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Research Article
Assessment of Hurdle Technology to Preserve Nile Tilapia Fillets During Refrigeration with the Application of Marjoram Oil/Polyphosphates Dipping

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Abstract

Objective: Keeping food safety and quality among consumers is of high importance. In this regard, naturally occurring antimicrobial, antioxidant and flavoring agents were preferred. **Methodology:** Therefore, the preservative effects of marjoram essential oil (MEO, 0.5%) and sodium tripolyphosphate (STPP, 2%) or their combination on the quality changes of raw Nile tilapia fillets during 15 days refrigerated storage (4±1°C) were investigated. **Results:** Physicochemical evaluation of tilapia fillets revealed that MEO batch showed significantly ($p<0.05$) lower pH, TBARS and TVB-N values, whereas STPP treated samples gave higher constant ($p<0.05$) pH values, WHC, moisture retention and weight gain with the least cooking loss as compared to other fillet batches. Microbiological analyses indicated that Total Viable Counts (TVC) for fresh *Oreochromis niloticus* stored aerobically exceeded 6 log CFU g^{-1} after 6 days, while STPP and MEO treatments reached the same value after 9 and 12 days, respectively. In contrast, MEO+STPP treated samples did not reach this value throughout the 15 days. Psychrotrophic counts (PTC) of MEO treated samples were significantly ($p<0.05$) lower compared to STPP and control samples during storage period. Throughout the cold storage, phosphate pretreatment showed the synergistic effect with MEO on reduction of microbiological proliferation, lipid oxidation, protein breakdown and sensorial changes of tilapia fillets till the end of storage. As regards sensory evaluation, preference of panelists was focused on MEO applied fillets, while slight improvement in sensory quality was noticed in samples treated with STPP as compared with control samples. Shelf-life of tilapia fillet was longest for MEO+STPP batch (15 days), followed by MEO (12 days), STPP (9 days) and control samples (6 days). **Conclusion:** Due to concerns regarding the safety and toxicity of synthetic preservatives, combined dipping pretreatment (MEO+STPP) may prove useful as safe, natural application to fish industry, to preserve their good quality characteristics, delay the spoilage, extend the shelf-life and ensure safe consumption of such fish product.

Key words: Hurdle technology, Nile tilapia fillet, marjoram oil, phosphate, quality characteristics, shelf-life

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the most important economic freshwater fish of Egypt, which due to its high nutritional quality and excellent sensory properties is preferred and hence, consumption of fish in Egypt rose from 8.5-15.4 kg/person/year between 1996 and 2008[1]. However, Egypt is considered the largest farmed tilapia producer in Africa and the second globally after China[2].

Consumers perceive fresh seafood to be a superior product to its frozen equivalent[3]. Being highly perishable, fresh seafood has a limited shelf-life due to their biological composition[4]. Spoilage of fish results from oxidation of lipids, autolytic enzymes and the metabolic activities of microorganisms[5]. Even if refrigeration can be applied to the products, these activities although slower, will over time lead to a shorter shelf-life and a poorer safety and quality of fish products and consequently represent a high risk for consumer health and economic loss, therefore enhancing shelf-life of fish products with natural preservatives is an important issue to eliminate economic losses and provide safe and good quality seafood to consumer[6].

Synthetic preservatives have been confirmed for their toxicological and carcinogenic effects[7]. Recently the interest of industry and researchers is directed towards the use of natural ingredients such as essential oils and phosphates as an alternative to synthetic chemicals and preservatives[8,9]. They have the advantage of being accepted by consumers and can inhibit microbial growth, reduce health hazards, minimize lipid oxidation, decrease drip and cooking losses, with consequent increase of the safety, acceptability and shelf-life of refrigerated fish products[4,10].

Phosphates are natural components in almost all foods, they are multi-purpose, generally recognized as safe (GRAS). Polyphosphates are a common cryoprotectant and legally permitted additives that are widely used to improve eating quality of fish products, increase Water Holding Capacity (WHC), decrease drip and cooking losses, retard oxidative rancidity, yielding better color and supply protection against microbial growth[11-13]. They can improve texture, tenderness, control and retain moisture during harvest, processing and storage[14,15]. The changes in the surface protein layers, pH value and metal ions chelating may contribute to the overall antioxidant, antimicrobial and functional properties of various phosphates[16-18]. Therefore, the use of such materials in refrigeration can play an effective role in maintaining fish quality.

Essential Oils (EOs) offer multiple advantages to the fish industry and the consumers. They have been applied in many fish species to prolong shelf-life and improve sensory properties during refrigeration[19,8]. *Origanum majorana* L., of Lamiaceae family is one of the most familiar kitchen herbs, which contains up to 3% of marjoram oil. The MEO is a natural product classified as GRAS and well known for its high phenolic compounds content including carvacrol, thymol, p-cymene and γ-terpinene with strong antioxidant and antimicrobial activities[20]. Other compounds like flavonoid, arbutin, tannins, caffeic acid, labiatic acid, rosmarinic acid, ursolicacid carnosic acid and carnosol can be found in the herb[19,21]. A purified component isolated from methanolic extract of marjoram, T3b, exhibited a better superoxide anion radical scavenger than BHT, BHA, α tocopherol, ascorbic acid and a variety of polyphenolic flavonoids[22].

