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EFFECT OF THYME EXTRACT AS A NATURAL PRESERVATIVE ON THE QUALITY OF SMOKED MACKEREL FISH

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ABSTRACT

Fresh and partially processed fish are subject to spoilage either by microbes or by fat oxidation, which gives a great attention to fish preservatives, especially natural preservatives, a great interest as alternatives to chemical preservatives. In this study, thyme extract has been used along with smoking and soaking fish fillets in brine solutions as an alternative method of preservation. Fish fillet samples were evaluated for their chemical composition (moisture, pH and protein contents), total bacterial count, and the numbers of isolated *Salmonella* spp., *Escherichia coli*, *S. aureus,* and *Pseudomonas* spp. The obtained results showed a decrease in pH values and moisture contents and an increase in protein contents in smoked fish fillet soaked in brine solution alone without thyme extract or with the addition of thyme extract. The thyme extract showed a high reduction rate in *Salmonella* spp., *E-coli* and *Pseudomonas* spp numbers in comparison with the control and the fish fillet samples soaked in brine solution. On the other hand, *S. aureus* showed high resistance rate. The results indicated that, thyme could be added to the brine solution used for the preparation of smoked fish as an antimicrobial agent.

Key words: Mackerel fish smoking, antimicrobial, thyme, quality.

INTRODUCTION

Fish is a good source of high quality proteins that constitutes about 14% of animal protein needs of human around the world **(Abolagba and Melle, 2008**). Also, it was reported as one of the main sources of vitamins and minerals and was cleared as essential nutrients in infant and adult daily food **(Abdullahi *et al.*, 2001)**.

Mackerel Fish is one of the healthiest and cheapest sources of protein; it contains 21.34% protein and 63.36% moisture **(Oyelese, 2008)**. This much of water encourage the spoilage of fish by microorganisms. Additionally, the presence of enzymes in fish accelerates fat auto-oxidation of fish meat tissues and leads to quality deterioration and spoilage **(Desrosier, 1977)**. Muscles of healthy animals including fish do not contain microorganisms and contamination always happen during slaughtering, handling, processing and transportation **(Ercolini *et al.*, 2006).** Fish and their products, in respect of pathogen content, natural toxins and possible contaminants is considered a high source of hazard **(Gram, 1993)**.

Clostridium botulinum, *Mycobacteium* spp, *Streptococcus* spp., *Vibrio* spp., *Aeromonas* spp., *Salmonella* spp., *Pseudomonas* spp, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus* spp, yeasts and moulds are among the most important microorganisms that contribute to the biological hazards in fish either raw or treated or even processed **(Lipp and Rose, 1997; Nishimori *et al.*, 2000; Okonta and Ekelemu, 2005; Adebayo *et al.*, 2012; Al-**

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Jasser and Al-Jasass, 2014). *E-coli* is naturally found in fish gills, skin, intestine and muscles but it is unable to neither grow nor cause spoilage **(Yagoub, 2009)**.

The contamination with *Salmonella* spp is considered a low risk infection **(Heinitz and Johnson, 1998)** and never found in smoked sardine **(Nyarko *et al.,* 2011)**. Fish products are preserved using many methods including chilling, freezing, caning, smoking, drying, radiation and others, in addition to some chemical preservatives as sodium benzoate, citric acid, sorbate and others used to extend its shelf-life **(Espejo-Hermes, 1998)**. Fish smoking is an old food preservation method, widely used in fish processing, and smoked fish represents 15% of the total European fish market **(Stolyhwo and Sikorski, 2005)**.

Generally speaking, smoking is used to improve the consumers' acceptability including all characteristics such as taste and odor and extends the shelf-life, inhibit oxidative reactions, lower pH, and acts as spoilage agents antagonist **(Sengor *et al.,* 2004).**Three different methods are used to smoke fish; cold smoking, hot smoking and liquid smoking **(Goulas and Kontominas, 2005)**.

Using salting with smoking in fish preservation prevent or reduce the postharvest losses, remove water, reduce water activity and inhibits both bacterial growth and enzymatic activities **(Mustafa *et al.,* 2009; Kumolu-Johnson *et al.,* 2010)**. Salting, brining and the type of wood used for the smoking fire are all factors affect the smoked fish quality and reduces the microbial spoilage for up to 6 months **(Omojowo *et al.*, 2008)**.

