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Black seeds (Nigella sativa) essential oil impact on the microbiological and oxidative stability of Nile tilapia (*Oreochromis niloticus*) fish kofta

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ABSTRACT

Nowadays, the customer's approach towards naturally preserved fish meat products gained interest to overcome health risks of synthetic preservatives. So, our investigation aimed to study the effectiveness of natural preservatives for controlling spoilage in fish product. The potential of different concentrations of black seeds oil (1%, 1.5% and 2%) to act as antioxidant and antibacterial in tilapia kofta was evaluated. The count of Aerobic plat (APC), *Staphylococcal*, *Psychrotrophic*, *Pseudomonas* and *Aeromonas* counts recorded in control group were significantly differing between different concentrations of black seed ($P< 0.05$) throughout days of chilling. These results highlighted the ability black seed oil to increase shelf life of chilled fish kofta and improve sensory attributes (color, odor, texture and over all acceptability) and physicochemical characteristics (pH, TBA TVB-N and TMA) stored up to 14 days at 4°C. These results imply that the black seed oil especially with concentration 2% alternate option to be utilized as a food additive to increase the shelf life and acceptability in Tilapia kofta.

1. INTRODUCTION

Globally, more than a billion people depend on fish for their food and the production of fisheries employs an estimated 59 million people (FAO, 2020). Fish also, are a good source of macro- and micronutrients that are crucial for human health and are a staple diet in coastal locations (Rifat et al., 2023). Due to the oxidation of lipids and microbiological spoilage, which cause fish to degrade when stored at low temperatures or under refrigeration, fish is regarded as a highly perishable commodity that spoils rapidly after it has passed away (Pereira et al. 2010). So, employing chemical preservatives, various methods are used to increase quality and safety of fish and fish-related products. But, nowadays, the customer's approach towards naturally preserved fish meat products gained interest to overcome health risks of synthetic preservatives (Elsabagh et al. 2023).Oily, volatile substances are essential oils with potent, frequently enticing scents that are derived from various plant parts. The FDA has designated the majority of essential oils as GRAS (Generally Recognized as Safe). Additionally, according to Gutierrez et al. (2008), the European Union has permitted their usage as flavouring agents. According to the global agricultural production, 607.3 million metric tonnes of black seed oil are produced globally each year (Sands and Suttles 2022). Black seed (Nigella sativa) has also been shown to be particularly effective in treating a wide range of medical conditions. According to Indian medical tradition, black seeds can be used to treat a variety of ailments, including jaundice and skin problems (Ahmad et al. 2013 and Warrier et al. 2004).

They can be used as a medicinal agent as well as anti-bacterial and anti-fungal agent (Ahmad et al. 2013). An innovative new source of edible oils is black seed oil. Additionally, according to Kabir et al. (2020), the chemical variety and functional qualities of the flavonoids and phenolic chemicals found in black seeds have garnered considerable interest. P-cymene (28.61%), Thymoqunone (38.23%), thujene (3.88%), and carvacrol (2.31%) are the primary flavonoids and phenolic chemicals in black seeds. These substances have demonstrated pharmacological and antibacterial effects (Liu et al. 2013), the biological benefits of these flavonoids and phenolic substances included anti-inflammatory, anti-bacterial, and antioxidant activity. The relationship between food's antioxidant qualities and health has lately undergone substantial research (Tibebe et al. 2023). Therefore, the goal of our study was done to assess the efficacy of a natural preservative (black seed oil) in various concentrations for enhancing the quality of Nile Tilapia kofta and extending its shelf life during cold storage.

2. MATERIAL AND METHODS

2.1. Tilapia kofta preparation

Samples of Nile Tilapia (*Oreochromisniloticus*) fillets were procured from fish markets in El-Sharkia governorate and were directly minced using mincing machine then packed in sterile polyethylene bags. The bags were then transported directly in an insulated ice container to microbiology laboratory for further treatment and analysis

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2.2. Essential oil preparation

The ready-made black seed (Nigella sativa L.) herbal oil used in this study was acquired from the National Research Centre, Dokki, Cairo, Egypt, which delivered it using the hydro-distillation method. It was purchased in its purest form and was free of antioxidants or preservatives. Before usage, these oils were kept in amber-colored bottles at 4°C.