In recent years, the microbial safety, lipid stability and the sensory quality of most seafood are based on an application of combined preservative factors called hurdle technology[23]. It seems that combining EOs with small amounts of other natural or existing chemical preservatives might minimize their doses and reduce unsatisfactory effect in food products[24,25]. The effects of essential oils or sodium tripolyphosphate have been studied extensively and reported in a variety of meat types including seafood[4,9,10,13,26,27]. To researchers knowledge, no available literature regarding the combined effect of Marjoram Essential Oil (MEO) and food grade sodium tripolyphosphate (STPP) to improve the safety and quality of fish fillets.

The aim of this study is to consider the antioxidant and antimicrobial activities of Marjoram Essential Oil (MEO) and food grade sodium tripolyphosphat (STPP) to determine the freshness quality attributes and shelf-life of raw Nile tilapia (*Oreochromis niloticus*) fillets pretreated by MEO and/or STPP dipping prior to refrigeration by monitoring microbiological, physicochemical and sensorial changes throughout the refrigerated storage at 4±1°C under aerobic packaging for 15 days. Proximate composition of raw fresh tilapia fillets was also investigated.

MATERIALS AND METHODS

Raw materials and chemicals

Fish source: A total of 30 kg fresh Nile tilapia (*Orechromis niloticus*) fish samples of 260-290 g each were purchased a live directly from local fishermen at El-Monib, Giza, Egypt in September, 2015. They were put in ice boxes and rapidly under complete aseptic conditions transported to the Laboratory of Food Technology, National Research Centre, within 1 h.

Powder marjoram leaves: Air-dried powder marjoram leaves were purchased from Al-Dahlia Co., Egypt and stored at 4±1°C until use.

Chemicals: Food grade sodium tripolyphosphate (STPP- China/made) was purchased from Matrix International Company, Dokki, Egypt. Magnesium oxide, 2-thiobarbituric acid, sodium chloride, methyl red, bromocresol green, BHT were from Sigma Aldrich. Plate Count Agar (PCA) and peptone water were purchased from Oxoid (Hampshire, UK). All other solvents and chemicals (glacial acetic acid, sulphuric acid, boric acid, hydrochloric acid, methanol) were from ADWIC, Egypt.

Methods

Preparation of Marjoram Essential Oil (MEO): Two hundred grams of air-dried marjoram leaves were hydro-distilled in a clevenger type apparatus for 3 h until no further increase in the oil was observed. After finishing the distillation process the apparatus was left to be cooled, then the essential oil was collected and dried over anhydrous sodium sulphate before held in dark sealed glass vials and stored at 4±1°C until use.

Preparation of bolti fillets: Upon arrival to the laboratory, fish samples were immediately weighed, gutted, headed, washed, filleted into two pieces (using gloved fingers to avoid cross-contamination) and rewashed with clean water. The samples were divided into four equal groups and stored in refrigeration until further treatments (~30 min).

Dipping treatments of fish fillets: Fillet batches were soaked separately in four different cold solutions (4±1°C), the 1st untreated control (C) was dipped into distilled water containing neither phosphate nor essential oil, while the 2nd batch was dipped into food grade sodium tripolyphosphate solution, 2% w/v (STPP), the 3rd was dipped into marjoram essential oil solution, 0.5% v/v (MEO) and the 4th was dipped into MEO 0.5% + STPP 2% solution as a combined application (MEO+STPP).

Fresh fillets were immersed once every time in 2 L cold (4±1°C) tested solutions for 10 min inside a refrigerator with moderate agitation, then fillets were removed from the treatment solutions with a strainer (drained well for 1 min) prior to packaging. The concentrations of 2% STPP and 0.5% MEO solutions are based on the previous successful pretreatment studies by Ozogul *et al.*[10], Badee *et al.*[19] and Wangtueai *et al.*[27], with the potential to extend the shelf life and improve the quality of perishable muscle origin food.

Packaging, storage and analysis: The soaked and drained (treated) fillets from each batch were then individually labeled and aerobically packaged in polyethylene bags, with 3 fillet pieces in each bag and stored at 4±1°C for 15 days. The treated batches were subjected to microbiological, physicochemical and sensory assessment at day zero, then periodically every 3 days until decomposition or up to 15 days of refrigerated storage. Three bags of each group (9 fish fillets) were withdrawn at each intervals of storage, 7 fish fillets were subjected to raw and cooked organoleptic assessment and the remaining two fillets were used for bacteriological and physicochemical determinations, which were made on finely ground samples. Averages of three replicates were considered.

Analytical techniques

Microbiological examination: Total Viable Count (TVC) and psychrotrophic count (PTC) of fillet samples were determined by Plate Count Agar (PCA) according to Tajik *et al.*[28]. Samples were aseptically taken and homogenized with sterile peptone water. After decimal dilutions, 0.1 mL of each dilution was spread on PCA. Then, all plates were prepared in triplicate and incubated for 24 h at 35°C for TVC and 7 days at 7°C for PTC. After specific incubation periods plates showing 25-250 colonies were counted. The number of colonies was multiplied by the reciprocal of the respective dilution and expressed as log CFU g^{-1}.

