



# QUALITY IMPROVEMENT OF REFRIGERATED NILE TILAPIA (*OREOCHROMIS NILOTICUS*) PATTIES USING GARLIC (*ALLIUM SATIVUM L.*) AND GINGER (*ZINGIBER OFFICINALE*) EXTRACTS

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## Abstract

**Background and Objective:** Fish, unlike other muscle foods, is highly susceptible to both microbiological and chemical deterioration. The present study evaluated the effect of incorporating ginger (*Zingiber officinale*; 1%) extract and garlic (*Allium sativum*; 0.05%) extract on the quality characteristics and shelf-life extension of Nile tilapia (*Oreochromis niloticus*) fish patty samples under refrigeration ( $4\pm 1^{\circ}\text{C}$ ), for a period of 12 days. Fish patties were refrigerated to be periodically examined for their sensory quality, chemical indices and bacteriological status, until appearance of signs of spoilage. Results indicated that addition of ginger and garlic extracts to Nile tilapia fish patties formulas gave a pleasant odor, appearance and taste and extended the shelf-life of the product without undesirable changes of sensory and chemical quality when compared to control patties. Results also revealed that garlic (0.05%) extract provided the highest significant ( $P<0.05$ ) antioxidant and antimicrobial properties and the lowest ( $P<0.05$ ) protein degradation (TVB-N) and pH followed by ginger (1%), then control samples during cold storage. This study demonstrates the potential use of ginger (1%) and garlic (0.05%) extracts to improve the microbial quality, retard lipid oxidation, maintain the quality indices and extended the shelf-life of treated Nile tilapia patty samples by 3-6 days over that of control (6 days) as confirmed by microbiological, chemical indices and organoleptic analyses, and could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the fish industry.

**Key words:** Nile tilapia, Antioxidant, Antimicrobial, Ginger, Garlic, Quality, Shelf-life.

## Introduction

Fish, unlike other muscle foods, is highly susceptible to both microbiological and chemical deterioration. High levels of moisture, proximate composition and natural pH render fish an easily perishable product, often going bad after a short period post-harvest (Li *et al.*, 2012). Quality and shelf life of fish and fish products are often enhanced by using various food additives during handling, processing and storage. Due to potential health hazards, synthetic additives are being widely replaced (Naveena *et al.*, 2008), by their natural counter parts. Plant extracts such as garlic and gingers containing bioactive compounds have shown remarkable in-vitro antioxidant and antimicrobial activities (Viji *et al.*, 2017).

Garlic (*Allium sativum*) extract is the most prevalent and is mainly applied to processed meat and chicken products in the form of natural antimicrobials, flavorings and antioxidants (Sallam *et al.*, 2004; Nurwantoro *et al.*,

2015; Tareq *et al.*, 2018 and Xiong *et al.*, 2018). Zhang *et al.*, (2014) found that after feeding the rats for seven days by garlic extract, the serum levels of total cholesterol and low-density lipoprotein decreased significantly, while high density lipoprotein level increased. Organosulfur compounds (alliin, diallylsulfide, allylsulfide and propylsulfide) have the capability as fat antioxidants (Hassanin and El-Daly, 2013 and Kirkpinar *et al.*, 2014). Garlic also contains antioxidant compounds such as tocopherol (Gorinstein *et al.*, 2005), selenium (Gheisari and Ranjbar, 2012), butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ) (Dewi *et al.*, 2010). Thus, the use of garlic extract is hence attractive for keeping the quality of refrigerated fish patties.

Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world. *Z. officinale* is widely used as spice, as a tenderizing agent and food seasoning due to sweet aroma and pungent taste (Chen,

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2008 and He *et al.*, 2015). Ginger is relatively inexpensive; it has also “Generally Recognized as Safe” (GRAS). The rhizome is rich in the secondary metabolites such as phenolic compounds (gingerol, paradol and shogaol), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral) (Ali *et al.*, 2008). Previous studies have demonstrated that plant extracts from *Z. officinale* possess strong antioxidant as free radical scavenging properties (Takahashi *et al.*, 2011; Bellik, 2013; Emir Çoban, 2013), antibacterial, antifungal, anticancer and anti-inflammatory effects (Nikoliæ *et al.*, 2014; Islam *et al.*, 2014; Riaz *et al.*, 2015). Hence, there is a great interest to enhance safety and quality of refrigerated fish patties by using ginger extract.