2.3. Experimental design

Fish dough was shaped into round balls manually under hygienic conditions to get raw fish kofta, then was divided into four groups , the first group was untreated (control), and the rest of groups were well homogenized with different concentrations of black seeds (*Nigella sativa* L.) oil (1% - 1.5% - 2%). Samples were separately packed in polyethylene bags, labeled and stored at 4 ºC. They were analyzed for physicochemical, bacteriological and sensory properties promptly at 3 days intervals during storage period (at 0 day, 3rd, 6th, 9th, 12th and 15th days) as the experiment was conducted in triplicate.

2.4. Microbiological quality evaluation

Tilapia kofta samples were examined throughout storage periodin order to evaluate the Aerobic Plate Count (APC) using the pour plate surface plate method at 35°C (ISO 4833-1, 2013), *Staphylococcal* count on Baird Parker ager medium incubated at 37°C for 48 hours (FDA, 2001), *Psychotrophic* count on aerobic plate count at40°C (FDA, 2001), and the Pseudomonas count according to ISO, (2004) on Pseudomonas media at 25 °C for 48 hours after which all developed colonies (greenish yellow colonies) were enumerated and *Aeromonas* count performed according to ISO (2004) on specific media of *Aeromonas* species (Aeromonas Agar Media, Lab M) and for 18 to 24 hours, inoculation plates were incubated at 37°C.

2.5. Physico-chemical evaluation

The pH values were calculated using the procedure outlined in Zenebon et al. (2008), and the TVB-N content was assessed using the procedure outlined in AOAC (2005) and reported as mg N/100 g of sample. Trimethylamine (TMA) and thiobarbituric acid reactive substances (TBARS) values, as per AOAC (2005), were used to determine the samples' oxidative condition

2.6. Sensory Evaluation performed according to Fan et al. (2008)

Fish quality is mostly determined by sensory qualities, which are the key factors that buyers can consider when buying fresh fish. The color, odour, texture and over all acceptability are all examples of sensory attributes. Sensory evaluation was based on a five-point scale to determine

2.7. Statistical analysis

Utilizing the graph pad prism programme (version 8.0.2), data were analyzed. All data values were subjected to analysis of variance (two-way ANOVA), with means and standard deviations being provided. At p <0.05, significant F-values were determined.

3. RESULTS

In a challenge, the examined oil applied on chilled fish kofta to evaluate its impacts on physic-chemical quality. Results in figure (1) showed the significance variation (P<0.05) between physic-chemical characters of chilled fish kofta in control and those in treated with black seed oil. Oil of black seeds showed the higher impacts on enhancing values of pH, TVB-N, TBA and TMA that reflect freshness of chilled fish kofta samples and shelf life of it. As, control group showed higher values of pH, TBA, TMA reflects its spoilage from day 6th of refrigerated storage, while treated samples with 2% black seeds stayed with in accepted range till 14thday of refrigerated storage

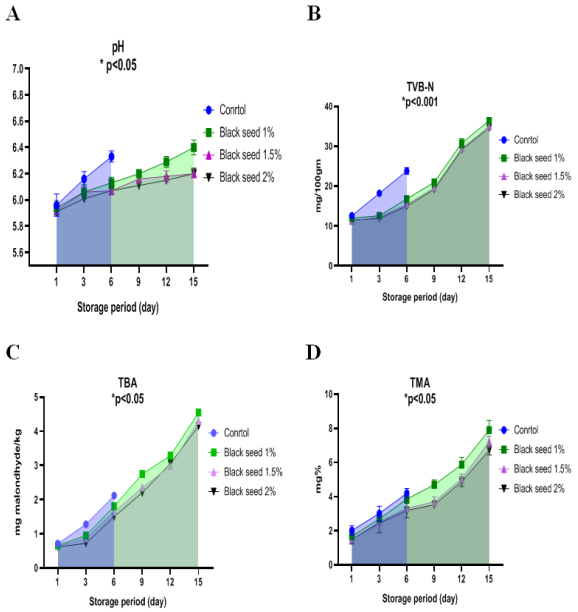

Fig (1) Impacts of different concentration of black seeds *(Nigella sativa*) oil on pH (A), TBA (B), TVB (C), and TMA (D) values in chilled Tilapia kofta. Values are represented as the mean of triplicates ±SD. The significance at P< 0.05 with confidence level 95%

