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

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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 has increased. So 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%, sage2%, and mixtures (garlic: sage) has increased till 15th day of chilled storage compared to control group (untreated) that rejected at day 6th 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 *Staph*ylococcus count. It was concluded to that using EOs especially garlic and/or sage is a promising solution to overcome risk of chemical preservatives.

**Keywords:** Fish kofta, Tilapia, microbial quality, chemical, and sensory attributes

**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 refrigerator 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 implicated in many out breaks due to different food-borne pathogen.

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 has been focused to reducing chemical preservatives and replace it 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 has 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 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 *Staph*ylococcus count) of chilled fish balls (Kofta).

**MATERIAL 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 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 (G3).

Koftasamples were separately packed in polyethylene bags, labeled, and stored at 4ºC. Kofta samples were analyzed for sensory, chemical, and bacteriological properties promptly at 3days intervals during the storage period (at 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 was 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 raw fish fillet 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 was evaluated on pH meter following E.S 63/11, (2006), while TMA was applied according to Muray 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, (2006), and calculated by formula: TVN/l00g = (mls H2 So4 n 0.1 for sample – ml H2 So4 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,(2006) 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 following;

Total bacterial count (TBC) was evaluated Using pour plate surface plate technique following ISO 4833-1, (2013) at 35̊C. While, Psychrotrophic count was determined following 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 inoculation at 25 °C for 48 hours. While, *Aeromonas hydrophila* count was counted on a particular Aeromonas species' media (Aeromonas Agar Media, Lab M) at 37°C/ 24hrs. *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 Nile tilapia kofta samples which concluded by the mean values of overall acceptability (5 points) on sensory acceptability provided by control samples, samples treated with Garlic2%, Sage2% and samples treated with Garlic: Sage 1:1 at different refrigerator 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 fish kofta to be accepted till 15th day of storage in contrast to that of control group that rejected at 6th day of storage based on sensory evaluation.

**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 detect 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 at 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 at 6th day of storage. The higher impact on enhancement of chemical quality was detected in chilled 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 figure (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 at 1st day of experiment to reach 8.9± 0.01cfu/ mg at 6th gay of storage.

**Impact of essential oils on psychrotrophic count:**

Concerning the impacts of EOs on psychrotrophic counts, results in figure (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), that decrease counts from 6.73± 0.2 cfu/mg at 1st day to reach4.93± 0.18 cfu/ mg at 15th day of 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 decrease count of pseudomonas from 4.8± 0.28 cfu/ mg in 1st day to3.02 ± 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 refrigerator storage time starting with a mean value of 4.9± 0.07 on the first to became 5.68± 0.12 cfu/ mg at 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/gm 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 in 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 at 6th day of storage.

**DISCUSSION**

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

Sensory evaluation of sampled 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 mixtures delayed the spoilage of chilled fish kofta, enhanced shelf life to reach 15 day compared to 6 day in control untrated 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 use of 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. Extention 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 mixtures 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 also, positively impacts bacteriological quality of chilled tilapia fish kofta.

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