend shelf life, and satisfy the growing customer demand for clean-label items that are fresh and free of chemical preservatives (Hassoun and Oban, 2017). These EOs have antimicrobial capacity in fish (Guerino *et al*., 2019), prevent Gram-positive and Gram-negative food-borne pathogens in vitro tests (Guerino *et al*., 2019). Moreover, EOs have antibacterial, antifungal, antiviral, and anticancer activities, and extend shelf life of food (Salgueiro *et al*., 2010; Nikoli´c *et al*., 2013; Bukvi˘cki *et al*., 2014; Pereira *et al*., 2014). The positive impacts of these compounds are mainly related to its phenolic acid secondary metabolites (Basavegowda & Baek, 2021; Huang *et al*., 2021).

Numerous studies were applied to evaluate the impacts of EOs on fish quality, but few were recorded about its using in fish products as fish kofta. So, The goal of the current study was to evaluate the natural preservation effects of sage (*Salvia officinalis*) and garlic (*Allium sativum*) essential oils on the shelf life, sensory attributes, chemical quality (pH, TMA, TVB-N, TBA), and bacteriological quality (total bacterial counts, Psychrotrophic count, Pseudomonas count, Aeromonas count, and *Staphylococcus* count) of chilled fish balls (Kofta).

Materials and methods

Tilapia fish Kofta preparation

Samples of Nile tilapia (*Oreochromis niloticus*) fish fillet were collected from fish markets in El-Sharkia governorate and were directly minced using a mincing machine and packed in sterile polyethylene bags. The bags were then transported directly in insulated ice containers to the microbiology lab. (Animal Health Research Institute) under hygienic conditions for further treatment and analysis.

Fish dough was shaped into round balls manually under hygienic conditions to get raw fish kofta, then was divided into four groups as following; The first group (G1) was untreated (control), and the rest of the groups were well homogenized with garlic oil 2% (G2) and sage oil 2% (G3), and a mixture of the two oils at 1% each (G4).

Kofta samples were separately packed in polyethylene bags, labeled,

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and stored at 4ºC. Kofta samples were analyzed for sensory, chemical, and bacteriological properties promptly at 3 days intervals during the storage period on 0, 3rd, 6th, 9th, 12th, and 15th days. The experiment was conducted in triplicate.

Essential oils

The ready-made herbal oils of garlic (*Allium sativum*) and sage (*Salvia officinalis*) used in this study in pure state, free from preservatives or antioxidant substances, were purchased from the National Research Center, Dokki, Cairo, Egypt, which were provided by hydro-distillation method. These oils were stored in amber-colored bottles at 4°C until use.

Sensory Evaluation

Using the scoring test, the sensory evaluation of the tilapia fish kofta was conducted (Fan *et al*., 2008). On a five-point scale, the following sensory attributes of the samples were evaluated: odor (5, extremely desirable; 1, extremely unacceptable); color discoloration (5, no discoloration; 1, extreme discoloration); texture (5, firm; 1, very soft); and overall appearance (5, extremely desirable; 1, extremely unacceptable). The samples were excluded when the sensory characteristics fell below 4.0.

Chemical analysis

Values of pH were evaluated on pH meter following E.S 63/11, (ES, 2006a), while TMA was applied according to Murray and Gibson (1972) to assess the TMA concentration of marine and aqua-cultured fish species. While Total Volatile Nitrogen "TVN" Determination detected by spectrophotometer following E.S: 63/9, (ES, 2006b), and calculated by formula: TVN/l00g = (mls H2 So4 n 0.1 for sample-ml H_2So_4 n 0.1 for Blank) x 14. Concerning Determination of Thiobarbituric Acid Number "TBA", malonaldehyde (MDA) as an end product of lipid peroxidation was detected according to ES: 63/10, (ES, 2006c) by measuring the absorbance of the sample using a Spectrophotometer (UNICAM969AA Spectronic, USA) under wavelength 538.

TBA value= absorbance of sample x 7.8 (malonaldehyde (mg) /Kg

Bacteriological examination

Examined fish kofta was prepared according to ISO, (2017), to be examined bacteriologically as follow; total bacterial count (TBC) was evaluated using pour plate technique following ISO 4833-1, (ISO, 2013) at 35°C. While, Psychrotrophic count was determined following ISO, (ISO, 2002), at 7°C on plates with 30-300 colonies, the total psychrotrophic count/g was determined.