Table (1): Effect of different concentration of black seed *(Nigella sativa*) oil supplemented to Tilapia kofta on Aerobic plate count (log_{10}cfu/g) during cold storage at 4 ^{O}C for 15 days

Day	Groups			
	Control	Black seed 1%	Black seed 1.5%	Black seed 2%
1st	7.90 ± 0.04^{A,a}	7.31 ± 0.04 ^{A,a}	7.16 ± 0.06 ^{A,a}	7.07 ± 0.07 ^{B,a}
3rd	8.32 ± 0.14 ^{A,b}	6.92 ± 0.08^{B,a}	6.31 ± 0.13 ^{B,b}	6.06 ± 0.06 ^{B,b}
6th	8.71 ± 0.06 ^{A,c}	6.25 ± 0.2 ^{B,b}	5.12 ± 0.16 ^{C,c}	4.91 ± 0.13 ^{D,c}
9th	S	6.06 ± 0.07 ^{A,b}	4.87 ± 0.09 ^{B,d}	4.16 ± 0.07 ^{C,d}
12th	S	5.87 ± 0.04 ^{A,c}	4.33 ± 0.12^{B,d}	3.93 ± 0.11 ^{C,e}
15th	S	5.75 ± 0.06 ^{A,c}	3.98 ± 0.04 ^{B,e}	3.56 ± 0.05 ^{C,f}

The results expressed as mean ± SD, small letter means that results in rows are significant (P<0.05) while, capital letters means that results in columns are significant (P< 0.05)

Table (2): Effect of different concentrationsof black seed *(Nigella sativa*) oil supplemented to Tilapia kofta on Staphylococcal count (log_{10}cfu/g) during cold storage at 4 ^{O}C for 15 days

Day	Groups			
	Control	Black seed 1%	Black seed 1.5%	Black seed 2%
1st	5.75 ± 0.07^{A,a}	5.59 ± 0.11 ^{A,a}	5.16 ± 0.06 ^{A,a}	5.10 ± 0.12 ^{A,a}
3rd	6.37 ± 0.03 ^{A,b}	4.99 ± 0.11 ^{B,b}	4.72 ± 0.14 ^{B,b}	4.11 ± 0.13 ^{B,b}
6th	6.74 ± 0.09 ^{A,c}	4.38 ± 0.09^{B,b}	4.05 ± 0.06 ^{B,c}	3.63 ± 0.30 ^{C,c}
9th	S	4.06 ± 0.07 ^{A,c}	3.70± 0.14 ^{B,d}	3.31 ± 011 ^{C,d}
12th	S	4.71 ± 0.09 ^{A,d}	3.22 ± 0.28 ^{B,e}	2.71 ± 0.14 ^{C,e}
15th	S	3.11 ± 0.12 ^{A,e}	2.90 ± 0.11 ^{B,f}	2.05 ± 0.06 ^{C,f}

The results expressed as mean ± SD, small letter means that results in rows are significant (P<0.05) while, capital letters means that results in columns are significant (P< 0.05)

Furthermore, the data in table (1) showed that APC (log_{10}/cfu) increase in control (untreated) group while, decrease in treated groups with different concentrations of black seeds with significance value (P<0.05). At 6 days of chilled storage of Tilapia kofta, the control sample's APC

value of 7.90 ± 0.04(log_{10}/cfu) increased to 8.71 ± 0.06 (log_{10}/cfu) then spoiled when compared with the other treatments. At the end of storage period (15day) APC increased from 7.31 ± 0.04, 7.16 ± 0.06, 7.07 ± 0.07 (log_{10}/cfu) to 5.75 ± 0.06, 3.98 ± 0.04, 3.56 ± 0.05 (log_{10}/cfu). *Staphylococcal* count that presented in table (2) showed increase the count of control (untreated) group while, decrease in treated groups with different concentrations of black seed oil. At 6 days of chilling of Tilapia kofta, the control sample's value of 5.75 ± 0.07 (log_{10}/cfu) increased to 6.74 ± 0.09 (log_{10}/cfu) then spoiled when compared with the other treatments. At the end of storage period (15day) *staphylococcal* count increased from 5.59 ± 0.11, 5.16 ± 0.06, 5.10 ± 0.12 to3.11 ± 0.12, 2.90 ± 0.11, 2.05 ± 0.06 (log_{10}/cfu) Up to the end of the chilling period of storage, changes in the *Psychrotrophic*, *Pseudomonas* and *Aeromonas* counts (log_{10}/cfu) in chilled Tilapia kofta are generally revealed significantly higher (P<0.05) levels in all of this study's treatments (Fig. 2)