There is a lack of study on the effect of incorporation Ginger (*Zingiber officinale*) extract or Garlic (*Allium sativum*) extract on the overall quality of fish patties. Thus, the purpose of this study was to evaluate the antimicrobial and antioxidant effects of Garlic (*Allium sativum*) extract (0.05%) and (*Zingiber officinale*) extract (1%) on the quality characteristics and shelf-life extension of fish patties stored at  $4\pm 1^{\circ}\text{C}$  up to 12 days by evaluating certain sensorial attributes (appearance, odor and taste), physicochemical criteria (pH, total volatile basic nitrogen “TVB-N” and thiobarbutric acid reactive substances “TBARS”) and microbiological status (Total viable count “TVC”, Psychrotrophic “PTC” and Enterobacteriaceae “EBC”). Proximate chemical composition of raw fresh Nile tilapia fillets and patties was also determined.

## Materials and Methods

### Chemicals and reagents:

Plate count agar (PCA), violet red bile glucose agar (VRBGA) and peptone water were purchased from Oxoid (Hampshire, UK). Methyl red, magnesium oxide, 2-thiobarbituric acid, bromocresol green, BHT and TCA were from Sigma-Aldrich (Germany). Ethanol and other solvents and chemicals used were of analytical grade or the highest grade available.

### Plant Materials:

Garlic (*Allium sativum*) devoid of any diseases, insect injuries or mechanical damage was obtained from vegetable market at Giza. Fresh Ginger (*Zingiber officinale*) was purchased during October, 2019 from Saudi market at Dokki, Giza, Egypt. The tested garlic and ginger were first washed, peeled, sliced and re-washed from extraneous matter and properly then dried in hot air-oven at  $45\pm 1^{\circ}\text{C}$  for 48 h. The dried materials were ground in a blender to form fine powder. Thereafter, powdered samples were stored separately in polyethylene

bags in a dark and dry place at  $4\pm 1^{\circ}\text{C}$  until extractions.

### Preparation of Extracts:

According to the extraction method of Nikoliæ *et al.* (2014), 20 g each of the powdered plant samples were percolated at room temperature ( $25^{\circ}\text{C}$ ) with 400 ml 97% ethanol for ginger and garlic, respectively in 500 ml beakers for 24 h at room temperature in the dark (thus achieving 1:20 ratio). These were prepared in multiples to ensure enough extractions for the study. The beakers were covered with foil paper. The extract centrifuged at  $3000\times g$  for 10 min at  $20^{\circ}\text{C}$ , the resultant was then filtered through Whatman No. 1 filter paper and the residue re extracted and filtered. The filtrate was concentrated separately in a rotary evaporator (Heidolph Instruments Germany) to remove the solvent at  $38^{\circ}\text{C}$  under reduced pressure. The dried crude extract residue was stored at  $-20^{\circ}\text{C}$  until use.

### Fish source:

A total of 18 kg fresh Nile tilapia (*Oreochromis niloticus*) fish samples of 350 – 500 g each were purchased from fishermen at Imbaba, Giza, Egypt at the same day of harvesting in September 2019, and rapidly transported under complete aseptic conditions in ice boxes to the laboratory of Food Technology, National Research Centre, within 1 h. Upon arrival to the laboratory, each sample was gutted, deheaded, cleaned and filleted into two pieces of about 100 g weight for each piece and re-washed with clean water. The obtained Nile tilapia fillets were minced twice with a pore size of 4.0 mm. The minced Nile tilapia samples were tempered in a freezer  $\sim 60$  min before treatments and processing into patties.

### Niletilapia patties processing and treatments:

Nile tilapia patties were prepared without seasonings using a simple traditional formulation: 85% minced Nile tilapia, 7% corn flour, 4% wheat flour, 2.2% bread crumbs and 1.8 % salt were added as ingredients, afterward, 10% ice water was added, thoroughly mixed by hand for five minutes, then divided into three equal portions (3kg each) and separately comminuted again through a 3-mm mincer steel plate. First portion was used as control containing neither garlic extract (GE) nor zingiber extract (ZE), while the other portions were either mixed with garlic extract (0.05% GE) and zingiber extract (1% ZE). After treatments each group was separately mixed well to ensure uniform distribution of garlic extract and zingiber extract added, the obtained pastes were formed into  $50\pm 3$  g Nile tilapia patties using a Petri dish in the laboratory.

### Nile tilapia patties packaging, storage and analysis:

Nile tilapia patties from each group were then

individually packaged in polyethylene bags (with three patties in each bag), then, they were stored at  $4\pm 1^\circ\text{C}$  for 12 days and analyzed periodically on days zero, 1, 3, 6, 9 and 12 for sensory assessment (taste, odor and appearance), microbial growth (TVC, PTC and EBC), and chemical criteria indices (pH, TVB-N and TBARS). Utilization of 0.05% garlic extract and 1% of zingiber was based on the results of previous studies (Emir Çoban, 2013 and Kuzelov *et al.*, 2016). Three bags of each group (9 Nile tilapia patties) were withdrawn at each intervals of cold storage, six Nile tilapia patties were fried before subjected to organoleptic assessment and the remaining three patties were used for chemical determinations after sampling for microbiological purpose, which were made on finely ground samples. All experiments were repeated three times in order to remove effects deriving from the initial quality of raw material.