Proximate composition and physiochemical analyses: Proximate composition in terms of moisture, ash, crude lipid and total nitrogen were determined according to the methods described in the AOAC[29]. The total volatile basic nitrogen (TVB-N) expressed as mg TVB-N per 100 g muscle of fillets was determined according to the method of Parvaneh[30]. A Thiobarbituric acid reactive substance (TBARS) was estimated by using macrokjeldahl distillation apparatus according to Kilinc *et al.*[12]. The TBARS content was expressed as mg of malondialdehyde (MDA)/kg fillets. For pH determination 10 g of fillet samples were homogenized in 100 mL distilled water for 1 min in a warring blender and the pH values of the slurry were measured at room temperature using pH meter (JENWAY, 3510;UK) as described by Ozyurt *et al.*[4]. Percentage of Water Holding Capacity (WHC%) of fillet samples were evaluated using centrifugal technique described by Zhuang *et al.*[31]. Weight gain (yield %) was calculated according to the modification method of Wangtueai *et al.*[27]. For cooking loss calculation, fillet samples were grilled for 3 min per each side using a stainless steel grill (a HG 230 Kenwood multi cooker, thermostat was set at 200°C), with

turning every 3 min until cooked to an internal temperature of 72°C. Cooking loss percentage was calculated according to Goncalves and Ribeiro[32] as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Weight after soaking and draining} - \text{Weight after grilling}}{\text{Weight after soaking and draining}} \times 100$$

Sensory assessment of raw and cooked fillets: Modified acceptance test with 10 non-trained panel members of the laboratory staff was carried out using 9-points hedonic scales, according to Mexis *et al.*[33]. The whole three fish fillets were taken from each group at regular intervals and immediately served to the panelists to evaluate the color and odor attributes of raw fillets. On day zero only for safety precautions, the panel members were also asked to state whether the freshly cooked fillet samples were acceptable or not. Another four fish fillets from each batch were cooked (grilled at 200°C, 3 min/each side, in similar way to cooking loss procedure), each fillet was cut into 30×30×20 mm cubes, 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. Sensory attributes (appearance, flavor, texture and overall acceptability) of cooked fillets were scored. The 9-points hedonic scales were 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. A score of 5 was taken as the lower limit of acceptability.

Statistical analysis: Results were expressed as means and standard deviation (Mean±SD) from triplicate determinations. Analysis of variance (ANOVA) was performed to compare the effect of dipping treatments. Significant differences were defined as $p<0.05$, according to PC-STAT[34].

RESULTS AND DISCUSSION

Proximate composition of Nile tilapia fillet: Mean value for the proximate composition (Mean±SD) of raw Nile tilapia fillet was the following: 18.04±0.54% protein, 2.29±0. 36% lipid, 78.12±0.65% moisture, 1.18±0.13% ash and 0.37±0.06 carbohydrate-by difference (on wet basis), respectively. The proximate composition revealed that fresh raw Nile tilapia fillet is high in proteins and minerals and low in fat content, that encourages the consumption of tilapia fish. It is worth mentioning that fish muscle tissue contains only very few amounts of carbohydrates which limit the degree of post mortem glycolysis (acidification) of the tissue, hence the pH remained high between 6.2-6.5 as compared to low average values (pH 5.5) of the bovine muscles[35]. Variations in chemical composition of tilapia, mainly in lipid and moisture were reported[36]. However, the differences in the chemical composition of fish are related to nutrition, living area, fish size, catching season and sexual variations[37].

Sensory evaluation of cooked fish fillets: In the present study, the sensorial criteria (appearance, flavor, texture and overall acceptability) of the freshly cooked tilapia fillet samples were evaluated and presented in Table 1. Data reveals that all fresh cooked fillet samples were acceptable as evidenced by the higher ($p<0.05$) overall acceptability scores. Results also indicate that soaking fillet samples in cold solutions containing 2% STPP, 0.5% MEO and/or their combined application did not cause any undesired changes in the sensory characteristics of seafood product but a partial significant ($p<0.05$) improvement in appearance, flavor and texture scores were achieved in the treated fillet samples as compared to control one (Table 1). However, the highest improvement ($p<0.05$) in all sensory criteria under investigation was achieved by MEO+STPP dipping treatment.

Results of Table 1 also indicate that phosphates slightly enhanced sensory scores as was previously reported by Goncalves and Riberio[32] with shrimp. No significant ($p<0.05$) differences in appearance scores between MEO and STPP treated fillets. Phosphates may improve flavor by chelation of heavy metals[17]. Panelists judged MEO samples better ($p<0.05$) in flavor scores as compared to other treatments, which confirmed the antioxidant, antimicrobial and flavoring properties of EOs in food application[10,19]. Significant differences were also observed ($p<0.05$) between texture scores of STPP treated samples as compared to MEO and control samples (Table 1). Our results confirmed the findings of Goncalves *et al.*[26] who reported that phosphates were able to retain moisture and enhanced the ability to hold water in cooked fish fillets which resulted in higher texture scores. Similar results were achieved by other authors[9,27].