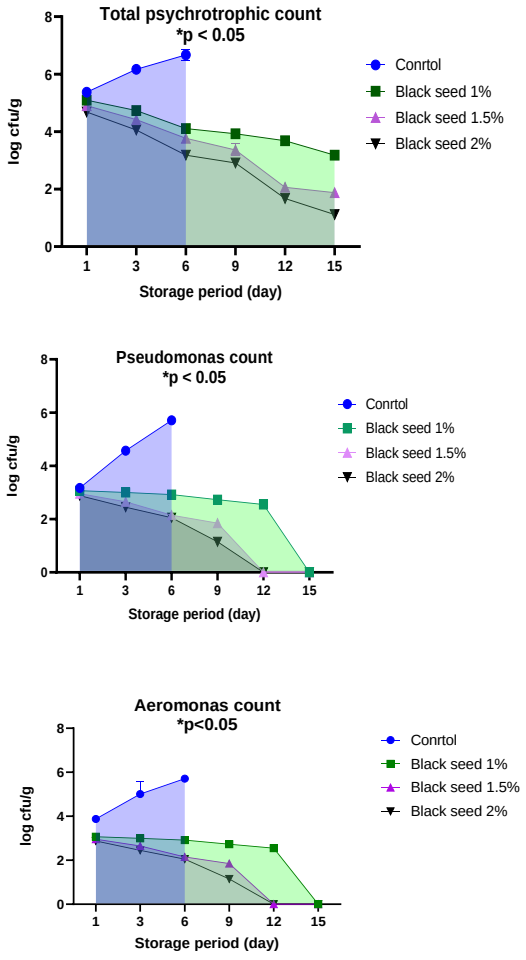

Fig (2) Impacts of different concentration of black seeds *(Nigella sativa*) oil on *Psychrotrophic* count, *Pseudomonas* count and *Aeromonas* count (log_{10}/cfu) in chilled Tilapia kofta. Values are represented as the mean of triplicates ± SD. The significance at P< 0.05 with confidence level 95%

The percent of 1.5% and 2% of oil completely inhibit *Pseudomonas* and *Aeromonas* counts after 12 days of chilling storage while at1%concentration oil inhibited the count of *Pseudomonas* and *Aeromonas* after 14day of cold storage. Concerning sensory attributes of chilled Tilabia kofta, results in fig. (3) Showed the impacts of different oil concentrations (1%, 1.5% and 2%) on sensory attributes (odor, color, texture and over all acceptability) and shelf life of chilled Tilapia kofta

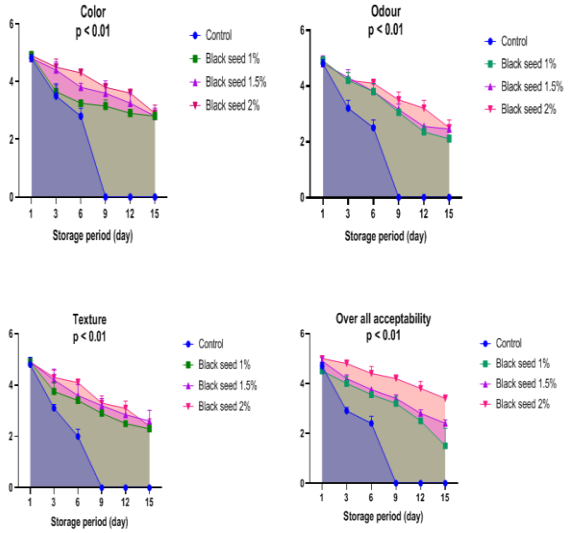

Fig (3) Impacts of different concentration of *Nigella sativa* oil on sensory attributes including color, odour, texture and over all acceptability in chilled Tilapia kofta. Values are represented as the mean of triplicates ± SD. The significance at P< 0.05 with confidence level 95%