#### **Chemical Assessments:**

Chemical analyses were made on finely ground fish fillets and patties samples. Analyses were conducted in triplicate. Proximate composition in terms of moisture, ash, crude lipid and total nitrogen of fish fillets and patties were determined according to the methods described in the A.O.A.C., (1995). For pH determination 10 g of fish patties samples were homogenized in 90 mL distilled water for 1 min in a warring blender, and the pH value of the slurry was measured at room temperature using pH meter (JENWAY, 3510; UK). The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g fish patty samples was determined according to the method described by Parvaneh (2007). A Thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA)/kg fish patties was estimated according to Kilinc (2007).

#### **Microbiological Analysis:**

Twenty five grams were aseptically excised from fish patty samples and homogenized in 225 ml of sterile buffered 0.1% peptone water for 3 min. From this homogenate, decimal serial dilutions were made in the same sterile peptone water and used for microbiological analyses of the fish patty samples at appropriate time intervals during refrigerated storage. On each of the predetermined sampling days, 0.1 ml of each dilution was pipetted onto the surface of plate count agar to determine total viable counts (TVC) and psychrotrophic counts (PTC); while enterobacteriaceae counts (EBC) were determined by using violet red bile glucose agar. Then, all plates were prepared in triplicate and incubated for 2 days at  $30^\circ\text{C}$  for TVC and EBC; and 10 days at  $5^\circ\text{C}$  for PTC (Ozogul and Uçar, 2013). After specific incubation

periods plates showing 25-250 colonies were counted. The number of colonies were multiplied by the reciprocal of the respective dilution and expressed as log CFU per gram.

#### **Sensory evaluation of grilled Nile tilapia patties:**

The sensory evaluation of grilled Nile tilapia patties was done by 10 non-trained panel members of the laboratory staff who were familiar with fish characteristics. To conduct sensory analyses, three Nile tilapia patties were taken from each group at regular intervals, the fish patties were separately grilled ( $250^\circ\text{C}$ ) for 3 min for each side (until cooked to an internal temperature of  $72^\circ\text{C}$ ), before being presented to the panelists. Grilled Nile tilapia patty samples from each group were cut into four rectangular pieces from the center, packed in small plastic cups, then labeled and served warm to the panelists at room temperature in random order; water was served for rinsing the mouth between samples. The panelists were asked to evaluate grilled fish patties acceptability with respect to their appearance, taste, and odor using 5-points hedonic scales, ranging from very poor (1) to very good (5), where: 1 – very poor, 2 – poor, 3 – normal, 4 – good and 5 – very good, a score of 3 was taken as the lower limit of acceptability (Kurtcan and Gonul, 1987).

#### **Statistical Analysis:**

Results were expressed as means and standard deviation ( $M\pm SD$ ) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of garlic extract or ginger extract treatments on fish patties quality. Significant differences were defined as  $P < 0.05$ ; according to Rao and Blane (1985) using PC-STAT program.

## **Results and Discussion**

#### **Proximate Composition of Nile tilapia (Fillets and Patties):**

The proximate analysis is important from many aspects including nutritional value, processing and preservation. So, the proximate composition of fresh Nile tilapia (fillets and patties) were evaluated and presented in Table 1. From which it is apparent that the proximate composition of fresh Nile tilapia (*Oreochromis niloticus*) fillets showed 74.16% moisture, 20.18% crude protein, 3.34% crude fat, 1.21% crude ash and 1.11% carbohydrates (on fresh weight basis); respectively. The fish species examined belonged to high-protein (15 to 20%) and low-fat (<4%) category (Ackman, 1989). The proximate analysis as shown in Table 1 was within the normal values for the specie and season, and is in agreement with other researchers (Anani and Agbeko,

2018 and Desta *et al.*, 2019). Chemical composition of the Nile tilapia fillets reported in different studies (Tsegay *et al.*, 2016; Olopade *et al.*, 2016) showed some degree of differences, especially for the lipid and moisture contents. Such variations in the chemical composition of fish are greatly related to the nutrition, catching season, sexual variation, and fish size (Ackman, 1989).