Herbal plants are rich in antioxidants and some of them have antimicrobial effects that help in preserving food against oxidation and microbial growth **(Sampels *et al*., 2010).** Thyme, garlic, lemongrass and cinnamon showed an antimicrobial activity and are used to preserve Tilapia and other fish types **(Alsaid *et al*., 2010).** Ginger extract was used against bacteria in smoked mackerel fish during chilled storage **(Iheagwara, 2013)** and Coriander seeds and garlic showed an antibacterial activity against *Salmonella typhi* **(Belguith *et al.*, 2009; Matasyoh *et al*., 2009**). In one study, Berry Marinades prolonged the shelf-life of Herring Fillets fish and that might be due to the oxidation inhibition effect of the herb **(Sampels *et al.,* 2010)**. On the other hand, the treatments of mackerel fish using pomegranate peels and green tea extracts inhibited the microbial growth and biochemical attributes during ice storage **(Shinde *et al.,* 2015)**.

Herbs not only prevent microbial growth but also prevent lipid oxidation; Marjoram oil improved the peroxide value of lipids extracted from frozen beef during storage at -18°C **(Shelbaya *et al.,* 2014).** Rosemary, thyme, sage and other plants are used to control lipid oxidation in fish meat, as reflected in thiobarbituric acid (TBA) reactive substance values **(Yu *et al.,* 2002)**.

Thyme enhanced the chemical and microbiological properties of fresh rainbow trout fillets during storage under refrigeration conditions **(Angis and Oguzhan, 2013)**. It was used to help preserving smoked rainbow trout by inhibiting the growth of any available microorganism **(Erkan, 2012)**.

Ethanolic extracts of thyme, thyme essential oil, and thymol showed inhibition against *Salmonella sonnei, E. coli, Shigella* spp; *Pseudomonas* spp; *Streptococcus* spp. and *Staphylococcus aureus* **(Cosentino *et al.*, 1999; Fan and Chen, 2001; Yasar *et al*., 2005; Mohammad and Ali, 2006; Akrayi and Abdulrahman, 2013; Al-Mohana and Al-Hussein, 2014**). Thyme extract was able to maintain acceptable microbial limit which indicates that it is a good additive to extend the shelf life of refrigerated fish **(Corbo *et al*., 2008)**.

The phenolic components of thyme are reported to play a big role as antibacterial and antioxidant in rainbow trout fillets **(Mexis *et al*., 2009)**. **Ilhak and Guran, (2014**) used thymol and sodium lactate to reduce the growth of *Salmonella typhi* in

fish patty. Thymol and carvacrol rich plants have a significant antioxidant effect on the process of the lard oxidation **(Tsimidou and Boskou, 1994)**.

The present study investigated the possible use of thyme as a natural preservative and antibacterial agent in mackerel fish due to its antimicrobial activity and its lipid oxidation inhibition properties. Integrated effects of light salting, thyme extracts (7%) and smoking on chemical analysis (pH-value, moisture and protein contents) and growth rate of *Staphylococcus aureus, Salmonella* spp., *E. coli,* and *Pseudomonas* spp. in processed mackerel fish were analyzed.

MATERIALS AND METHODS

Preparation of Thyme Aqueous Extract

Aqueous extract of thyme was prepared according to the method described by **Vaishnavi *et al.*, (2007)**. Thyme leaves was dried at shade, crushed to a coarse powder in a mechanical blender, a 100g of powder were extracted in500 ml of distilled water at room temperature for 24 hours. Mixture was then filtered using Whatman No1 filter paper and the filtrate was separated in glass dishes and dried in a vacuum oven at 40°C for 48 hours.

A 7% thyme in brine solution (5% salt) was prepared for the salting process by the addition of 70g/L thyme powder in brine water.

Brining and Smoking Processes

Fish samples (90 fish) with average weigh 200-250g were selected. Fish were carefully gutted, dressed, filleted by hand and washed with tap water. Fillets were divided into 3 groups, control (thawed than frozen mackerel without brine or extract added brine treatment), BS (soaked in brine solution 5% only), and BS + TE (soaked in brine solution with the addition of 7% thyme powder for 30min at 4^{o}C. Ratio of brine to fish was 2:1 (w: w) as shown in Table (1). Wood sawdust (white wood and some other wood types), purchased from carpentry shops, was used to smoke fish for 2 hours and products were packed in transparent polyethylene bags and sealed with a sealing machine to reduce microbial contamination **(Salán *et al.*, 2006)**.

