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Research Article

Combined Effect of *Origanum majorana* and Sodium Acetate on the Quality Attributes of Catfish (*Clarias gariepinus*) Patties During Refrigerated Storage

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Abstract

Background and Objective: In view of increasing the consumer demand for natural preservative seafood, the present study aimed to evaluate the effect of using Powdered Marjoram Leaves (PML, 1% w/w) alone or in combination with Sodium Acetate (SA, 2% w/w) on the quality characteristics and shelf-life extension of African catfish (*Clarias gariepinus*) patties under refrigeration (4±1°C) for a period of 16 days. **Materials and Methods:** Chemical indices (pH, Total Volatile Base Nitrogen "TVBN" and Thiobarbituric acid reactive substances "TBARS"), Sensorial properties (appearance, taste and odor) and Microbiological status (Total Viable Count "TVC" and Psychotrophic count "PTC") were evaluated during refrigerated storage. Data were subjected to one-way analysis of variance (ANOVA) to compare the effect of PML and/or SA treatments. **Results:** The results indicate that 1% PML was more effective ($p<0.05$) for maintaining oxidative stability, whereas 2% of the SA treated samples were significantly ($p<0.05$) lower in TVC, PTC, pH values and TVB-N content as compared to the other treatments. Throughout the cold storage, acetate pretreatment showed the synergistic effect with marjoram on catfish patties quality till the end of the storage process. The sensory scores gradually decreased during the storage period with a higher rate ($p<0.05$) in the control patties as compared to the treated samples. Overall, the study indicated the potential use of natural herb (PML) in combination with organic preservative (SA) to develop novel healthy fishery products with improved shelf-life and superior quality. **Conclusion:** Inclusion of PML and/or SA in catfish patties formulas gave a pleasant odor and taste and extended the shelf-life of the product without undesirable changes in their qualities. Based on chemical indices, microbial analyses and sensorial change, the shelf-lives of catfish patty were the longest for PML+SA- (16 days), followed by 2% SA- (14 days), 1% PML- (12 days) and Control samples (about 6-7 days).

Key words: Catfish patty, quality deterioration, chemical indices, shelf-life, sodium acetate, powdered marjoram leaves

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

INTRODUCTION

Fish and fishery products contain high-quality protein and other essential nutrients, they are also few in saturated fatty acids and cholesterol content, whereas they are many in ω-3 and unsaturated fatty acids content. Therefore, the addition of fish in the diet does not only improve the nutritional quality but also increases health benefits to consumers[1]. Egypt has many fish sources and water areas but the local production cannot cover the domestic consumption[2]. Very rapid developments have occurred in the Aquaculture Sector of Egypt in recent years for narrowing the current gap between production and consumption of fish. Several species (Fig. 1a) of marine and freshwater fish, such as Nile tilapia, mullet, carps, sea bream, African catfish, sea bass, meager, penaeid shrimp and freshwater prawn are being farmed in order to meet the increasing demand of consumers for fresh fish[3].

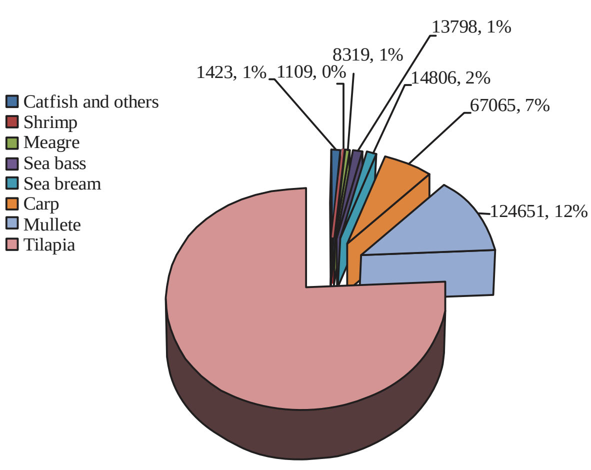
African catfish (*Clarias gariepinus*) farmed in the country are promising and are becoming a more important thanks to their remarkable growth rate. They are generally considered some of the most important tropical catfish species for aquaculture[4,5]. Processing and value-addition to low-cost materials such as catfish fillet are needed in order to make them more profitable. The flesh of the African catfish fillet is a valuable and cheap source of easily digestible high-quality protein. It is pink in color and almost boneless (Fig. 1b) and tasty, with delicate flavor and firm texture when cooked. Their flesh can be prepared in various ways; it can be fried, grilled and broiled and that is why it is very popular and suitable for producing various mince-based products[6,7].

Recently, the increasing number of the worlds working women, in addition to some various socioeconomic changes, has caused that increase of the consumers preference for ready-to-cook/eat fish and fishery products[8]. Fresh fish (including catfish) are more susceptible to both microbiological and chemical deteriorations, compared to chicken and red meat, due to their high water activity, large amounts of free amino acids and volatile nitrogen bases, the presence of autolytic enzymes and a higher final pH that limiting the shelf life of the product[9].

Minced fish meat, especially its industrial products, faces serious problems in terms of processing and storage. Unsaturated lipids, fine grinding, incorporation of air, haem pigments, metal contact and the high temperature during processing contribute to lipid oxidation and microbial deterioration[10]. More literature review has proved that even refrigerated minced fish based products can undergo undesirable quality changes due to microbial growth, which gives rise to quality reduction, fish spoilage and economic loss. Refrigerated minced fish products are also susceptible to lipid oxidation that can deteriorate color, texture, flavor and the safety of these products[11].