Table 1: Sensory scores of freshly cooked control and treated Nile tilapia fillet samples (at zero time)

Dipping treatment	Appearance	Flavor	Texture	Overall acceptability
Control (C)	8.47±0.15^{b}	7.56±0.24^{c}	6.95±0.16^{c}	7.66±0.11^{d}
STPP (2%)	8.63±0.13^{ab}	8.18±0.18^{b}	8.86±0.10^{a}	8.56±0.14^{c}
MEO (0.5%)	8.76±0.21^{a}	8.90±0.14^{a}	8.45±0.11^{b}	8.70±0.18^{b}
MEO+STPP	8.77±0.12^{a}	8.92±0.17^{a}	8.73±0.14^{a}	8.80±0.12^{a}

All values reflect the mean and standard deviation, (n = 10). Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$)

Table 2: Sensory scores of raw Nile tilapia fillets during refrigerated storage at 4±1°C for 15 days

Treatment/day	0	3	6	9	12	15
Color scores						
Control (C)	8.90±0.46^{b}	7.64±0.35^{d}	5.82±0.71^{d}	4.15±0.71^{d}	3.22±0.52^{d}	2.43±0.26^{d}
STPP (2%)	8.82±0.36^{c}	7.90±0.66^{c}	6.56±0.59^{c}	5.34±0.40^{c}	4.54±0.61^{c}	3.42±0.30^{c}
MEO (0.5%)	8.90±0.54^{a}	8.26±0.36^{b}	7.18±0.52^{b}	6.25±0.35^{b}	5.62±0.36^{b}	4.35±0.15^{b}
MEO+STPP	8.90±0.44^{b}	8.45±0.48^{a}	7.72±0.24^{a}	6.80±0.38^{a}	6.38±0.24^{a}	5.40±0.32^{a}
Odor scores						
Control (C)	8.73±0.63^{b}	7.12±0.30^{d}	5.21±0.53^{d}	3.92±0.55^{d}	2.80±0.19^{d}	2.00±0.56^{d}
STPP (2%)	8.75±0.41^{c}	7.61±0.42^{c}	6.24±0.56^{c}	5.12±0.46^{c}	4.36±0.63^{c}	3. 28±0.35^{c}
MEO (0.5%)	8.90±0.56^{a}	8.00±0.22^{a}	7.10±0.39^{a}	6.08±0.48^{a}	5.46±0.40^{b}	4.140±0.48^{b}
MEO+STPP	8.90±0.25^{b}	8.36±0.35^{b}	7.48±0.24^{b}	7.00±0.27^{b}	6.21±0.57^{a}	5.180±0.29^{a}

All values reflect the mean and standard deviation, (n = 10). Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$), MEO: Marjoram essential oil, STPP: Sodium tripolyphosphate

Sensory changes of raw tilapia fillet: Changes in the sensory attribute scores of raw control and treated fish fillets samples during refrigerated storage at 4±1°C are depicted in Table 2. Fresh Nile tilapia fillets were generally considered to possess very high acceptability. All samples developed a fishy odor as the storage time increased with significantly differences ($p<0.05$) between treatments (Table 2). For the control samples, the deterioration occurred after 6 days of storage as evidenced by strong fishy and putrid odor. Also, the deterioration in color occurred after 6 days during storage at 4±1°C. Samples pre-treated with STPP and MEO exhibited higher scores ($p<0.05$) for odor and color and exhibited no negative effect on sensory characteristics during storage as compared with control samples. For such batches, extended shelf life of tilapia fillets to 9 and 12 days, respectively was observed.

Results of Table 2 also indicate that soaking tilapia fillet samples in cold solution containing 0.5% MEO plus 2% STPP prior to air packaging and refrigeration process effectively retarded off-odor, off-flavor, maintained good color and extended the shelf-life of fillet samples to 15 days with the highest acceptability scores ($p<0.05$) and pleasant herbal flavor. Similar trend of tilapia fillet shelf-life was achieved during refrigerated storage by other authors[38,39]. However, the protective effects of MEO and/or STPP reflect strong antioxidant and antimicrobial activities of such natural materials. While, undesired off-odors and flavors, color changes, slime formation and texture deterioration occurring during fillet spoilage are mainly caused by products of bacterial growth and metabolism[5]. The present results indicate that sensory scores correlated well with the increase in TBARS, TVBN values and microbiological counts (TVC, PTC) of the same samples.

Microbiological count changes: Microbiological evaluation, together with chemical indices has been used extensively to assess the quality and shelf-life of fish products[33]. Total Viable Count (TVC) and psychrotrophic counts (PTC) of raw Nile tilapia fillet under investigation were evaluated and the mean counts (as log CFU g^{-1}) were presented in Fig. 1 and 2, which reveal that a slight higher in TVC and PTC was noticed in control samples when compared with other treatments at zero time of cold storage, indicating that marjoram essential oils (MEO), sodium tripolyphosphate (STPP) or their combined dipping application (MEO+STPP) caused sudden lethal effect for the tested microorganisms immediately after soaking process.

Total Viable Count (TVC) of raw fillet samples steadily increased as the time of cold storage progressed (Fig. 1). The increment noticed for control samples was significantly ($p<0.05$) higher than those found in pretreatment groups. Similar findings have been previously given[19,9,39]. According to the permissible limit of TVC in chilled fish (6 log CFU g^{-1}) recommended by Egyptian Organization for Standardization (EOS)[40], it was evident from Fig. 1 that all fillet groups exceeded such limit at the day when it became unacceptable by the sensory evaluation results. Regarding the upper acceptability limit recommended by ICMSF[41] for total viable count in fresh fish (7 log CFU g^{-1} flesh), it could be observed that control group exceeded such limit at 9th day of storage, while such limit has not exceeded by treated groups until time of spoilage.