Chemical composition of fillet

Crude protein and moisture contents of fish samples were determined according to the official methods **(AOAC, 2002).** For pH determination, 10 gm of fillet samples were homogenized with 100 mL distilled water for 1 min, and the pH values of the slurry were measured at room temperature (**Hayes *et al.*, 2010)** using pH meter Suntex-T-s-l 911005942/ Taiwan) with calibrated probe type (Ingold 406-M6-DXk-S7/25).

Microbiological Analysis

Total Plate Count (TPC)

10 grams of fish sample are transferred to a stomacher bag and 90ml of sterile Salt Peptone Solution (SPS) as diluents was added (20g peptone/1000ml distilled water). The sample was homogenized in a stomacher (Seward 400 Stomacher Lab Blender /Stock 36001) for 90 seconds to obtain 1:10 (10^{-1}) dilution. Serial dilutions (10^{-2} to 10^{-5}) of the homogenized samples were made using sterile distilled water.100µLof each dilution was placed on the surface of media. Plates were incubated at 37^{o}C for 18-24 h. Viable count was calculated as colony forming unit CFU/g sample **(ICMSF, 1978)**.

Table (1): Preparation of samples and treatments.

Sample	Soaking in brine solution 5%	Thyme extract addition 7%
Control	-	-
BS	Soaked for 30 minutes	No addition
BS + TE	Soaked for 30 minutes	Added

BS = soaked in brine solution, BS + TE= soaked in brine solution with thyme extract added.

Isolation and Enumeration of Pathogenic Bacteria

Baird-Parker Agar (BPA) medium was used to isolate and enumerate *Staphylococcus.* Diluted samples were plated and incubated as previously described for 24-48 h at 37°C. Colonies of *S. aureus* appeared as black colonies with clear to opaque zones **(ISO, 1999**).Xylose Lysine Desoxycholate Agar (XLD) was used to isolate and enumerate *Salmonella*, pink colonies with or without black center showed the presence of Eosin Methylene Blue *Salmonella* spp.

(ISO, 1993). Agar (EMP) was used for the identification and enumeration of *E. coli*. Colonies with green metallic sheen indicate the presence of *Escherichia coli* **(FAD, 2001)**. Pseudomonas Agar Base (PAB) was used to isolate and enumerate *Pseudomonas* in samples.

Samples are diluted and plated as previously described and Plates were incubated for24-48 h at 25-37°C. Blue-green or brown pigmentation colonies were counted as *Pseudomonas* **(FAD, 2001)**.

The bacterial isolates were characterized based on microscopic examinations and Gram staining reactions according to the methods described by **Fawole and Osho, (2002)**, as well as appropriate biochemical tests, example Kligler's Iron Agar (KIA) test, Indole production test, Methyl Red (MR) test, Vogues-Proskauer test, Citrate utilization test and carbohydrate fermentation test **(Oyeleke and Manga, 2008)**.

Statistical Analysis

Statistical analyses are performed using SPSS V.15.0 for Windows. Analysis of Bergius (ANOVA) was used at $p<0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Prepared Smoked Mackerel Fillet

Data in Table (2) presents the chemical composition of the prepared fish fillet without treatments (control) and smoked fish fillet treated with 5% brine solution (BS) without thyme extract (TE) and treated with 5% brine solution with 7% thyme extract.

The moisture contents of mackerel fillets showed that, the control sample of mackerel fillet had high moisture content (67.13%) compared to other treatments.

The smoked mackerel fillet with 5% brine solution (BS treatment) showed less moisture content (58.11%) compared to the control. However, the moisture content of mackerel samples treated with 5% brine solution and 7% thyme extract was lower than that of the other 2 samples (56.70%).

The high moisture content of the fillet treated with brine solution might be due to the presence of NaCl in solution, which cause an osmotic pressure that lead to the loss of water from low salt concentration in the fillet to the high salt concentration in the liquid.