According to the results of Table 1, there were statistically significant differences ( $P < 0.05$ ) between Nile tilapia fillet and its minced passed product (Nile tilapia patty) for moisture, fat and ash contents, due to patty formula ingredients. Table 1 also indicate higher amount of carbohydrate content has been found in Nile tilapia patties as compared to Nile tilapia fillet, which might be derived from flour, bread and plant extracts. Similar findings were reported in minced fish passed products by other researchers (Emir Çoban, 2013 and Ehsani *et al.*, 2013). Generally, formula ingredients, fat level as well as method of processing affect to a great extent the proximate composition and quality attributes of formulated patties (Emir Çoban, 2013).

#### Sensory evaluation of grilled fish patty:

Sensory quality changes of grilled Nile tilapia patties in terms of appearance, odor and taste were evaluated and the mean score values are presented in table 2. Owing to development of off-odor and off-flavor in raw Nile tilapia patties associated with spoiling fish (Results not shown); the control (C) and ginger-treated patties samples were halted (discontinued) from sensory evaluation after

9, 12 and 12 days of refrigerated storage, respectively. Garlic-treated samples, however, were evaluated until the end of the storage period (day 12). Results of table 2 indicate that no, significant ( $P < 0.05$ ) differences in the sensory scores were detected between control and treated samples at the beginning of storage (0 time). Moreover, panel preference for treated samples over stored untreated controls table 2, indicating that both ginger and garlic extracts are potent preservatives having better function, which enhanced the pleasant odor, lipid and microbial safety; essential for maintaining the sensory attributes of fishery products.

On advancement of storage period, the overall mean scores of sensory attributes (appearance, odor and taste) of control, ginger and garlic extracts treatments were sharply decreased ( $P < 0.05$ ) irrespective of treatment, as a result of microbial spoilage, oxidation of lipid and degradation of protein in the fish patty samples (Gram and Huss, 1996), to be below the lowest acceptability limit (3) according to Kurtcan and Gonul, (1987), after 6, 9 and 12 days of storage, respectively. The treatment of ginger extract to the Nile tilapia patties stored at  $4 \pm 1^\circ\text{C}$  led to an improvement in the appearance, odor and taste of the patty samples, which received higher scores than those of the control ( $P < 0.05$ ) until day 9 of storage table 2. Similarly, inclusion of garlic extract led to an improvement in the appearance, odor and taste of patty samples under investigation till the end of chilling course (12 days). Ginger and Garlic extracts retarding development of lipid oxidation and rancid odors allowing

**Table 1:** Proximate chemical composition of Nile tilapia fillet and patties (on fresh wt. basis).

RAW material	Moisture %	Protein %	Fat %	Ash %	Carbohydrates%
N.tilapia fillets	74.16 $\pm$ 0.17 <sup>a</sup>	20.18 $\pm$ 0.28 <sup>a</sup>	3.34 $\pm$ 0.16 <sup>b</sup>	1.21 $\pm$ 0.24 <sup>b</sup>	1.11 $\pm$ 2.35 <sup>b</sup>
N. tilapia patties	65.43 $\pm$ 0.58 <sup>b</sup>	17.14 $\pm$ 0.42 <sup>b</sup>	6.62 $\pm$ 0.21 <sup>a</sup>	3.51 $\pm$ 0.12 <sup>a</sup>	7.30 $\pm$ 0.18 <sup>a</sup>

All values reflect the mean and standard deviation (SD), are mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ( $P < 0.05$ ). Nile tilapia fillets, Nile tilapia patties.

an extension of 3-6 days for the shelf life over that of control samples (6 days). Similar results were obtained from the other studies (Sallam *et al.*, 2004; Ucak *et al.*, 2011; Khalafalla *et al.*, 2015; Babatunde and Adewumi, 2015; Kuzelov *et al.*, 2016; Tareq *et al.*, 2018 and Draszanowska *et al.*, 2019).

#### Microbiological count changes:

**Table 2:** Sensory scores of grilled Nile tilapia patties during refrigerated storage at  $4 \pm 1^\circ\text{C}$  for 12 days.