Moreover, total Pseudomonas count on Pseudomonas selective agar medium supplemented with glycerol (ISO, 2004) was detected by counting the greenish-yellow colonies after the incubation at 25°C for 48 h. While *Aeromonas hydrophila* count was counted on a particular Aeromonas species' media (Aeromonas Agar Media, Lab M) at 37°C/ 24 h. Total *Staphylococcus aureus* count was assessed using Baird Parker agar medium with incubation for 48 hours at 37°C (FDA, 2001).

Statistical analysis

Using the SPSS program and the one-way ANOVA test, the findings were statistically assessed (Feldman *et al*., 2003).

Results

Sensory evaluation

As shown in Fig. 1, the scores of essential oils' impacts on odor, color, Consistency, and appearance (5 points, for each) of tilapia fish kofta samples which concluded by the mean values of overall acceptability (5 points) on sensory acceptability provided by control samples, samples treated with Garlic 2%, Sage 2% and samples treated with Garlic: Sage 1:1 at different refrigerated storage time. A significant difference ($p<0.05$) between groups indicated that the samples treated with Sage 2% had the highest overall acceptability, followed by samples treated with Garlic: Sage 1:1%, and finally samples treated with Garlic 2%. So, EOs enhanced the sensory attributes of tilapia fish kofta to be accepted till the 15th day of storage in contrast to that of control group that rejected on the 6th day of storage based on sensory evaluation.

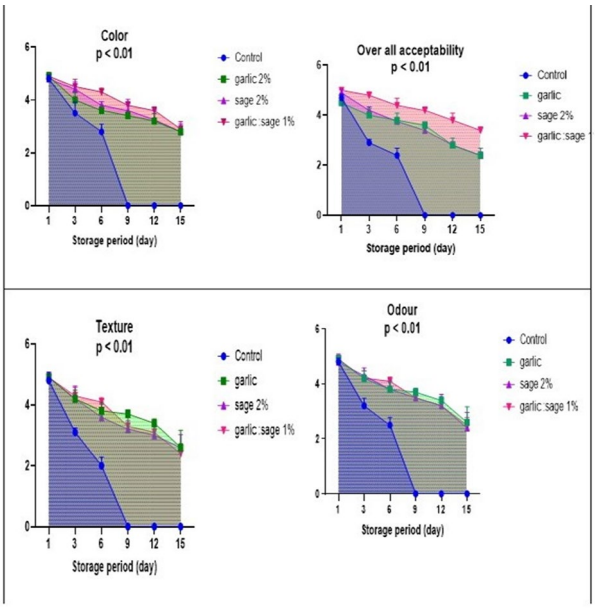

Fig. 1. Impacts of garlic EO, sage EO, and mixture of them on sensory attributes (color, texture, odor, and overall acceptability) of tilapia fish kofta during refrigerated storage.

Chemical evaluation

Impacts of used EOs on chemical quality were showed on Fig. 2, with a significant difference ($p<0.05$) between treated and control groups was detected in each indices (pH, TMA, TVB-N, and TBA). Mean values of pH, TMA, TVB-N, and TBA indicated that EOs treated samples delayed the chemical deterioration of samples to be with in accepted range till spoilage of samples on the 15th day of chilled storage. While in control untreated groups, chemical indices (pH, TMA, TVB-N, and TBA) increased rabidly to indicate incipient spoilage of control samples on the 6th day of storage. The higher impact on enhancement of chemical quality was detected in chilled tilapia fish kofta treated with garlic at 2%, followed by sage at 2%, and finally garlic: sage at 1:1%.

Bacteriological evaluation

Impact of essential oils on total bacterial count

Results in Fig. 3 showed the antimicrobial impact of used EOs on bacterial quality of chilled fish kofta. A significant difference ($p<0.05$) was shown between treated and control group. Fish kofta preserved by garlic 2%, sage 2%, and mixture showed a gradual decrease in mean values of bacterial counts to reach 6.13±0.1, 5.95±0.07 and 4.98±0.03 cfu/ mg, respectively in day 15 of refrigerated storage. While those of control untreated group increased from 8.3±0.02ccfu/mg on the 1st day of experiment to reach 8.9±0.01 cfu/ mg on the 6th day of storage.