According to the results illustrated in Fig. 1, it could be observed that control samples exceed the upper limit of acceptability for fresh fish after 6 days of storage, comparing to 9 days noted for STPP treated fillets and 12 days for MEO batch samples, whereas MEO+STPP pre-treated fillet samples contained TVC (5.84 log CFU g^{-1}) below the critical value at 15th day. The obtained results indicate that STPP and MEO possess antimicrobial properties which help for prolonging shelf-life of pretreated fish products and their combination might have synergistic effect on the retardation of bacterial growth during cold stored fish, leading to safer products.

Data shown in Fig. 2 indicate that the psychrotrophic counts (PTC) in raw fish fillet were slightly lower than the corresponding TVC, this was true for all of the four groups

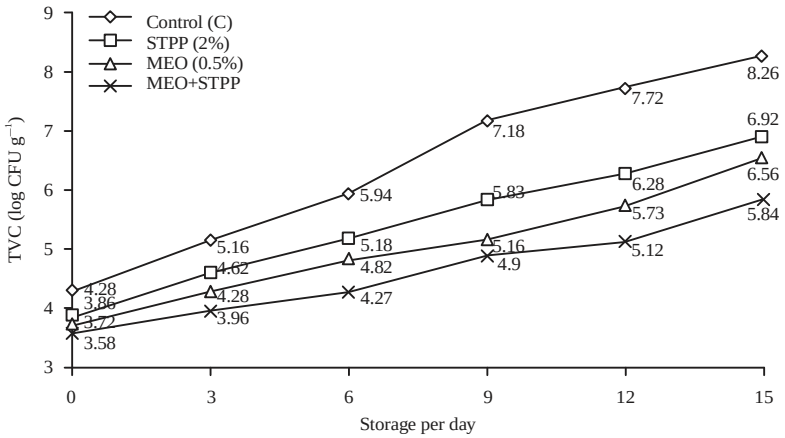

Fig. 1: TVC (as log CFU g^{-1}) of raw Nile tilapia fillets during refrigerated storage at 4±1°C for 15 days

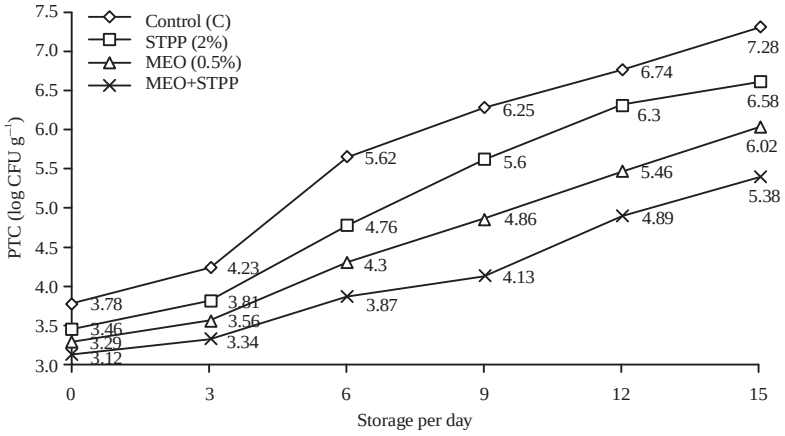

Fig. 2: PTC (as log CFU g^{-1}) of raw Nile tilapia fillets during refrigerated storage at 4±1°C for 15 days

analyzed. During chilling storage the incline of PTC was found to be similar to that of TVC at a higher rate. Psychrotrophic bacteria are very important since they cause most of the changes in odor, texture and flavor as a result of production of different metabolic compounds such as ketones, aldehydes, volatile compounds and biogenic amines[42,4]. Based on PTC number (Fig. 2) the shelf-life of refrigerated (4±1°C) control, STPP, MEO and MEO+STPP fillet samples were 6, 9, 12 and 15 days, respectively. The present results regarding PTC confirmed the findings obtained by other researchers[9,36,39] in their frameworks on fish fillet treated with natural essential oils or sodium tripolyphosphate and subjected to cold storage. It is important to state that changes in bacterial counts of samples (TVC, PTC) are correlated well with sensory quality changes (Table 2) of the same samples.

Results of Fig. 1 and 2 also reveal that, at any given time of refrigerated storage, MEO dipping treatment was significantly ($p<0.05$) more effective against microbial growth (TVC, PTC) than STPP pre-treatment, which could be due to the fact that MEO is well known for its high phenolic compounds content including carvacrol, thymol, p-cymene and γ-terpinene with strong antimicrobial activities against a wide range of spoilage and pathogenic microorganisms[20,21]. Antimicrobial action of phenolic compounds was related to the inactivation of cellular enzymes, which depended on the rate of penetration of the substance into the cell and on weakening or destruction of the permeability of cell membranes, those results in lethal damage to the bacterial cell[43,44]. On the other hand, pretreatment by soaking tilapia fillets in polyphosphate solution prior to storage was more effective in reducing microbial numbers compared with control samples. Polyphosphates may suppress the growth of bacteria by chelating metal ions essential for cell division[17] or by changing the cellular morphology[45].