4. DISCUSSION

Fresh fish has a shorter shelf life (even when refrigerated) than other animal protein meals like meat and eggs because of its neutral pH, high moisture content, and higher levels of nitrogen from non-protein molecules, all of which are excellent for bacterial and chemical deterioration (Martin et al. 2023).In order to avoid side effects, natural herbs with antioxidant and antibacterial properties should be utilised instead of artificial ones (Mansour et al. 2023). An annual blooming plant known as black seed (Nigella sativa) is primarily found in the western part of Asia, the Mediterranean region along the North Sea, and southwestern and southern Europe (Rahim et al.2022). In both the untreated (control) and treated Tilapia kofta samples, the values of the physicochemical parameters increased over the course of storage. The physicochemical characteristics increased more in the untreated sample than in the treated samples, nevertheless. Black seed oil (2%), followed by (1.5%) and (1%), displayed the least modification among the treated Tilapia kofta samples. Cholesterol, carvone, campesterol, palmitoleic, oleic and citronellol are some of the other components of black seed oil (Rahim et al.2022). Additionally, oleic, and palmitic acids; carvone, and p-cymene are among the volatile oils (Nickavar et al. 2003). The volatiles in black seeds have higher concentrations of thymoquinone than do those in the oil, which also contains a sizeable amount of volatile oils. The active components of black seed are thymoquinone, flavonoids, alkaloids, and tannins, according to (Gali-Muhtasib et al. 2006). Therefore, their antioxidant and antibacterial activities may be responsible for the low pH in the treated group during storage. Fish with a pH between 6.8 and 7 are considered to be fresh; fish with a pH above 7 are thought to be less fresh (Mendes et al. 2019). Additionally, nitrogen-based components (mostly proteins) deteriorate during fish preservation due to volatile basic chemicals. Fish proteins breakdown in ammonia and TMA provide the TVB-N value. Among these, TMA is a biogenic amine that is typically linked to fish odour (and is thought to be produced by fish that is not fresh) and is produced when bacteria and natural enzymes break down the TMAO in fish muscle (Prabhakar

et al. 2020 and Sivertsvik et al. 2002). TMA levels in fresh fish are extremely low or nonexistent. The concurrent TVB-N and TMA levels are widely used as indicators of fish deterioration. However, some studies have revealed that the TMA and/or TVB-N used as indicators of fish freshness are not always accurate (Prabhakar et al. 2020). TMA has been shown to be a poor predictor of degradation in the early stages of spoiling for some fish species. TVB-N is recognized as a better marker than TMA, however results can only be used after 10 days of storage, much like TMA. Therefore, for this measure, only observations of advanced stages of spoiling are significant (Sykes et al. 2009). The key elements limiting seafood's shelf life are microbial growth and activity. Indeed, fish rotting frequently happens as a result of bacterial metabolism-related off-odors and off-flavors (Gramme and Huss 1996). Since spoiling can be seen when the total viable *psychrotrophic* count surpasses 10^7–10^8cfu/g, a total *psychrotrophic* count of 10^6cfu (colony forming units)/g is often the top limit recognized for fresh fish (Broekaert et al. 2011).In fact, only a small percentage of bacteria actually produce these undesirable tastes and odours. As a result, seafood rotting is not necessarily correlated with the total number of microorganisms. The proliferation of particular spoilage microbes is better connected with fish rotting. Because these bacteria negatively affect fish during cold storage, the current study's goal was to investigate their total counts. The findings showed that 2% of black seeds totally suppress these microorganisms. Black seed hence enhance Tilapia kofta's quality and lengthen its shelf life. Due to its distinctive flavour and scent, black seed (Nigella sativa) is a widely used spice in a wide range of cuisines (Sireswar et al. 2017). The seeds have been discovered to be utilized as a spice and flavouring element in Indian and Middle Eastern cuisine, claim Bono and Badalucco (2012). The sensory qualities are improved, which are the primary indicators of fish quality and the primary indicators that consumers might consider when choosing fresh fish (Zhang et al. 2022).

5. CONCLUSION

Results of this study concluded that black seed (*Nigella sativa)* oil improves all aspects of Nile Tilapia kofta as physicochemical, microbiological and sensory attributes. The concentration of 2% proved to be higher in potency than 1% and 1.5 % of black seed oil.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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