Impact of essential oils on psychrotrophic count

Concerning the impacts of EOs on psychrotrophic counts, results in Fig. 3 showed a marked decrease in its count in treated groups with garlic

2%, sage 2%, and mixture compared to those of control group. The higher positive impacts detected in those samples treated with mixture (garlic: sage), decreased from 6.73±0.2 cfu/mg on the 1st day to reach 4.93±0.18 cfu/ mg on the 15th day of storage.

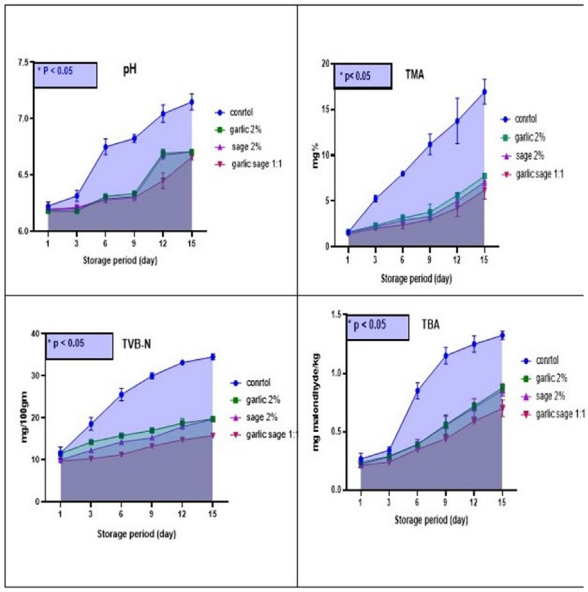

Fig. 2. Impacts of garlic EO, sage EO, and mixture of them on chemical indices (pH, TMA, TVB-N, TBA) of tilapia fish kofta during refrigerated storage.

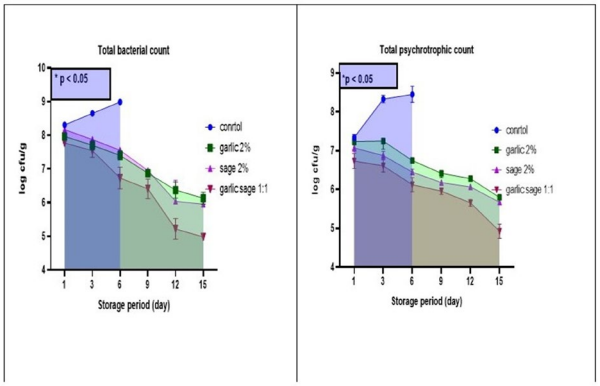

Fig. 3. Impacts of garlic EO, sage EO, and mixture of them on total bacterial counts, and Psychrotrophic count of tilapia fish kofta during refrigerated storage.

Impact of essential oils on *Pseudomonas* count

Concerning the antimicrobial effects of EOs on *Pseudomonas*, results in Fig. 4 showed a gradual decrease of its count in contrast to that of control untreated group that markedly increased with a significant difference (P<0.05). The higher impact was recorded by mixture of garlic and sage that decreased count of *Pseudomonas* from 4.8±0.28 cfu/ mg on the 1st day to 3.02±0.32 cfu/ mg at end of experiment.

Impact of essential oils on *Aeromonas hydrophila*

The recorded results revealed that the initial counts of *Aeromonas hydrophila* in control samples increased gradually during the refrigerated storage time starting with a mean value of 4.9±0.07 on the first to become 5.68±0.12 cfu/ mg on the 6th day of storage (sample rejected chemically and sensory. While samples treated with garlic 2%, sage 2%, and mixture (garlic: sage) showed a positive impact on decreasing *Aeromonas hydrophila* count to reach 3.42±0.32, 3.1±0.28 and 2.2±0.14 cfu/g at day 15 of storage (Fig. 4).

Impact of essential oils on Staph Count

Results in Fig. 4 showed the antimicrobial effect of EOs under experiments on staphylococcal count. Garlic 2%, sage 2% and mixture of them (garlic and sage) decreased staphylococcal count on the 15th day of storage to reach 4.05±0.07, 3.95±0.06 and 3.64±0.2 cfu/ mg, respectively to show a significant difference with those of control that reach 6.3±0.15 cfu/ mg on the 6th day of storage.