Physicochemical changes

Weight gain, moisture retention and cooking loss: Some technological properties (water uptake ability, moisture after soaking in test solutions, moisture retention after grilling

and cooking loss) of Nile tilapia fillet samples were calculated and the results are shown in Table 3. As expected, soaking treatments with all solution caused significant ($p<0.05$) increase in weight of bolti fillet samples depending on nature of the pretreatment, due to a net increase in moisture content as a consequence of water binding properties of muscle proteins. Meanwhile, cooking loss reflects the WHC[27].

Referring to Table 3, it could be observed that an increase of weights was recorded for Nile tilapia fillets after immersion in water (3.27±1.15%), in STPP solution (6.48±1.63%) and in Marjoram Essential Oil (MEO) solution (4.83±1.87%), respectively. The combined applied solution promotes more weight increase (5.16±1.34%) than MEO alone or control groups (Table 3). Proximate analyses in the present study indicate that moisture content of raw Nile tilapia fillet before soaking was 78.12±0.65%, it increased to 79.32±0.86, 80.85±0.58, 79.84±0.40 and 80.12±0.76 % after soaking in cold water (C), STPP, MEO and MEO+STPP solutions, respectively. Phosphate-treated (STPP) fish fillets showed the lowest ($p<0.05$) cooking loss percentages (17.23±1.24) and the highest ($p<0.05$) moisture content after soaking (80.85±0.58) as well as the highest moisture retention after grilling (70.18±2.10), when compared with other treatments. In addition fillet samples soaked in Marjoram Essential Oil (MEO) also exhibited higher percentages of the previous technological properties as compared to control samples. Whereas fillet samples treated with MEO+STPP in combination significantly ($p<0.05$) improved the above mentioned values which reduced the economic loss. These results confirmed the findings of Goncalves *et al.*[26] and Moawad *et al.*[13].

Some researchers have been demonstrated that consumer prefers cooked seafood with high moisture content[26,32]. These studies help to explain why in the present study the panelist preferred treated samples compared to control group (water). The retention of moisture and ability to hold water in the cooked product can provide a consumer benefit in terms of texture (higher sensorial responses).

However, the antioxidant activities of both STPP and MEO improves lipid stability in muscle origin food and consequently reduces the formation of lipid oxidation products which render fish proteins less soluble, hence the pre-treatments would increase moisture retention and reduce the amount of drip loss and cooking loss[14]. Another explanation is the ability of phosphate to interact with fish proteins producing a surface film, such a film would seal in fluids and thus not only reduce cooking loss but also minimize moisture loss during cooking[16], hence the hydration characteristics of protein increased and consequently better retention of the flavor and better texture[26].

Table 3: Technological properties control and treated Nile tilapia fillet samples (at zero time)

Dipping treatment	Weight gain (%)	Moisture raw (%)	Moisture cooked (%)	Cooking loss (%)
Control (C)	3.27±1.15^{d}	79.32±0.86^{b}	65.45±1.16^{d}	26.35±1.16^{a}
STPP (2%)	6.48±1.63^{a}	80.85±0.58^{a}	70.18±2.10^{a}	17.23±1.24^{d}
MEO (0.5%)	4.83±1.87^{c}	79.84±0.40^{b}	68.82±0.93^{c}	20.18±1.35^{b}
MEO+STPP	5.16±1.34^{b}	80.12±0.76^{a}	69.17±1.24^{b}	18.64±1.12^{c}

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 ($p<0.05$)

Water holding capacity changes: A useful tool for describing quality changes in the muscle post mortem is by measuring the Water Holding Capacity (WHC) of muscle[31,46]. The muscles ability to retain water is regarded as an essential quality parameter and a high WHC is of great importance both to the industry and the consumers[47,48]. The WHC of Nile tilapia fillets are shown in Table 4. The initial WHC for raw control, STPP, MEO and MEO+STPP soaked fillet samples were 74.05±0.18, 76.54±0.34, 75.48±0.21 and 76.42±0.42% these values were significantly decreased ($p<0.05$) with increasing storage time, reaching values of 40.57±0.14, 52.32±0.12, 46.54±0.32 and 51.43±0.18%, respectively in end of refrigerated storage (15 days). A similar trend WHC change of R.f. kutum fillets was observed during refrigerated storage by Etemadian *et al.*[9]. However, WHC directly affects product appearance, production efficacy/profitability and consumption quality such as juiciness[31].

Results of Table 4 also reveal that The highest WHC (%) were exhibited for the group treated with polyphosphate (STPP), followed by essential oil (MEO) treated batch, then for control fillet samples in that order, respectively. No significant difference ($p<0.05$) was noticed between WHC (%) of MEO+STPP and STPP treated fillets. Polyphosphates (STPP)

Table 4: Water holding capacity (%) changes of raw tilapia fillets during refrigerated storage at 4±1 °C for 15 days

Storage per day	0	3	6	9	12	15
Control (C)	74.05±0.18^{c}	67.86±0.12^{c}	61.23±0.12^{c}	54.64±0.16^{c}	46.12±0.11^{c}	40.57±0.14^{c}
STPP (2%)	76.54±0.34^{a}	72.70±0.10^{a}	70.46±0.15^{a}	66.38±0.12^{a}	58.29±0.18^{a}	52.32±0.12^{a}
MEO (0.5%)	75.48±0.21^{b}	70.62±0.13^{b}	65.80±0.14^{b}	60.06±0.34^{b}	53.42±0.28^{b}	46.54±0.32^{b}
MEO+STPP	76.42±0.17^{a}	72.59±0.16^{a}	70.76±0.10^{a}	66.54±0.13^{a}	57.92±0.12^{a}	51.43±0.18^{a}