Smoking itself increase the loss of water from the fish body due to the high temperature which led to moisture vaporization from fish fillet surfaces. Similar results have been reported before by **Cho *et al.*, (2014)** who stated that, smoked fish fillet showed lower moisture contents compare to fresh fillet. **Huong (2014)** also showed a decrease in moisture content after soaking in brine 10% NaCl and smoking by wood in fillets samples.

From data presented in Table (2), it could be noticed that smoking with soaking of fish in BS alone or with the addition of TE reduced the pH values of mackerel fillet.

The maximum pH value was observed in control fillet samples with a score of 6.36 while the treated fillet samples with BS and BS with TE showed lower pH values (6.29 and 6.25 respectively). These findings were similar to the published results by **Adeyemi *et al.* (2013)** who attributed the decrease in the pH values to the smoking process and also in line with the findings of **Tsai *et al.* (2005)** who indicated that pH values of fresh mackerel were higher than the pH values of the treated fish when 8% brine solution was used.

Table (2): Chemical composition of mackerel fish with and without treatments (soaking in brine alone or with thyme extract).

Sample	Moisture (%)	pH values	Protein (%)
Control	67.13a ± 0.727*	6.36a ± 0.095	18.60c ± 0.690
BS	58.11b ± 0.613	6.29b ± 0.074	20.78b ± 0.577
BS + TE	56.70c ± 0.605	6.25c ± 0.070	21.65a ± 0.815

The low decrease in pH values may be attributed to the effect of natural antioxidant of thyme that reduce or inhibit the oxidation of lipids, antimicrobial effects of salt and of thyme, and low moisture contents in mackerel fish due to smoking treatments.

The maximum protein content (21.65%) was noticed with smoked mackerel fish treated with TE and BS. The smoked fillet samples soaked in brine solution only (treatment BS) contained 20.78% of protein, and both treatments were quite high compare to the control sample which had lower protein content (18.60%).

This high protein content might be due to the reduction of moisture during smoking, which increased the relative percentage of protein contents. Higher protein contents may also due to the effects of the salt (5%) preservatives effect which slow down autolysis in fish muscles and slow down the protein break down.

Higher protein contents noted in the brine and thyme addition was also due to the antimicrobial effect of phenolic components in thyme extract as carvone, thymol and carvacrol which may inhibit the enzyme activity and protect protein from degradation **(Ayala-Zavala *et al.*, 2007)**. These findings were in accordance with those reported by **Koru *et al.* (2007)** and **Omojowo *et al.* (2010)** who observed an increase in protein contents in fish fillet after soaking in brine and smoking processes.

Total Plate count of Mackerel Fillet

Changes in total bacteria count (TBC) of bacteria was determined by aerobic plate count and measured as $\log_{10}$ CFU/g (Table 3).

Effects of smoking and soaking in brine solution alone or with the addition of thyme extract on total bacterial count of mackerel fish fillet compared to control are shown in Figure (1). Soaked mackerel fillet in brine solution with and without thyme extract decreased the total microbial count of fillet samples compared to the control.

Total microbial count measured by aerobic plate count method showed that, control mackerel fillet contained higher number of microbes (4.39 Log_{10} CFU/g) compared to samples of soaked mackerel fillet in brine solution with and without thyme extract (3.47 and 3.69 Log_{10} CFU/g).

The reduction of microbial count in the smoked fish fillet without and with the addition of thyme extract might be due to the protective effects of salt against microbes, lower moisture contents with the application of smoking, and/or the high antioxidant activity of thyme which inhibits the growth of microbes. Similar results were obtained by **Degnon *et al.* (2014**) and **Abolagba and Igbinevbo (2010),** they reported that, salting and smoking reduced the total bacteria count in all fish samples.

Isolation and Enumeration of Pathogenic Bacteria

Table (4) shows the numbers of the isolated foodborne and/or pathogenic bacteria. The bacterial numbers were decreased as the bacteria showed sensitivity to our treatments. Fish smoking and soaking in brine solution without and with thyme extracts showed less numbers of isolated *Salmonella* spp., *E. coli*, *S. aureus*, and *Pseudomonas* spp. compare to the control which indicated that thyme extract has an antimicrobial effect and it was able to reduce the growth rate of these foodborne bacteria.

Table (3): Total microbial count of mackerel fish with and without thyme addition.