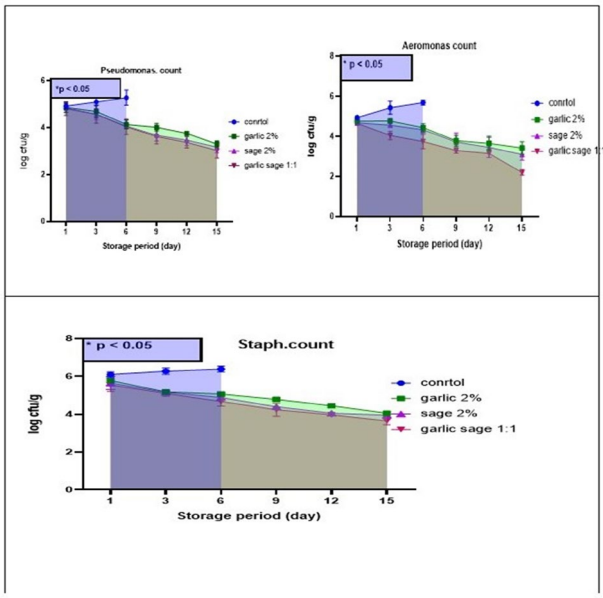

Fig. 4. Impacts of garlic EO, sage EO, and mixture of them on Pseudomonas count, Aeromonas count, and *Staphylococcus* count of tilapia fish kofta during refrigerated storage.

Discussion

Fish and fish products are considered one of the main sources of food and protein worldwide (Golden *et al*., 2021). However, fish products rapidly autolyzed and spoiled (Nie *et al*., 2022). In this study, impacts of garlic, sage, and mixture of them on chilled tilapia fish kofta were evaluated.

Sensory evaluation of the samples was evaluated, as it is the main requirement of consumer after safety of products. It depends on organoleptic qualities like color, odor, texture, and general acceptability of the product (Haq *et al*. 2013). sensory evaluation also, evaluate freshness of products (Reineccius, 1990). From results, garlic, sage, and mixture of them delayed the spoilage of chilled tilapia fish kofta, enhanced shelf life to reach 15 days compared to 6 days in control untreated groups. These results agree with those of Kuzgun (2019), who prolong the shelf life of trout with garlic essential oils and matches with those of Gomma *et al*. (2019), who used sage essential oils to enhance shelf life of anchovy fish burgers. It was found that, Results of sensory evaluation agrees with those of chemical profile's results and confirmed enhancement of shelf life of Tilapia fish kofta to reach 15 days under refrigerated storage. Extension of shelf life using bioactive compounds was previously reported by Elsabagh *et al*. (2023). Chemical profile of fish kofta (pH, TMA, TVN, and TBA) proofed the antioxidant effect of used EOs (garlic, and sage). Antioxidant effect of garlic and sage is owned to its content of phenolic and flavonoid compounds. sage has many phytochemicals, such as phenolic acids, polyphenols, anthocyanins, sesquiterpenoids, diterpenoids, flavonoid glycosides, sesterterpenes, and triterpenes (Sepahvand *et al*. 2014) that retard lipid oxidation in meat and fish products (Mizi *et al*., 2019). Also, garlic rich in sulfur-containing compound (Kirrella *et al*., 2021).

Results of chemical profiles (pH, TBA, and TVN) agree with those of Kuzgun *et al*. (2019) in *Oncorhynchus* fillet samples by garlic essential oil. Moreover, results of TMA agree with those by Hussein *et al*. (2023).

Results of this study proofed that garlic EO, sage EO, or mixture of them have not only antioxidant and improve shelf life, but also, positively impacts bacteriological quality of chilled Tilapia fish kofta.

Examined EOs showed a reduction effect to total bacterial count, and total psychotropic count. Moreover, a marked antibacterial effect of EOs against *Pseudomonas aeromonas*, *Staphylococcus* has shown by reduction of their counts. The higher antimicrobials from examined EOs was garlic, this agree with Kirrella *et al*. (2021) against *Aeromonas hydrophila*, Pranoto *et al*. (2005) against *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Bacillus cereus*, and also, with Hussein *et al*. (2023) against staphylococci.

Conclusion

It was concluded that using of EOs especially garlic EO, and/or sage

EO enhanced shelf life, sensory attributes, chemical profile and, positively impacts bacteriological quality of chilled tilapia fish kofta.

Conflict of interest

The authors declare that they have no conflict of interest.

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