Nile tilapia/Day		0	1	3	6	9	12
Control ( C )	App.	4.90 $\pm$ 0.15 <sup>a</sup>	4.32 $\pm$ 0.17 <sup>c</sup>	3.85 $\pm$ 0.10 <sup>c</sup>	3.14 $\pm$ 0.17 <sup>c</sup>	NA	NA
Ginger Ext.		4.83 $\pm$ 0.17 <sup>a</sup>	4.50 $\pm$ 0.21 <sup>b</sup>	4.17 $\pm$ 0.25 <sup>b</sup>	3.74 $\pm$ 0.15 <sup>b</sup>	3.25 $\pm$ 0.12 <sup>b</sup>	NA
Garlic Ext.		4.88 $\pm$ 0.25 <sup>a</sup>	4.72 $\pm$ 0.13 <sup>a</sup>	4.38 $\pm$ 0.12 <sup>a</sup>	4.00 $\pm$ 0.16 <sup>a</sup>	3.72 $\pm$ 0.15 <sup>a</sup>	3.21 $\pm$ 0.24 <sup>a</sup>
Control ( C )	Odor	4.75 $\pm$ 0.19 <sup>a</sup>	4.26 $\pm$ 0.15 <sup>c</sup>	3.56 $\pm$ 0.15 <sup>c</sup>	3.10 $\pm$ 0.10 <sup>c</sup>	NA	NA
Ginger Ext.		4.78 $\pm$ 0.13 <sup>a</sup>	4.42 $\pm$ 0.17 <sup>b</sup>	4.00 $\pm$ 0.32 <sup>b</sup>	3.68 $\pm$ 0.14 <sup>b</sup>	3.15 $\pm$ 0.13 <sup>b</sup>	NA
Garlic Ext.		4.70 $\pm$ 0.32 <sup>a</sup>	4.58 $\pm$ 0.24 <sup>a</sup>	4.23 $\pm$ 0.18 <sup>a</sup>	3.85 $\pm$ 0.15 <sup>a</sup>	3.46 $\pm$ 0.19 <sup>a</sup>	3.10 $\pm$ 0.35 <sup>a</sup>
Control ( C )	Taste	5.00 $\pm$ 0.42 <sup>a</sup>	4.36 $\pm$ 0.21 <sup>c</sup>	3.70 $\pm$ 0.14 <sup>c</sup>	3.25 $\pm$ 0.32 <sup>c</sup>	NA	NA
Ginger Ext.		4.90 $\pm$ 0.35 <sup>a</sup>	4.52 $\pm$ 0.53 <sup>b</sup>	3.86 $\pm$ 0.17 <sup>b</sup>	3.47 $\pm$ 0.25 <sup>b</sup>	3.18 $\pm$ 0.25 <sup>b</sup>	NA
Garlic Ext.		4.95 $\pm$ 0.64 <sup>a</sup>	4.71 $\pm$ 0.36 <sup>a</sup>	4.15 $\pm$ 0.45 <sup>a</sup>	3.76 $\pm$ 0.18 <sup>a</sup>	3.53 $\pm$ 0.36 <sup>a</sup>	3.17 $\pm$ 0.11 <sup>a</sup>

Odor – Taste – and Appearance scores values reflect the mean and standard deviation, (n=10).

Mean values in the same column bearing the same superscript do not differ significantly ( $P < 0.05$ ). NA: not analysis. Ginger extract (1%; *Zingiber officinale*) – Garlic extract (0.05%; *Ga Ext.*).

The antimicrobial activity of ginger extract and garlic extract in Nile tilapia patty samples stored at  $4\pm 1^{\circ}\text{C}$  for 12 days, are shown in table 3. TVC are used as an acceptability index for fish products because of the effect of bacteria in spoilage. The statistical analysis of TVC revealed significant difference ( $P>0.05$ ) between control and extract treated fish patty samples during refrigeration storage, while the increase in the storage time produce significant proliferations in TVC ( $P<0.05$ ), whatever the treatment conditions. However, TVC reached and exceeded a value of 6 log cfu/g, considered as the upper microbiological limit for good quality fish patties, as defined by the EOS, (2005) after day 6 for the control Nile tilapia samples. The use of the garlic extract was the most effective, achieving the greatest reduction and TVC reached the limit of 6 log cfu/g after 12 days' storage period, with regard to ginger extract resulted in a shelf-life of 9 days as compared to control samples (6 days). Similar extension periods have been reported in muscle origin food by other authors (Sallam *et al.*, 2004; Nikolai *et al.*, 2014; Islam *et al.*, 2014; Paul *et al.*, 2015; Kuzelov *et al.*, 2016; Riaz *et al.*, 2015; Terefe, 2017 and Tareq *et al.*, 2018).