All values determination±standard deviation (SD) are mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$)

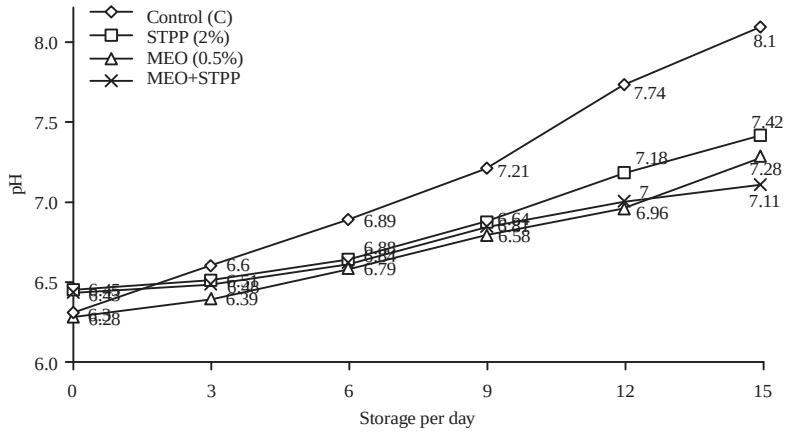

Fig. 3: pH changes of Nile tilapia fillets during refrigerated storage at 4±1°C for 15 days

may interact with the positive charges of the protein molecule to increase the net negative charge, resulting in the increased WHC, leading to the increased water retention[15]. On the other hand, a decreased WHC of muscle has often been described as an effect of structural alterations in the muscle post mortem. Such alterations could be shrinkage of the myofilament lattice, myosin denaturation[49] and increased extracellular space[50]. The present results confirmed the findings obtained by Carneiro *et al.*[51] who reported that the pH increase with the use of phosphate is an important factor to increase the fish WHC. In this respect Goncalves *et al.*[26] reported that, the most important advantages of seafood phosphate treatment are increase water holding capacity, reduction of drip losses, nutrient retention and improve texture and tenderness.

pH changes: Figure 3 shows pH values for raw Nile tilapia fillet as a function of treatment and storage time at 4±1°C. Initial (day 0) pH value in fresh control fillet samples was 6.30 and are consistent with results reported for tilapia fillet[38,39]. For the samples pretreated with phosphates, the increase in pH (6.45) was observed. In contrast, MEO dipping treatments exhibited the lowest pH values (6.28) as compared with STPP and control treatments. Our results (Fig. 3) confirmed the findings of other authors[9,11] in their frameworks on fish fillets.

Figure 3 further shows that during cold storage, pH of tilapia fillet samples increased throughout the storage time, presumably due to the production of basic amines as a result of decomposition of nitrogenous compounds caused primarily by microbial activity. Such an increase in pH of fish could indicate bacterial growth, the reduction of quality and ultimately spoilage of fish[4,42]. The sharp increase was observed in pH of control samples to end day of storage. The pH of STPP treated samples was quite increased throughout the storage. This quite increase might be due to the buffering activity of phosphate which could maintain the pH of muscle[52]. On the other hand, the lower pH values for MEO treated samples indicating strong antimicrobial activity of marjoram oil[19,22,44].

Display of data demonstrated in Fig. 3 it is obvious that starting from the third day of cold storage control samples exhibit significantly higher ($p<0.05$) pH values than treated samples, conversely MEO treatments had the lowest pH increment during the course of refrigerated storage. No significant differences were noticed between pH values of STPP and combined application of MEO+STPP treated samples, except the late period of refrigerated storage. Figure 3 also obvious that control tilapia fillet were of good and acceptable quality with regard to pH value up to 6 days in comparison to 9 and 12 days noticed for STPP and MEO treated fillet samples, respectively. In addition, MEO+STPP samples reached the critical pH value (7.11) at 15th day of refrigerated storage. These results are in accordance with that found by Ozyurt *et al.*[4], who reported that pH values above 7.1 are indicative of decomposition in fish.

TVBN changes: The TVB-N, which is mainly composed of ammonia and primary, secondary and tertiary amines, resulted from degradation of proteins and non-protein nitrogenous compounds is well documented as a good index for the quality and shelf-life of fish and fish products because its increase is related to spoilage by the activity of endogenous enzymes and bacterial growth[4,53]. The initial (day 0) TVB-N values (Fig. 4) of control, STPP, MEO and MEO+STPP tilapia fish fillets were 11.25±0.12, 10.64±0.10, 10.43±0.11 and 10.35±0.14 mgN/100 g flesh, respectively. These values are indicative of good quality raw fillets used in this assay and they are in agreement to those found by Ibrahim and El-Sherif[36]. Higher values of TVB-N at day zero of chilled storage were reported by Ozogul *et al.*[10], while lower values were achieved by other researchers[9,39].