Minimizing product contamination, delaying or inhibiting the growth of pathogenic and spoilage organisms, retarding lipid oxidation and the formation of toxic oxidation products, through the use of naturally combined preservative factors known as hurdle technology[12] are very important objectives of food technologists and microbiologists. Natural preservatives have been preferably employed in muscle origin products because of their potential health benefits and safety compared to synthetic preservatives whose use is confirmed for their carcinogenic potential and toxicity for consumers[13]. Hence, the use of sweet marjoram in combination with sodium acetate seems to be considerable influence on industrial applications and scientific research[14,15].

(a)



(b)

Fig. 1(a-b): (a) Aquaculture production in Egypt[3]. Carp includes common, silver and large head, (b) Flesh of African catfish

Sodium acetate as an organic decontaminating agent, in addition to general hygienic practice, is an approved[16] flavoring and pH control agent. It is widely available, economical and is Generally Recognized As Safe (GRAS). Furthermore, it was noticed that Sodium Acetate (SA, 2%) was effectively used to control the growth of food spoilage and food-borne pathogens bacteria. It was also found out that it helps to improve sensory attributes, ameliorate color stability and extend the shelf-life of various refrigerated fishery products[17-19]. The decrease in water activity (a_w), pH value and the chelation of pro-oxidant metal ions may contribute to the overall antioxidant and antimicrobial properties of sodium acetate[20,21]. Therefore, sodium acetate can be utilized as a safe organic preservative under refrigerated storage for maintaining fish quality[22].

Marjoram of *Lamiaceae* family is a natural familiar kitchen herb; it is widely distributed in the country and was known in ancient Egypt for its flavoring qualities and healing properties. Marjoram is classified as GRAS[23]. It is well known for its high phenolic compounds, such as carvacrol, thymol, p-cymene, γ-terpinene, flavonoid, arbutin, tannins and phenolic acids with strong antioxidant and antimicrobial activities[24,25]. Moreover, a purified component "T3b" isolated from marjoram methanolic extract exhibited a better superoxide anion radical scavenger than BHT, BHA, α tocopherol and ascorbic acid[26]. However, using it in foods requires detailed characterization of its compounds.

Most of the studies that have dealt with marjoram extracts and essential oil tackled the effect of Powdered Marjoram Leaves (PML) on the quality of perishable foods[27]. Therefore, it would be economically more convenient to use powdered herbs as ingredients rather than their essential oils or extracts to preserve food. To the researchers knowledge, there is no available literature regarding the effect of Powdered Marjoram Leaves (PML) and food grade Sodium Acetate (SA) to improve the safety and quality of catfish patties. Thus, the objective of the present work was to determine the effect of SA (2%) and PML (1%) or their combination on microbiological status, chemical criteria, sensorial qualities and the shelf-life of raw catfish patties with a new flavor (marjoram) during the refrigerated storage process at 4$\pm$1°C for 16 days. In addition, the proximate analysis is important from many perspectives including nutritional value, processing and preservation. Hence, the proximate composition of PML, catfish fillets and catfish patties was also investigated.

MATERIALS AND METHODS

Experimental materials, chemicals and supplies: A total of 25 kg fresh African catfish (*Clarias gariepinus*) samples of 1350-1600 g each; were purchased directly from fishermen in Imbaba, Giza, Egypt, in November, 2016 and rapidly transported under complete aseptic conditions in ice boxes to the laboratory of Food Technology, National Research Centre, within 1 h. Sodium acetate (CH_3COONa, Anhydrous 99% extra pure), was purchased from Alpha Chemika (India). Sodium chloride and magnesium oxide were from El-Nasr Pharm. and Chem. Co., (Egypt). Plate Count Agar (PCA) and peptone water were from Oxoid (UK). 2-Thiobarbituric acid, methyl red, bromocresol green and BHT were from Sigma Aldrich. All other solvents and chemicals (glacial acetic acid, sulphuric acid, boric acid, hydrochloric acid and methanol) were from ADWIC (Egypt). Air-dried marjoram leaves prepared for export were purchased from Al-Dahlia Co., Egypt and stored at 4$\pm$1°C, until they were used. Wheat flour, bread crumbs and corn starch were purchased from the local market in Dokki, Giza.

Methods

Preparation of minced catfish fillets and PML: Upon arrival to the laboratory, catfish samples were immediately weighed, slaughtered by pitching them, carefully beheaded, gutted and washed. Then, they were aseptically filleted into two pieces (Fig. 1b) by using gloved fingers, sterile cutting boards and knives and rewashed with clean water. The obtained catfish fillets were minced twice with a kitchen meat mincer with a pore size of 6.0 mm. The minced catfish samples were tempered in a freezer for 60 min before treating and processing them into patties. Dry marjoram leaves were crushed by hands and ground by in an electric mill for 1 min. The obtained PML was packed in sealed polyethylene bags, stored at 4$\pm$1°C and immediately used for patties processing.