The changes in psychrotrophic count were approximately similar to those of TVC, with control also being the highest followed by samples treated with ginger extract and much lower counts was detected in samples treated with garlic extract. The psychrotrophic counts in Nile tilapia patty samples were slightly lower than the TVC. This was true for all of the three groups analyzed. As the storage time increased, the PTC steadily increased. The *Enterobacteriaceae* were the most affected organisms, where ginger- and garlic-treatment caused great reduction in their counts in fish patty. At the end of the storage period (day 12), treated fish patties exhibited much lower ( $P<0.05$ ) counts as compared with control samples table 3. These findings could be attributed to antibacterial impacts of ethanolic extracts of ginger and garlic (Ucak *et al.*, 2011; Emir Çoban, 2013;

Khalafalla *et al.*, 2015; Babatunde and Adewumi, 2015 and Salem *et al.*, 2018). The rhizome is rich in the secondary metabolites such as phenolic compounds (gingerol, paradol and shogaol), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral) (Ali *et al.*, 2008). Garlic (*Allium sativum*) extract is also used as natural antimicrobials (Sallam *et al.*, 2004; Nurwantoro *et al.*, 2015 and Xiong *et al.*, 2018), in muscle origin foods.

#### pH changes:

pH values of control and extract treated fish patties were depicted in table 4, from which it is apparent that inclusion of ginger and garlic extracts reduced the initial pH values of fish patty samples when compared to control samples, but the difference is not significant ( $P < 0.05$ ). The pH value of all fish patty samples slightly decreased during the first 3 days of storage, by more time of refrigeration storage pH values increased in different degrees within untreated and treated Nile tilapia patty samples as shown in table 4. This decrease indicates that some fermentation occurs during storage. The last pH values increase might have been due to the liberation of ammonia compounds as a result of enzyme activity or the proteolytic microbial flora present in the raw fish patty samples (Gram and Huss, 1996).

The increase in the storage time, produce significant increase in pH values ( $P<0.05$ ), whatever the treatment conditions. Treatments of natural extracts (ginger and garlic) resulted in significant ( $P < 0.05$ ) reduction in pH values when compared with control fish patties during storage. The pH value of control non-treated patties was above the acceptable limit (7.1) with objective signs of deterioration after the 6th day of storage. The pH values of fish patties treated with ginger and garlic extracts were higher than the acceptable limit at the 9<sup>th</sup> and 12<sup>th</sup> day of storage at  $4\pm 1^{\circ}\text{C}$ , respectively table 4. These results were in good agreement with previous authors after treatment of different meat products with natural extracts (He *et*

**Table 3:** TVC, PTC and EBC (as logCFU/g) of raw fish patties during cold storage for 12 days.

Treatment/Day	0	1	3	6	9	12
Control (C)	4.23±0.15 <sup>a</sup>	4.52±0.14 <sup>a</sup>	5.14±0.12 <sup>a</sup>	5.85±0.13 <sup>a</sup>	6.38±0.15 <sup>a</sup>	7.16±0.13 <sup>a</sup>
MLE (2 %)	4.06±0.19 <sup>b</sup>	4.28±0.17 <sup>b</sup>	4.83±0.18 <sup>b</sup>	5.27±0.19 <sup>b</sup>	5.85±0.17 <sup>b</sup>	6.52±0.14 <sup>b</sup>
OLE (2 %)	3.85±0.13 <sup>c</sup>	4.10±0.23 <sup>c</sup>	4.47±0.23 <sup>c</sup>	4.90±0.24 <sup>c</sup>	5.42±0.24 <sup>c</sup>	5.90±0.35 <sup>c</sup>
Control (C)	3.85±0.12 <sup>a</sup>	3.92±0.11 <sup>a</sup>	4.45±0.18 <sup>a</sup>	5.36±0.36 <sup>a</sup>	6.26±0.13 <sup>a</sup>	6.85±0.14 <sup>a</sup>
MLE (2 %)	3.67±0.21 <sup>b</sup>	3.80±0.25 <sup>b</sup>	4.19±0.27 <sup>b</sup>	4.87±0.12 <sup>b</sup>	5.47±0.17 <sup>b</sup>	6.41±0.27 <sup>b</sup>
OLE (2 %)	3.58±0.32 <sup>c</sup>	3.64±0.16 <sup>c</sup>	4.00±0.12 <sup>c</sup>	4.56±0.16 <sup>c</sup>	5.10±0.24 <sup>c</sup>	5.72±0.42 <sup>c</sup>
Control (C)	2.78±0.27 <sup>a</sup>	2.85±0.23 <sup>a</sup>	2.98±0.21 <sup>a</sup>	3.27±0.27 <sup>a</sup>	3.48±0.12 <sup>a</sup>	3.62±0.13 <sup>a</sup>
MLE (2 %)	2.62±0.18 <sup>b</sup>	2.72±0.32 <sup>b</sup>	2.84±0.18 <sup>b</sup>	3.11±0.35 <sup>b</sup>	3.24±0.16 <sup>b</sup>	3.46±0.21 <sup>b</sup>
OLE (2 %)	2.47±0.22 <sup>c</sup>	2.58±0.21 <sup>c</sup>	2.70±0.15 <sup>c</sup>	2.87±0.22 <sup>c</sup>	3.10±0.28 <sup>c</sup>	3.25±0.15 <sup>c</sup>

All values reflect the mean and standard deviation are mean of triplicate determinations.