Sample	Aerobic plate count Log 10 CFU/g
Control	4.39a ± 0.417
BS	3.69 b ± 0.293
BS + TE	3.47c ± 0.398

Control = thawed mackerel, filleted, then frozen, BS= soaked in brine solution (5%), BS + TE= soaked in brine solution with thyme extract (7%).

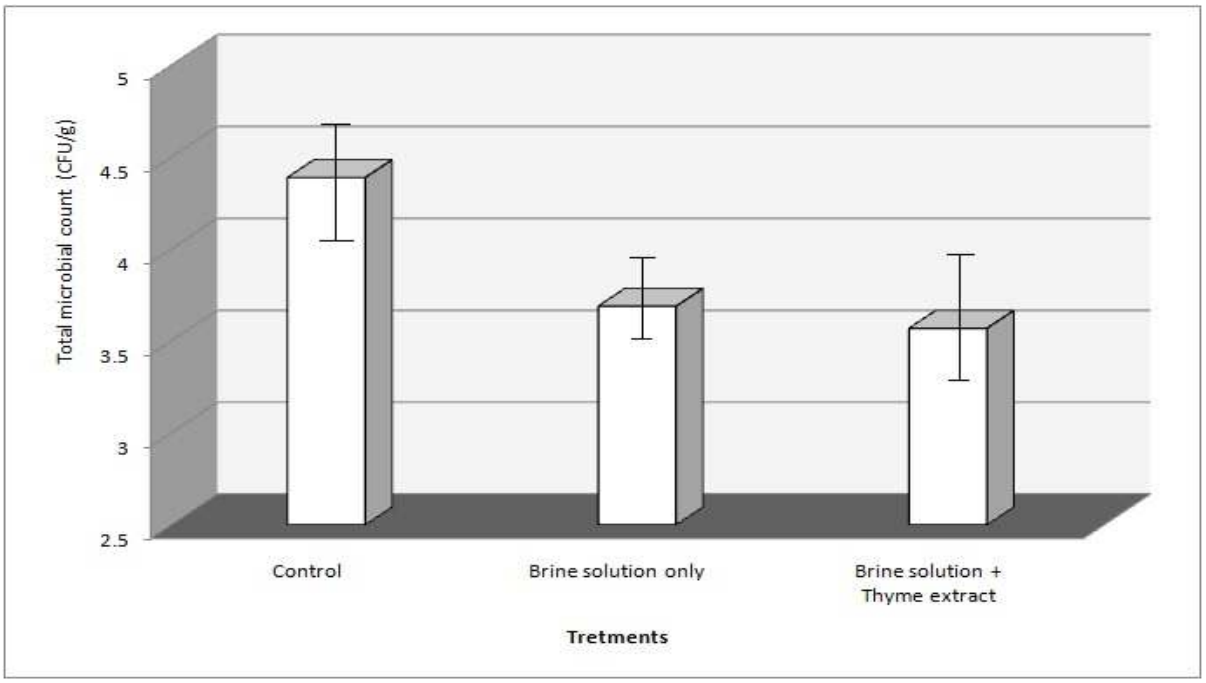

Figure (1): Total bacterial count of mackerel fillet samples.

Table (4): Number of the tested pathogenic bacteria in mackerel fillets.

Sample	Bacterial count (CFU/log_{10})			
	***Salmonella* spp.**	***E. coli***	***S. aureus***	***Pseudomonas* spp.**
Control	2.61a ± 0.387	2.89a ± 0.261	3.03a ± 0.257	2.82a ± 0.390
BS	1.84b ± 0.480	2.05b ± 0.232	2.48b ± 0.233	2.00b ± 0.360
BS + TE	1.02 c ± 0.20	1.87c ± 0.367	2.31c ± 0.473	1.70c ± 0.318

The control samples were positive to *Salmonella* spp. with average number 2.61 $\log_{10}$ CFU/g. The treated samples with brine solution and thyme extract showed lower numbers of *Salmonella* spp. (1.84 and 1.02 $\log_{10}$ CFU/g, respectively) compared to control samples. It is clear that, thyme extract with salt and smoking process reduced the numbers of *Salmonella*, significantly ($p < 0.05$) compared to the control.