Catfish patties processing and treatments: Catfish patties were prepared without any seasonings using a simple traditional formulation, according to the method of Tokur *et al.*[28], with 85% minced catfish, 7% corn flour, 4% wheat flour, 2.2% bread crumbs and 1.8% salt as ingredients. Subsequently, 10% ice water was added, thoroughly mixed by hand for 5 min, then divided into 4 equal portions (3 kg each) and separately comminuted again through a 3 mm mincer steel plate. The first portion was used as a control containing neither marjoram nor acetate (C), while the other portions were either mixed with Powder Marjoram Leaves (1% PML), Sodium Acetate (2% SA) or their combinations (1% PML+2% SA), respectively. After treatments, each group was separately mixed well to ensure the uniform distribution of dry marjoram and/or the added sodium acetate. The obtained pastes were formed into 50±3 g catfish patties (54 patties×8.5 cm diameter and 1.2 cm thickness) from each group and prepared using a Petri dish in the laboratory[29].

Catfish patties packaging, storage and analysis: Catfish patties from each group were then individually packaged in polyethylene bags (with three patties in each bag) and then they were stored at 4±1°C for 16 days and periodically analyzed on days 0, 6, 9, 12, 14 and 16 for sensory assessment (taste, odor and appearance), Microbial growth (TVC and PTC) and Chemical criteria indices (pH, TVB-N and TBARS). Utilization of powder marjoram and sodium acetate in combination was based on the results of previous studies[15,30,31]. Three bags of each group (9 catfish patties) were withdrawn at each interval of cold storage, six catfish patties were fried before being subjected to organoleptic assessment and the remaining 3 patties were used for chemical determinations after being sampled for microbiological purpose. All experiments were repeated 3 times in order to eliminate the effects of the initial quality of raw material.

Analytical techniques

Proximate composition and chemical criteria analyses: Proximate compositions in terms of moisture, ash, crude lipid and total nitrogen were determined according to the methods described in the AOAC[32]. Carbohydrate content was determined by the percentage differences of other contents. The Total Volatile Basic Nitrogen (TVB-N) expressed as mg TVB-N per 100 g catfish flesh was measured by steam-distillation according to the method of Parvaneh[33]. A thiobarbituric acid reactive substance (TBARS) as mg of malondialdehyde (MDA kg^{-1}) catfish flesh, was estimated according to Kilinc *et al.*[34]. However, the method described by Ozyurt *et al.*[35], was used to measure pH values of patty samples.

Microbiological examination: Total Viable Count (TVC) and psychrotrophic count (PTC) of catfish patty samples were determined in triplicate by Plate Count Agar (PCA) as described by Tajik *et al.*[36]. 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}.

Sensory evaluation of fried catfish patties: The sensory evaluation of grilled catfish patties was done by 10 non-trained panel members of the laboratory staff who were familiar with the characteristics of fish. In order to conduct sensory analyses, six catfish patties were taken from each group at regular intervals; the fish patties were separately grilled (250°C) for 3 min for each side (until cooked to an internal temperature of 72°C) before being presented to the panelists. Grilled catfish patty samples from each group were cut into four rectangular pieces from the center, packed in small plastic cups, labeled and then served warm to the panelists at room temperature in a random order. Water was served for rinsing the mouth between samples. The panelists were asked to evaluate the 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[37].

Statistical analysis: Results were expressed as Means and Standard Deviation (M±SD) from triplicate determinations. Data were subjected to one-way analysis of variance (ANOVA) to compare the effect of PML and/or SA treatments. Significant differences were defined as $p<0.05$, according to PC-STAT[38], Version I A Copyright, the University of Georgia, USA.

RESULTS AND DISCUSSION

Yield of catfish fillets: Before considering the proximate composition of PML and catfish-based products, it is worth mentioning that the yield of catfish boneless pink flesh (Fig. 1b) in the present study was estimated as 52.84±1.12% (results not shown). The flesh of the African catfish fillet is a valuable and cheap source of easily digestible high-quality protein. It is pink in color and almost boneless (Fig. 1b) and tasty, with delicate flavor and firm texture when cooked[4,5,7].

Catfish fillet can also be prepared in various ways; it can be fried, grilled and broiled and that is why it is popular and suitable for producing various mince-based products[6,7]. Therefore, processing and value-addition to low-cost materials such as catfish fillet are needed in order to make it more profitable. Generally, the results of yield percentage of catfish fillet in this study confirmed the previous findings reported by other authors[39,40].

Table 1: Proximate chemical composition of Powder Marjoram Leaves (PML), catfish fillet and catfish patties (on fresh wet. basis)

RAW materials	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
Dry PML	8.10±0.15[d]	11.82±0.24[d]	6.18±0.06[b]	11.69±0.28[a]	62.21±2.46[a]
Catfish fillets	75.43±2.18[a]	19.14±0.82[a]	3.05±0.26[d]	1.28±0.34[d]	1.10±0.08[d]
Control, catfish patties	66.18±1.08[b]	18.46±0.64[b]	5.62±0.35[c]	3.51±0.42[c]	6.23±0.54[c]
PML-catfish patties	64.25±1.43[c]	17.82±0.34[c]	6.40±0.18[a]	4.02±0.10[b]	7.51±1.18[b]
SA-catfish patties	66.35±1.15[b]	18.37±0.21[b]	5.54±0.19[c]	3.62±0.65[c]	6.12±0.35[c]
PML+SA-patties	64.40±0.12[c]	17.71±0.27[c]	6.32±0.15[a]	4.13±0.21[b]	7.44±0.72[b]

All values reflect the mean and Standard Deviations (SD); they are triplicate determinations. The mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$). Where PML: 1% Powder Marjoram Leaves. SA: 2% Sodium Acetate, PML+SA: 1% Powder Marjoram Leaves +2% Sodium Acetate

Proximate composition of PML, catfish (fillets and patties): Mean value for the proximate composition of air shade-dried Marjoram leaves, raw fresh catfish fillet and patty formulas are shown in Table 1.