There is no significant difference ( $P>0.05$ ) between the values having the same superscripts in the same column. TVC: Total Viable Count – PTC: psychrotrophic count –EBC:Enterobacteriaceae count .Ginger Extract: (1 %; Zingiber officinale); Garlic Extract: (0.05%; Ga Ext.).

*al.*, 2015; Paul *et al.*, 2015; Babatunde and Adewumi, 2015; Mancini *et al.*, 2017; Tareq *et al.*, 2018 and Draszanowska *et al.*, 2019). However, the lower pH values of extract treated fish patty samples reflect protection properties of ginger and garlic extracts against microorganisms, which reduce the accumulation of basic substances (Khalafalla *et al.*, 2015; Majumdar *et al.*, 2017; Terefe, 2017 and Salem *et al.*, 2018).

#### Total volatile basic nitrogen (TVB-N):

Protein decomposition is one of the main causes for fish patties quality deterioration (Parvanch, 2007). The TVB-N values of control and extract treated Nile tilapia patties are shown in table 4. Fish patty samples treated with ginger and garlic extracts were significantly ( $P < 0.05$ ) lower TVB-N values than those of control fish patty samples after treatments and during storage. A sharp rise of TVB-N value was noticed in the control and treated patty samples during refrigerated storage at  $4\pm 1^\circ\text{C}$ . The TVB-N values of control Nile tilapia patties reached value 32.17 mg/100g (above the acceptable limit, 30 mg/100g) after the 6th day of storage with the objective signs of spoilage. Nile tilapia patty samples treated with ginger and garlic extracts revealed TVBN values higher than the acceptable limit of 30 mg/100g (EOS, 2005), at 9<sup>th</sup> and 12<sup>th</sup> days of storage, respectively table 4. These results indicated that natural extracts (ginger and garlic) have the ability to protect fish patties from protein degradation. This observation was in a good agreement with other authors (Ucak *et al.*, 2011; Hassanin and El-Daly, 2013; Paul *et al.*, 2015; Riazet *et al.*, 2015 and Khalafalla *et al.*, 2015), who reported that treatment of fish products by ginger, garlic or other extracts were very effective in inhibiting microbial growth, reduced chemical and enzymatic deterioration reactions and prolonged the shelf-life.

#### The thiobarbituric acid reactive substances (TBARS) changes:

TBARS value is used as an index of meat quality during storage (Kilinc *et al.*, 2007). Data presented in table 4 showed the changes that took place in TBARS values of raw Nile tilapia patty samples during refrigerated storage for 12 days. The TBARS values of fish patties treated with ginger and garlic extracts were significantly ( $P < 0.05$ ) lower than those of control fish patty samples after inclusion process and during cold storage. The TBARS values of fresh control and treated Nile tilapia patties were not significantly ( $P > 0.05$ ) different at the beginning of cold storage (zero time), indicating that oxidation of lipid occurred during refrigerated storage. The increase in the storage time, produce significant increase in TBARS values ( $P < 0.05$ ), whatever the treatment conditions with the control samples being the highest all sampling days, followed by 1% ginger extract and 0.05% garlic extract, respectively. This might be due to auto-oxidation of lipids over a period of low temperature storage. The TBARS values of control fish patty samples reached value 2.84 mg/kg (above the acceptable limit, 2.0 mg/kg) as defined by the EOS, 2005 after the 6th day of storage with the objective signs of spoilage.

Nile tilapia patty samples treated with ginger and garlic extracts remained higher than the acceptable limit, after 9<sup>th</sup> and 12<sup>th</sup> days of storage, respectively table 4. In the present study, the lowered lipid oxidation of fish patties treated with ginger and garlic extracts was in accordance with the lowered microbial growth table 3. These results indicated that ginger extract (1%) and garlic extract (0.05%) have antioxidant activities in fish patties (Babatunde and Adewumi, 2015; Nurwantoro *et al.*, 2015; Paul *et al.*, 2015; Kuzelov *et al.*, 2016; Majumdar *et al.*, 2017; Tareq *et al.*, 2018 and Xiong *et al.*, 2018). The antioxidant activity of natural extracts (ginger and garlic extracts) have been attributed to their phenolic compounds which act by terminating the free radical chain reaction by donating hydrogen or electrons to free radicals