These results are similar to those reported by **Diez (2012**) and **Olagunju *et al.* (2012)** who reported a decrease in *Salmonella* spp. counts when fish samples were smoked and they attributed that to the low moisture content due to the smoking process. The shelf-life of smoked fish depends on the time and temperature of heating along the manufacture process, on the decrease in water activity and on the antibacterial activity of smoke components as well as the concentration of the smoke may influence its antimicrobial effect **(Fretheim *et al.*, 1980)**.

This result indicated that *Salmonella* spp numbers were decreased after smoking and this may be due to the active phenolic compounds in wood and heat temperature of smoke that destroy or inactivate S*almonella* spp and reduce spoilage caused by enzymatic processes and microbial activity **Mailoa *et al.* (2013)**.

In present study, *Salmonella* spp. was positive in samples before and after treatment with smoking and this may be a cross contamination during all stages of fish production, handling and processing.

These results are similar to the results obtained by **Abbas (2014)** who showed that *Salmonella* spp. was positive in sample of raw and smoked fish. In mackarel, *Salmonella* spp. was isolated from fish samples before and after smoking **(Akinjogunla *et al.*, 2011)**.

The data in Table (4) show that thyme extract has a strong inhibitory effect on *Salmonella* spp. This reduction may be due to the effect of thyme extract which consists of high phenolic compounds (chavicol, eugenol, estragole carvone) that reduced the bacterial growth according to **Del Nobile *et al.* (2009)** who reported similar results with *Salmonella typhi* in mackerel fish during storage at 4°C. The smoked filets, showed lower *Salmonella* count after treatment with 5% rosemary extract in comparison to control **(Da Silva, 2002)**.

E. coli has been isolated before from fish samples such as *Tilapia zilli, Hemisynodontis membranacea, Clupea harengus* and *Scomber scombrus* (**Olagunju *et al.*, 2012; Elhadi and Alsamman, 2015)**.

Similar trends of results have been observed with both *E. coli* and *Pseudomonas* spp. A reduction in the growth of *E. coli* from 2.89 Log_{10} CFU/g in control fillet samples to 2.05 Log_{10} CFU/g in BS treatment may be due to the high salt concentration in the brine solution which may cause plasmolysis. Further reduction in *E. coli* numbers was observed in the BS and TE treatments where only 1.87 Log_{10} CFU/g was recorded.

The results indicated that the thyme extract that has been added to the brine solution had the best effect on reducing the *E. coli* numbers. Similar results indicated that pomegranate peels extracts (0.5% and 1%) and green tea extracts (3% and 5%), in addition to clove essential oil applied with smoking process successfully inhibited the growth of *E. coli* in mackerel fish (**Han *et al.*, 2000**; **Degnon *et al.*, 2014 and Shinde *et al.*, 2015)**.

A similar trend of reduction was noticed with *Pseudomonas* bacteria. Control sample had the highest numbers of *Pseudomonas* spp. (2.82 $\log_{10}$ CFU/g).

While the soaking treatment with brine solution and smoking reduced the numbers of *Pseudomonas* spp. in comparison with the control samples (2.0 Log_{10} CFU/g). Similar results were obtained by **Hassan *et al.* (2014)**, who found that, *Pseudomonas* numbers were reduced by washing fillet by

brine 1% NaCl. **Karabagias *et al.* (2011)** also found that, thyme herb was highly effective to reduce the numbers of *Pseudomonas* spp. in lamb meat at air-packaged conditions. On the other hand, *Staphylococcus aureus* showed a resistance pattern to both treatments in comparison to the control since we did not observe any reduction after both treatments.

The negative antimicrobial effect of thyme and brine solution may be because *S. aureus* is a Gram positive bacteria that have a thick peptidoglyc layers that may inhibit or reduce the penetration rate of the extract through the bacterial cell wall.

The tolerance rate of *S. aureus* may be higher than that of Gram negative bacteria. **Vishwanath *et al.* (1998)** reported that, *S. aureus* could grow well in low water activity and under saline conditions. From results of isolated pathogenic bacteria and the inhibition percentages of BS (brine solution alone with smoking) or BS+TE (brine solution with thyme extract and smoking) (Table 5).