It is apparent from the results shown in Table 1 that PML are characterized by their high protein, fat and ash contents and very high carbohydrates content, which gave the marjoram and other Lamiaceae herbs importance as a food ingredient[41,42]. It was also revealed by Table 1 that the proximate composition of dried health promoting marjoram leaves are within the normal limits for the species and are in agreement with those found early in the aromatic and medicinal plants literature for marjoram[43,44].

It was also revealed that the examined catfish *(Clarias gariepinus)* species belonged to high-protein (15-20%) and low-fat (<4%) category[45]. The proximate analysis as shown in Table 1 was within the normal values for the species and season and is in agreement with other analyses carried out by earlier researchers, such as Osibona *et al.*[46] and Chwastowska-Siwiecka *et al.*[47]. The chemical composition of the catfish fillets reported in different studies[48,49] showed some degrees of differences, especially for the lipid and moisture contents. Such variations in the chemical composition of fish are greatly related to nutrition, catching season, sexual variation and fish size[47].

It is obvious from the results of Table1 that there were statistically significant differences ($p<0.05$) between catfish fillet and its minced-based product (catfish patty) in terms of moisture, fat and ash contents, due to patty formula ingredients[7,50]. Moreover, the moisture content decreased while the crude fat and ash contents significantly increased ($p<0.05$) in the marjoram treated catfish patty samples as compared to the acetate or control patty samples. Results depicted in Table 1 also indicate that significant statistical differences in chemical composition were not found between control groups and sodium acetate groups.

It was also indicated in Table 1 that a higher amount of carbohydrate content has been found in catfish patties as compared to catfish fillet, which might be derived from flour, bread and PML. Similar findings were reported in minced fish-based products by other researchers[7,15,51].

Microbiological analysis: Under normal aerobic packaging conditions, the shelf-life of refrigerated fish and fish products is limited by the growth and biochemical activities of bacteria[9,52]. Figure 2a, b show the changes in the TVC and PTC as means log CFU g^{-1} of raw catfish patties samples during refrigerated storage. These results clearly indicate a slight significantly ($p<0.05$) reduction in the initial TVC and PTC was noticed in treated catfish patties samples when compared with control patties samples at zero time of cold storage; proving that 1% PML, 2% SA, or their combined application (PML+SA) caused significant ($p<0.05$) sudden lethal effect for the tested microorganisms immediately after formulating process. These results confirmed the findings obtained by Sallam[22].

Display of data demonstrated in Fig. 2a, b it is obvious that amongst the studied microbial categories, the population counts in control as well as in treated catfish patties followed the order: TVC >PTC throughout the storage period. Moreover, the increase in storage time produced significant steadily proliferations in TVC and PTC, regardless of the treatment conditions. However, the increment noticed for control samples was significantly ($p<0.05$) higher than those found in PML or SA pretreatment groups. These results confirmed the finding obtained by other authors[30,14,15,27], in fish and meat based products pretreated with natural herb and/or sodium acetate.

Total Viable Count (TVC): Total Viable Counts (TVC) are used as an acceptability index for seafood because of the effect of bacteria in spoilage. For fresh water and marine species, the microbiological limit recommended by Egyptian Standardization (ES)[53] is 6 log CFU g^{-1}. Results of Fig. 2a reveal that as may be expected, the increase in storage time produced significant proliferations in TVC, whatever the treatment conditions.

Further indicated by Fig. 2a that PML-, SA- and PML+SA-treated patties samples exhibited 1.24, 2.13 and 2.58 log CFU g^{-1} lower ($p<0.05$), respectively than the corresponding count in control patties samples at the end of cold storage duration (16 days). The present results confirmed the finding of Sallam[22], Attouchi and Sadok[30] and