**Table 4:** Chemical indices of raw Nile tilapia pattes during cold storage at  $4\pm 1^\circ\text{C}$  for 12 days.

Treatment/Day		0	1	3	6	9	12
Control (C)	pH	6.24±0.17 <sup>a</sup>	6.21±0.32 <sup>a</sup>	6.15±0.14 <sup>a</sup>	6.48±0.42 <sup>a</sup>	7.36±0.13 <sup>a</sup>	7.82±0.12 <sup>a</sup>
MLE (2 %)		6.18±0.23 <sup>a</sup>	6.14±0.18 <sup>a</sup>	6.10±0.25 <sup>a</sup>	6.32±0.21 <sup>b</sup>	6.82±0.27 <sup>b</sup>	7.46±0.16 <sup>b</sup>
OLE (2 %)		6.12±0.15 <sup>a</sup>	6.10±0.12 <sup>a</sup>	6.00±0.16 <sup>a</sup>	6.18±0.11 <sup>c</sup>	6.54±0.22 <sup>c</sup>	6.87±0.28 <sup>c</sup>
Control (C)	TVB-N	13.16±0.13 <sup>a</sup>	16.26±0.18 <sup>a</sup>	19.32±0.15 <sup>a</sup>	26.28±0.16 <sup>a</sup>	32.17±0.18 <sup>a</sup>	37.73±0.19 <sup>a</sup>
MLE (2 %)		11.28±0.21 <sup>b</sup>	14.57±0.32 <sup>b</sup>	17.45±0.23 <sup>b</sup>	21.62±0.21 <sup>b</sup>	27.00±0.15 <sup>b</sup>	33.35±0.14 <sup>b</sup>
OLE (2 %)		10.36±0.17 <sup>c</sup>	12.76±0.12 <sup>c</sup>	15.37±0.11 <sup>c</sup>	19.16±0.14 <sup>c</sup>	22.96±0.36 <sup>c</sup>	28.84±0.25 <sup>c</sup>
Control (C)	TBARS	1.06±0.15 <sup>a</sup>	1.38±0.17 <sup>a</sup>	1.76±0.11 <sup>a</sup>	1.96±0.18 <sup>a</sup>	2.84±0.23 <sup>a</sup>	3.73±0.13 <sup>a</sup>
MLE (2 %)		0.98±0.21 <sup>a</sup>	1.22±0.23 <sup>b</sup>	1.52±0.17 <sup>b</sup>	1.74±0.35 <sup>b</sup>	2.05±0.16 <sup>b</sup>	2.67±0.14 <sup>b</sup>
OLE (2 %)		0.94±0.13 <sup>a</sup>	1.18±0.18 <sup>b</sup>	1.27±0.32 <sup>c</sup>	1.43±0.14 <sup>c</sup>	1.76±0.12 <sup>c</sup>	1.92±0.24 <sup>c</sup>

All values reflect the mean and standard deviation are mean of triplicate determinations.

There is no significant difference ( $P > 0.05$ ) between the values having the same superscripts in the same column. Total volatile basic nitrogen (TVBN, as mg N/100g meat). Thiobarbituric acid reactive substances (TBARS, as mgMDA/kg meat. – Ginger Extract: (1 %; Zingiber officinale); Garlic Extract: (0.05%).

and converting them to more stable products. The antioxidant activities of these natural extracts have been observed previously in different meat products (Ucak *et al.*, 2011; Khalafalla *et al.*, 2015 and Salem *et al.*, 2018).

### Conclusions

It could be concluded from the current study that natural ginger extract and garlic extract have the ability to protect fresh Nile tilapia fish patties from protein deterioration and lipid oxidation. They also improve the chemical criteria indices of fish patty samples. They (ginger and garlic extracts) have antibacterial activities and can extend the shelf life of fish products. Garlic extract was superior to ginger extract as antioxidant and antibacterial agents and for extending the shelf life of fresh fish patties. The sensory attributes of all formulas treated with natural extracts were acceptable. Therefore, these natural additives could be safely used by fish processors to improve the quality and extend the shelf life of fish products.

### Conflict of Interest:

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

### Significance Statements:

This study demonstrates the potential use of ginger and garlic extracts to improve sensory characteristics, microbial quality, retard lipid oxidation, and maintain the quality indices of raw Nile tilapia fish patties during cold storage at  $4\pm 1^{\circ}\text{C}$  for 12 days. According to microbiological, organoleptic and chemical indices analyses it was also shown that the application of such natural components extended the shelf-life of treated fish patties by 3-6 days over that of control (6 days). Therefore, they could be a good replacement for the synthetic antimicrobials and antioxidants currently used by the fish industry.

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