From the results, it could be concluded that all tested bacterias except *S. aureus* showed 29.07-29.5% inhibition after treating mackerel fillet with brine only and smoking. The addition of thyme increased the inhibition percentages to reach 35.29-60.92%, with minimum inhibition against *S. aureus* (23.76%). The high rate of inhibition of the isolated pathogenic bacteria in case of the addition of thyme extract to the brine solution (BS+TE) might be due to its high antioxidant activity. **Tornuk *et al.*, (2011)** found that, thymol and carvacrol (main compounds in thyme) and thymol hydrosols affected the microbial growth in mackerel fish.

Thyme was effective against bacterial in hot smoked fish products when compared to the untreated fish fillet **(Erkan, 2012**).Additionally, the aqueous extracts of thyme were found to be effective in inhibiting the growth of bacteria **(Akrayi and Abdulrahman, 2013; Mehanna *et al.*, 2013)**. **Abdollahzadeh *et al.*, (2014)** used thyme essential oil in minced fish, and the results showed that, thyme had a strong antibacterial activity during storage.

Conclusion

In this study, thyme was used as a food additive to inhibit the foodborne bacteria in fish. The results indicated that soaking mackerel fish fillet in brine solution and brine solution with thyme extract managed to inhibit the growth of some pathogens including *Salmonella* spp., *E. coli, S.aureus and Pseudomonas* spp.

The total microbial count in fish fillet soaked in the added thyme to the brine solution or brine solutions alone were lower than that of control samples.

Moisture contents and pH values decreased while protein contents increased with the application of brine solution alone or with thyme extract, before getting through smoking process. The results encourage the use of thyme as a food additive to reduce the risk of pathogens in fish.

Table (5): Reduction rate of the tested pathogenic bacteria after treatments.

Samples	Bacterial count (%)			
	***Salmonella* spp.**	***E. coli***	***S. aureus***	***Pseudomonas* spp.**
BS	29.50	29.07	18.15	29.08
BS + TE	60.92	35.29	23.76	39.72

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الملخص العربي

تأثيـــر مستخلص الـــزعتـــر كمـــادة حافظـــة طبيعيـــة علـــى جـــودة ســمك المكــاريل المدخــن

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أجريت هذه الدراسة لتقيم تغيرات الجودة الكيميائية والبكتيريولوجيه لسمك المكاريل المنقوع في محلول ملحي والمستخلص المائي للزعتر مع إجراء معاملة التدخين كطريقة بديلة لحفظ شرائح سمك المكاريل. حيث تم عزل أربع أنواع بكتيرية من سمك المكاريل وهى السالمونيلا والاستاف اوريس والاى كولاى والبسيدوموناس ومن ثم إجراء ثلاث معاملات لهذه الأسماك وهى: كنترول (بدون إضافات)- الغمر بمحلول ملحى ٥% فقط - الغمر بمحلول ملحى ٥% + مستخلص الزعتر ٧% بنسب وزنيه (١:١) وغمرها لمدة ٣٠ دقيقة على درجة حرارة الغرفة ومن ثم إجراء معاملة التدخين لمدة ساعتين لجميع المعاملات وذلك لمعرفة مدى تأثير استخدام مستخلص الزعتر على التغيرات في الخصائص الكيميائية والبكتيريولوجية. وأوضحت النتائج المتحصل عليها أن معاملة مستخلص الزعتر بتركيز ٧% كان لها تأثير كبير في تقليل العدد الكلى للبكتيريا وتثبيط الأنواع البكتيرية المعزولة خاصة السالمونيلا والاى كولاى والاستاف اوريس مقارنة بمعاملة المحلول المحلى فقط والكنترول. وقد لوحظ أيضاً أن الاستاف اوريس كانت أكثر الانواع البكتيرية تحت الدراسة مقاومة لمستخلص الزعتر. كما وجد أن لمستخلص الزعتر أهمية كبيرة في التأثير على الخصائص الكيميائية لسمك المكاريل من خلال انخفاض رقم الحموضة والرطوبة مع زيادة بسيطة بالبروتين مقارنة بالكنترول وعلى ذلك يمكن التوصية باستخدام مستخلص الزعتر خلال عملية إعداد وتصنيع الأسماك كمادة طبيعية حافظة تتميز بأن لها خصائص مضادة للأكسدة وللميكروبات وبالتالي الحفاظ على جودة ومدة صلاحية هذه الأسماك.

الكلمات الإسترشادية: مستخلص الزعتر، المادة الحافظة الطبيعية، جودة، سمك المكاريل المدخن.