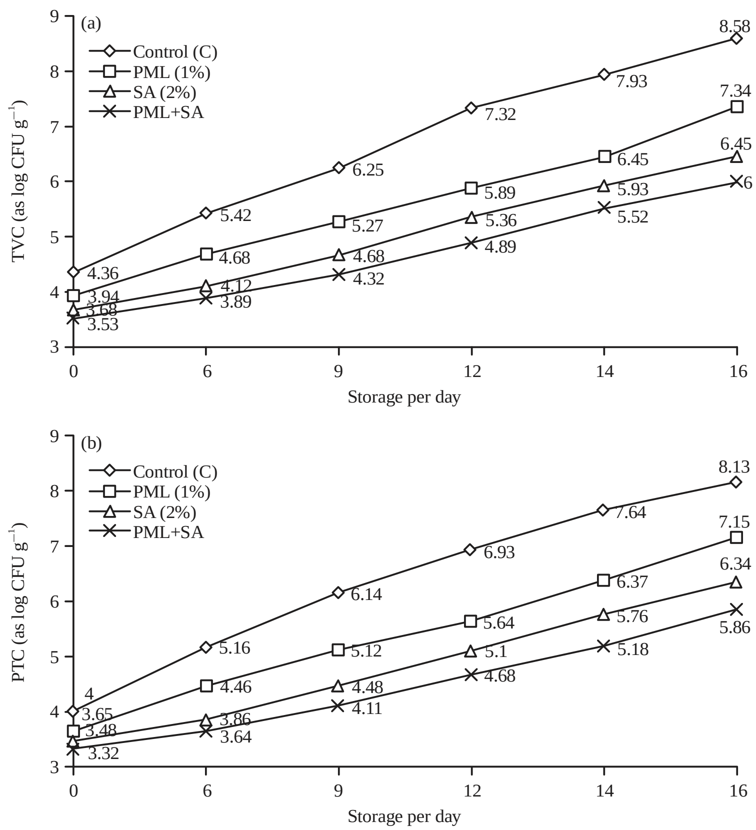


Fig. 2(a-b): (a) TVC (as log CFU g^{-1}) of raw catfish patties during refrigerated storage at 4±1°C for16daysdays, (b) PTC (as log CFU g^{-1}) of raw catfish patties during refrigerated storage at 4±1°C for 16 days

Ehsani *et al*.[15], who reported that sodium acetate (SA, 2%) either alone or in combination with natural herb oil/dried leaves was efficient against the proliferation of various categories of spoilage microorganisms as it delayed lipid oxidation and extended the shelf-life of the product by 4-7 days more than that of the control during refrigerated storage.

Psychrotrophic counts (PTC): The gram-negative psychrotrophic bacteria (PTC) are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish and fishery products at cold storage temperatures[52]. Data depicted in Fig. 2b indicate that during cold storage the incline of PTC was found to be similar to that of TVC (Fig. 2a) at a higher rate, indicating that fish bacterial flora is composed mainly of psychrotrophic bacteria[54]. The PTC of all previously mentioned catfish patties groups (Fig. 2b) continuously raised till the end of refrigerated storage. The same increase in bacterial count was observed by various researchers in different fish products stored in refrigerated condition[34,22,19,7].

Regarding the acceptability of cold stored fish, ICMSF[55] stated that the upper acceptability limit of the total viable bacterial count in fresh fish is 7 log CFU g^{-1} flesh and 6 log CFU g^{-1} is the maximum permissible limit of APC recommended by ES[53] in chilled fish. The obtained data of Fig. 2a, b reveal that control (C), PML-, SA- and PML+SA-containing catfish patties reached the upper limit of acceptability (<6 log CFU g^{-1}) after about 6-7, 12, 14 and 16 days of refrigerated (4±1°C) storage, respectively. The present results also indicate that SA and PML possess antimicrobial properties which help for prolonging shelf-life of pretreated fish products and their combination might have a synergistic effect on the retardation of bacterial growth during cold stored fish, leading to safer products.

Similar to shelf-life obtained in our results, Sallam[22] reported that 2.5% SA dipping, significantly delayed the microbial growth and extended the shelf life of salmon product up to 15 days versus 8 days for control. A shelf-life of approximately 6-7 days was noticed for fresh control rainbow trout during aerobic storage; the addition of 2% sodium acetate extended product shelf-life by 9-10 days[19]. Coban *et al*.[51] reported that the fish fingers contained 1% ginger oil can be stored for 17 days versus 5 days for control patties, at 4°C without undesirable changes of sensory and chemical quality. Guran *et al.*[6] found that the shelf life of patties made from the bonito fish was 4 days for the control group and 8, 10 and 14 days for the groups treated with thyme, clove and rosemary essential oil, respectively. Emir Coban and Tuna Keleştemur[7] reported that the shelf-life of the catfish burger was determined to be 6, 9 and 15 days for Control, 0.2 and 0.4% ZEO, respectively.

On the basis of the microbiological analyses reported in this study, the general order of antibacterial activity of the different treatments of fresh catfish patties was; (1% PML+2% SA) > 2% SA alone > 1% PML alone. The antimicrobial activity of SA is attributed to both the metabolic inhibition by the undissociated acetic molecules and to the depression of pH and water activity (a_w) below the growth range of many bacteria as well as acidifying the cytoplasm of the microorganism by penetrating the cell membrane[20,56,57]. Moreover, SA may suppress the growth of bacteria by chelating metal ions essential for cell division[21]. On the other hand, marjoram herb (PML) 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[24,14,27]. Antimicrobial action of phenolic compounds was related to their hydrophobicity, which enables their partition within the lipids of the bacterial cell membrane and mitochondria. Such activity disturbs cell structures, which causes an increased permeability and loss of cellular constituents[43]. Another explanation is due to the inactivation of cellular enzymes, which results in lethal damage to the bacterial cell[58].

Chemical criteria changes

pH changes: pH value is a critical determinant of microbial growth and food spoilage[52]. Mean pH values for raw catfish patties as a function of treatment and storage time at 4±1°C are shown in Table 2. Initial (0 day) means pH values in fresh control (C) catfish patties samples were 6.08 and are consistent with the results reported for fish patties[51,14]. For the samples pretreated with PML, the decrease in pH (5.92) was observed. In contrast, sodium acetate treatments exhibited lower (p<0.05) pH values (5.81) as compared with marjoram and control samples. The results (Table2) confirmed the findings of other authors[22,19,15] in their frameworks of fish products treated with natural herb and/or sodium acetate.