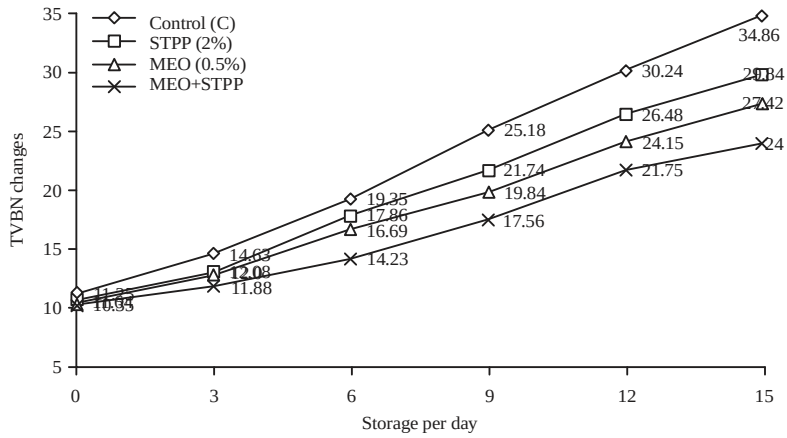

Fig. 4: The TVBN changes of Nile tilapia fillets during refrigerated storage at 4±1 °C for 15 days

Table 5: The TBARS values changes of raw tilapia fillets during refrigerated storage at 4±1 °C for 15 days

Storage per day	0	3	6	9	12	15
Control (C)	0.65±0.10[a]	0.86±0.12[a]	1.23±0.12[a]	1.64±0.17[a]	2.12±0.11[a]	2.57±0.14[a]
STPP (2%)	0.54±0.14[a]	0.70±0.10[b]	0.96±0.11[b]	1.38±0.12[b]	1.89±0.10[b]	2.32±0.12[b]
MEO (0.5%)	0.48±0.11[b]	0.62±0.13[c]	0.80±0.14[c]	1.06±0.11[c]	1.42±0.08[c]	1.94±0.10[c]
MEO+STPP	0.42±0.12[b]	0.59±0.11[c]	0.76±0.10[d]	0.98±0.13[d]	1.21±0.12[d]	1.43±0.15[d]

Mean±SD = All values determination±standard deviation (SD) are mean of triplicate determinations. Mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$)

From the same data of Fig. 4 it is apparent that TVBN of all samples gradually increased with different rates depending on the nature of the pretreatment and time of storage. The lowest TVBN value ($p<0.05$) was obtained from MEO+STPP followed by MEO, STPP and the control during storage period, respectively. The TVBN values of good quality fish are generally less 25 mg N/100 g muscle and above 25-30 mg TVB/100 g indicate that fish is decomposed and inedible[54,37]. Accordingly, the control fillet samples still acceptable for 6 days compared with 9 days for STPP batch and 12 days for fillets treated with MEO. Nile tilapia fillets soaked in MEO+STPP did not exceed the acceptability limit proposed for this study. Generally, when the TVB-N level exceeded the maximum value, samples were already refused by the panelists. Therefore, TVB-N values correlated well with the results of sensory analyses and microbiological examination, providing a good index for the assessment of freshness of Nile tilapia fillet during refrigerated storage.

TBARS changes: The TBARS is the second breakdown product of lipid oxidation and is widely used as an indicator of the degree of lipid oxidation[12]. The concentrations of TBARS (mg MDA/kg fillet) in raw Nile tilapia fillets are shown in Table 5. The initial (day 0) TBARS values of control, STPP, MEO and MEO+STPP tilapia fish fillets were 0.65±0.10, 0.54±0.14, 0.48±0.11 and 0.42±0.12 mg MDA/kg flesh, respectively. These values are indicative of good quality raw materials and are well below the limit level at which rancid flavors may become evident in fish. Similar MDA values have been reported at day zero of chilled storage by other authors[36,9,38]. The TBARS values for the control fillets significantly ($p<0.05$) increased, whereas it slightly increased in treatment groups as the storage time increased. The lowest TBARS values were obtained from the group treated with MEO+STPP as compared to other treatments (Table 5).

From the same given results of Table 5, it could be observed that marjoram oil was more effective ($p<0.05$) than STPP in retarding lipid oxidation. The high efficiencies of MEO were closely related to the high content of phenolic compounds, confirming the key role of phenolic compounds as scavengers of free radicals and as primary, chain breaking antioxidants[21,22]. Phosphates (STPP) potentially retarded the oxidation in fish through the chelation of pro-oxidant metal ions[11,17,18]. A level of 1.5 mg MDA/kg is usually regarded as the limit beyond which fish will develop an objectionable odor/taste[6,54,55]. Accordingly, the obtained data of Table 5 reveal that cold stored fillet samples exhibited low and acceptable TBARS value (less 1.5 mg MDA/kg fillet) up to 6 days for control, 9 days for STPP, 12 days for MEO fillet batch and 15 days for MEO+STPP treated samples. These results are in agreement with the findings achieved by Ozogul *et al.*[10] and Badee *et al.*[19], who reported that EOs incorporation retarded

lipid oxidation in muscle based products. In the present study TBARS values correlated well with the results of sensory analyses (Table 1, 2).

CONCLUSION

The MEO treatment was determined more effective than STPP application for maintaining quality aspects and extending shelf-life of tilapia fillets as supported by the results of microbiological, physicochemical and sensorial analyses. The effective retardation of quality deteriorations for cold stored Nile tilapia (*Oreochromis niloticus*) fillet could be achieved by pretreatment with MEO+STPP combined dipping application. The present results could arise as an interesting approach for the enhancement of food safety and quality using more natural procedures, considering the current demand of consumer and sensory quality of seafood products.

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