It is further showed in Table 2 that the pH of catfish patties samples increased significantly (p<0.05) throughout the storage time, presumably due to the production of volatile bases alkaline compounds, which can be attributed to that decomposition of nitrogenous compounds caused primarily by either endogenous or microbial enzymes[59]. The sharp (p<0.05) increase was observed in the pH values of control samples to end a day of storage. The pH of SA-treated samples quite increased throughout the storage up to 14 days, which might be due to the buffering and antimicrobial activities of acetate[17,18]. On the other hand, the catfish patty samples incorporated with 1% PML alone showed moderate and constant pH values up to 12 days of cold storage (Table 2), reflecting the strong antimicrobial activity of marjoram herb[41,14,27]. No significant differences were noticed between pH values of SA and combined application of PML+SA treated samples, except the end (16 days) of refrigerated storage. The similar increasing trend in pH values was observed in refrigerated fish products by other authors[22,51,6].

It is obvious from the data demonstrated in Table 2 that control catfish patty samples were of good and acceptable quality with regard to pH value up to about 6-7 days in comparison to PML- and SA- treated patties observed for 12 and 14 days, respectively, whereas PML+SA- treated samples did not exceed the critical pH value (> 7.1) even at the 16th day of refrigerated storage. These results are in agreement with that reached by Ehsani *et al.*[15] who reported that the pH of SA+ZEO- treated trout burger samples was shown to be more stable (p<0.05) in comparison with other treatments due to their antibacterial property and combined synergistic effect on delaying microbial growth and protein decomposition.

Protein degradation: TVB-N is one of the most widely used indices of fish and fish product quality. It is a general term which includes the measurement of trimethylamine, dimethyl-amine, ammonia and other volatile basic nitrogenous compounds associated with fish spoilage[52,35]. At the beginning of the storage period, TVB-N levels were ranged from 9.34-10.42 mg N/100 g. These values are indicative of good quality raw materials used in this assay and they are in agreement to those found by other researchers[60,14,6,7].

The results depicted in Table 2 also reveal that TVB-N levels of catfish patties samples progressively increased (p<0.05) with a time of storage at 4±1°C for all treatments. The present results also indicate that the 2% SA-treated samples were more effective at inhibiting enzymatic and microbial activity than the 1% PML-treated and control samples. Moreover, the lowest (p<0.05) TVBN value was exhibited for PML+SA. The present results confirmed the findings reached by other authors[22,15]. The reduction observed in TVBN levels of treated samples reflects the antimicrobial properties of both SA and PML under investigation, while an increase in TVB-N levels may be the result of deamination of free amino acids, oxidation of amines and degradation of

Table 2: Chemical criteria changes of raw catfish patties during the refrigerated storage process at 4±1°C for 16 days

		Cold storage per day					
Catfish patty	Criteria indices	0	6	9	12	14	16
Control	pH value	6.08±0.17[a]	6.69±0.13[a]	7.12±0.14[a]	7.38±0.10[a]	7.84±0.11[a]	8.17±0.14[a]
PML (1%)		5.92±0.10[b]	6.35±0.14[b]	6.74±0.10[b]	6.96±0.12[b]	7.40±0.17[b]	7.72±0.12[b]
SA (2%)		5.81±0.12[c]	6.11±0.21[c]	6.35±0.16[c]	6.70±0.11[c]	6.91±0.08[c]	7.34±0.24[c]
PML+SA		5.85±0.15[c]	6.04±0.18[c]	6.27±0.15[c]	6.62±0.15[c]	6.85±0.13[c]	7.10±0.10[d]
Control	TVBN (mg N/10)	10. 42±0.21[a]	19.73±0.13[a]	26.52±0.14[a]	29.48±0.11[a]	33.65±0.14[a]	38.16±0.12[a]
PML (1%)		10.13±0.12[b]	14.58±0.25[b]	19.36±0.18[b]	24.14±0.12[b]	27.48±0.15[b]	32.84±0.25[b]
SA (2%)		9.34±0.11[c]	12.40±0.13[c]	15.19±0.21[c]	19.64±0.15[c]	24.85±0.13[c]	28.72±0.18[c]
PML+SA		9.48±0.14[d]	11.82±0.14[d]	14.54±0.15[d]	17.26±0.17[d]	20.42±0.10[d]	24.12±0.13[d]
Control	TBARS (mg MDAI)	0.68±0.17[a]	1.37±0.18[a]	1.82±0.14[a]	2.14±0.11[a]	2.37±0.14[a]	2.76±0.12[a]
PML (1%)		0.45±0.12[ab]	0.72±0.45[b]	1.13±0.18[b]	1.32±0.12[b]	1.76±0.15[b]	2.13±0.25[b]
SA (2%)		0.57±0.11[b]	0.78±0.13[c]	1.21±0.21[c]	1.37±0.15[c]	1.52±0.21[c]	1.89±0.18[c]
PML+SA		0.51±0.14[b]	0.64±0.14[c]	0.98±0.15[c]	1.16±0.10[d]	1.35±0.10[d]	1.54±0.17[d]

All values reflect the mean and Standard Deviations (SD). They are of triplicate determinations. The mean values in the same column bearing the same superscript do not differ significantly ($p<0.05$). Where TVBN: Total volatile basic nitrogen as mg N/100 g flesh. TBARS: A thiobarbituric acid reactive substance) expressed as mg of malondialdehyde (MDA kg^{-1}) catfish flesh

nucleotides by autolytic enzymes and microbial activity[61]. TVB-N values in the present study confirmed the results of sensory analyses and microbiological examination (Fig. 2); they provide a good index for the assessment of freshness of catfish patties during the refrigerated storage process.

The TVBN values of good quality fish are generally less than 25 mg N/100 g muscle and above 25-30 mg N/100 g flesh which indicate that fish are decomposed and unsuitable for consumption[62,63]. In this respect, the Commission of the European Community (EEC)[64] stated that the upper acceptability limit of Total Volatile Basic Nitrogen (TVB-N) in fresh fish is 35 mg N/100 g of fish flesh and 20 mg N/100 g of fish flesh is the maximum permissible limit of TVB-N recommended by ES[53] in chilled fish. It is clear from Fig. 2 that the upper acceptability limit (25 mg N/100 g approximately) was reached by C, PML and SA catfish groups on almost 6-7th, 12th and 14th days of storage life, respectively. However, catfish patties pre-treated with PML+SA did not exceed the acceptability limit (25 mg N/100 g muscle) proposed for this study, even on 16th day of the refrigerated storage process (Table 2). These results are in agreement with those reported by other researchers[14,15,31], who found that Lamiaceae family herbs and/or SA pretreatments generally showed lower TVB-N values for refrigerated fish and fish products.

Lipid oxidation: TBARS is the second breakdown product of lipid oxidation and is widely used as an indicator of the degree of lipid oxidation in food products[34,65]. In this study, changes in TBARS values of raw catfish patties are shown in Table 2. At the beginning of the storage period, the amounts of TBARS ranged from 0.45-0.68 mg MDA kg^{-1}. 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 products. TBARS level significantly ($p<0.05$) increased in all groups during cold storage (Table 2). The increase of the TBARS value has been demonstrated for fish products by numerous authors[14,51,6,7]. The results in all of these studies are almost parallel to the results found in this experiment.

The data depicted in Table 2 clearly show that the increase in TBARS level was higher ($p<0.05$) in the control group than the acetate (SA) and marjoram (PML) added groups and that PML incorporation exhibited more oxidative stability than other treatments up to the 12th day of the refrigerated storage process. In contrast, PML+SA- treated patties exhibited the lowest TBARS values throughout the study. They also indicate the synergistic effect of sodium acetate and powdered marjoram. As shown in Table 2, it is deduced that the use of marjoram and/or SA slowly increased the formation of thiobarbituric acid reactive substances (TBARS) in refrigerated treated catfish patties, particularly in the late periods of storage time when compared with control samples. The high efficiencies of PML were closely related to the high content of phenolic compounds as thymol, carvacrol, cymene and γ-terpinene[26,24,14,27]. Acetate (SA) potentially retarded the oxidation in fish products through the chelation of pro-oxidant metal ions and due to antibacterial effects of sodium acetate on bacterial enzymatic reaction related to oxidation[21,17,19].

Concerning the permissible limit of TBARS value in fish and fish products (4.5 mg MDA kg^{-1}) recommended by ES[53], neither treated catfish patties nor control one exceeded such limit in any given time of the refrigerated storage process even after showing objectionable odors and flavor (Table 2). In this respect, Connell[66] and Tironi *et al.*[67] stated that the acceptability limit of fresh fish ranges from 1-2 mg MDA kg^{-1} of patties, while Shakila *et al.*[62] and Goulas and Kontominas[60]

Table 3: Mean sensory scores of grilled catfish patties after refrigerated storage at 4±1°C up to 16 days

		Cold storage per day					
Catfish patty	Sensory	0	6	9	12	14	16
Control	Appear. scores	4.90±0.18[a]	3.52±0.17[d]	2.43±0.10[d]	NA	NA	NA
PML (1%)		4.83±0.13[a]	4.17±0.21[c]	3.54±0.25[c]	3.14±0.15[c]	NA	NA
SA (2%)		4.88±0.25[a]	4.35±0.13[b]	3.90±0.12[b]	3.45±0.16[b]	3.00±0.21[b]	NA
PML+SA		4.90±0.16[a]	4.68±0.11[a]	4.23±0.15[a]	4.00±0.10[a]	3.64±0.11[a]	3.17±0.17[a]
Control	Odor scores	4.75±0.17[a]	3.36±0.15[d]	2.62±0.15[d]	NA	NA	NA
PML (1%)		4.78±0.13[a]	4.21±0.17[c]	3.84±0.32[c]	3.40±0.14[c]	NA	NA
SA (2%)		4.70±0.32[a]	4.06±0.24[b]	3.68±0.18[b]	3.27±0.15[b]	3.00±0.24[b]	NA
PML+SA		4.75±0.15[a]	4.37±0.16[a]	4.13±0.23[a]	3.78±0.24[a]	3.36±0.32[a]	3.00±0.12[a]
Control	Taste scores	5.00±0.42[a]	3.56±0.21[d]	2.36±0.14[d]	NA	NA	NA
PML (1%)		4.90±0.35[a]	3.85±0.53[c]	3.46±0.17[c]	3.00±0.25[c]	NA	NA
SA (2%)		4.95±0.64[a]	4.00±0.36[b]	3.62±0.45[b]	3.35±0.18[b]	3.00±0.25[b]	NA
PML+SA		4.92±0.28[a]	4.24±0.24[a]	3.90±0.26[a]	3.66±0.27[a]	3.35±0.19[a]	3..10±0.21[a]

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

stated that a level of 1.5 mg MDA kg^{-1} is regarded as the limit beyond which fish would develop an objectionable odor and taste. Thus, the acceptability limit proposed for this study is 1.5 mg MDA kg^{-1} and the obtained data in Table 2 reveal that cold stored catfish patties samples exhibited low and acceptable TBARS value (<1.5 mg MDA kg^{-1} flesh) up to about 6-7 days for control, 12 days for 1% PML, 14 days for 2% SA- and 16 days for PML+SA treated samples. These results are in good agreement with the findings achieved by[22,27,14,15].

Sensory assessment: Sensory quality changes of grilled catfish patties in terms of appearance, odor and taste were evaluated and the mean score values are presented in Table 3. Owing to the development of off-odor and off-flavor in raw catfish patties associated with spoiling fish (Results not shown) the control (C), PML- and SA-treated patties samples were halted (discontinued) from sensory evaluation after 9, 12 and 14 days of refrigerated storage, respectively. PML+SA-treated samples, however, were evaluated until the end of the storage period (16 days).

Results of Table 3 indicate that no differences in the sensory scores were detected between control and treated samples at the beginning of storage (0 times). Moreover, panel preference for treated samples over stored untreated controls (Table 3) indicated that both marjoram herb and sodium acetate are potent preservatives with better function and this enhanced the pleasant odor, lipid and microbial safety essential for maintaining the sensory attributes of fishery products.

The mean sensory scores of patties obtained from catfish as an alternate product in the present study, reveal that starting from the 6th days of refrigerated storage odor scores were significantly higher (p<0.05) in marjoram-treated batch than SA treated patties up to the 12th day. The use of marjoram herb, on the other hand, improved the sensory quality of fish patties due to its antimicrobial, antioxidant and flavoring properties. Similar results have been reported in the other muscle passed products treated with PML[27].

Results depicted in Table 3 also reveal that the SA-treated patties rated higher scores for appearance and taste as compared to PML alone and control samples for up to 14 days. This confirms its color and flavor stability characteristic[16]. This study results are in agreement with the findings of Sallam[22] and Ehsani *et al.*[15] in their frameworks of fish products pretreated with acetate either alone or in combination with herb oil. However, the sensorial scores decreased significantly (p<0.05) in all groups by increasing refrigerated storage time, probably due to microbial effect, oxidation of lipid and degradation of protein in the patties. Similar to these findings, various studies reported significant reductions in the sensory scores during the refrigerated storage process of different fish products[51,15,14,6,7].

The data depicted in Table 3 clearly show that appearance scores of control catfish patties were significantly lower (p<0.05), likely due to a brownish and watery appearance. In contrast PML+SA-treated samples exhibited the highest sensory scores throughout the cold storage. This fact confirms the synergistic effect of sodium acetate and marjoram herb. Based on sensory scores catfish patties exhibited shelf lives of about 6-7, 12, 14 and 16 days of refrigerated storage (4±1°C) for the control, 1% PML-, 2% SA- and PML+SA-treated patties samples, respectively. These results confirmed the findings reached by other researchers[22,15,51,6] who concluded that the shelf life of the treated fish products was extended by more 4-9 days than that of the control. The obtained sensory data were confirmed by the chemical and microbiological evaluations (Table 2 and Fig. 2).

It is important to state that it would be economically more suitable to use powdered spices or herbs as ingredients rather than their extracts or Essential Oils (EOs) to preserve food including catfish patties[30,31]. In the seafood sector such preservative compounds including spices/herbs were equally used to extend the shelf-life of fish products but at low levels to avoid strong flavors imparting unpleasant sensorial characteristics. One percent Powdered Marjoram Leaves (PML, 1%) used in the present study gave a pleasant odor to the treated catfish patties samples, being organoleptically desirable and pleasant until the 12th day of storage as judged by the sensory panel.

The use of higher concentrations than we used in the present study may result in a further increase of the shelf life of fish patties but high EO concentrations would probably impart unpleasant sensory effects (strong odor and flavor, etc.) on the quality of fish patties. Powdered Marjoram Leaves (PML) either alone or in combination with organic preservative (SA) can be used as an easily accessible source of natural antimicrobial and antioxidants in commercial food products as they have better consumer acceptance.

CONCLUSION

According to the results achieved, sodium acetate (SA; 2%) treatment was determined more effective than Powdered Marjoram Leaves (PML; 1%) application for maintaining microbiological quality and extending the shelf-life of catfish patties. Whereas, PML applications were more effective in enhancing the oxidative stability and sensory scores as supported by the results of microbiological, chemical indices and sensorial analyses. Due to concerns regarding the safety and toxicity of synthetic preservatives, combined pretreatment (PML+SA) may prove useful as safe, natural application to fish industry.

SIGNIFICANCE STATEMENTS

This study discovers the possible synergistic effect between Powdered Marjoram Leaves (PML; 1%) and Sodium Acetate (SA; 2%) combination that can be beneficial for successful reduction of lipid oxidation and microbial growth in catfish patties stored at 4 ± 1°C. The present results could show 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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