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Use of Some Essential Oils as Antimicrobial Agents to Control Pathogenic Bacteria in Beef Burger

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Abstract: Meat and meat products such as beef burger are subject to spoilage either by microbes or by fat oxidation. Accordingly, it is very important to protect them from spoilage by adding preservatives, especially natural preservatives to extend their shelf life and to improve their characteristics. The aim of this study was to evaluate the antioxidant activity and antibacterial effect of three different essential oils (thyme, cumin and parsley) at three different concentrations (0.3, 0.6 and 1.2 %) against *Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa* and *Escherichia coli* artificially inoculated in beef burger stored at 4±1°C for 4 days. Our results indicated that the high concentration (1.2 %) of essential oils reduced the values of pH, thiobarbituric acid (TBA) and total volatile nitrogen (TVN) in comparison to the control samples. The results of antimicrobial effects of those essential oils were variable. It has been observed that beef burger treated with parsley and thyme oil showed a better reduction effect than cumin on the bacterial count during storage at refrigeration temperature. The high concentrations of essential oils (0.6 and 1.2 %) had the highest inhibitory effect in comparison to the low concentration (0.3 %) with most bacteria during the first period of cold storage. The lowest inhibitory effect was observed with *P. aeruginosa*. These observations demonstrated that essential oils exhibited variable antibacterial activity against tested bacterial strains. In conclusion, the inhibitory effect of used essential oils could affect positively the quality of beef burger and extends its shelf-life.

Key words: Beef burger · Essential oils · Antioxidant and antibacterial properties · Pathogenic bacteria

INTRODUCTION

Meat and its products are highly perishable and can spoil very easily if they are not store properly. Once they spoiled, they become unhealthy to eat due to microbial growth and chemical changes by enzymes [1]. In general, lipid oxidation is considered one of the most important causes of quality deterioration in meat and it produces the off flavors and odors, in addition to the increase of the drip losses, loss of pigment and as a result decrease the consumer acceptability [2]. This deterioration is mainly due to the contamination of meat and its products during production, handling and consumption [3]. In addition to the cross contamination during processing, poor personal hygiene and inappropriate holding temperature also contribute to the contamination of ready to eat foods [4]. Because meat is very rich with protein, it is highly susceptible to microbial growth, which can cause its spoilage and contributes to food borne diseases in human, resulting in serious health problems [5]. The most important pathogenic bacteria associated with meat products are *Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Clostridium botulinum, Escherichia coli* O157:H7, *Pseudomonas, Acinetobacter, Enterobacter, Lactobacillus* spp., *Leuconostoc* spp. and *Proteus* spp. [6, 7].

It is expected that the growth of most bacteria in meat can be inhibited or completely stop if meat products are preserved by chilling and freezing [8] which keep the original characteristics of meat products [9]. Other methods such as the addition of sodium nitrite, potassium nitrite, sodium nitrate and potassium nitrate can be used to persevere the cured meat and poultry [10]. Sodium

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nitrite preserves the unique colors, textures and flavors of meat and poultry products which cannot be maintained by any other additives [11]. However, such chemical compounds can have bad effects on human health [12]. Meat preservation methods became a necessary to transport meat for long distances without spoiling and loss of nutritional value [13].

Essential oils and their extracts can be used as natural additives to reduce the use of chemical preservatives and reduce their risks. Such materials can extend the shelf life of meat and their products and control/inhibit the microbial growth [14]. Many of the essential oils have potential benefits in food production since they showed antibacterial, antifungal and antioxidant effects [15]. The essential oils are good sources of natural antioxidants such as phenolic, flavonoids, alkaloids, tannins and phenolic acids [16] and the TBA value is routinely used as an index of lipid oxidation in meat products [17]. The essential oils have different compounds with antibacterial activities such as; geraniol, menthol, cinnamyl alcohol, linalool, citronellol, carvacrol, cinnamaldehyde, eugenol, thymol, estragole, carvone and chavicol [18]. Different essential oils of different plants showed an effective antimicrobial effects on bacterial count when they are applied in meat products during storage such as; coriander [19], ginger and basil oil [20], rosemary, sage and thyme [21], grape seed extract and pine bark extract [22] and garlic and lemon grass oils [23].

Other plants and seeds such as cumin (*Cuminumcyminum*) are very rich with natural compounds such ascuminaldehyde, limonene and linalool that have both antimicrobial and antioxidant activity [24-26]. The extracted essential oils of cumin showed an antimicrobial effect against *E. coli, Salmonella, Pseudomonas aeruginosa, Staphylococcus aureus* and *L. monocytogenes* [27-30].

Thyme (*Thymus Vulgaris*) is also a good source of phenolic compounds as g-terpinene, 1, 8-cineole, thymol and eugenol that have antimicrobial effect on pathogenic bacteria [31]. The essential oils of thyme successfully inhibited the growth of *E. coli, Salmonella, Pseudomonas aeruginosa* and *Staphylococcus aureus* [32-35]. Thyme oil has also an excellent antioxidant activity when it is added to minced beef during storage and its high value decreases the values of the pH, TVN and TBA [23].

Parsley (*Petroselinum crispum*) is another herb which is very rich in vitamins, thiamin, carotene, organic minerals and phenolic compounds such as Cosmene, Limonene, Myristicin, α-Pinene and β-Pinene [36 & 37]. In addition to those organic compounds, parsley contains other compounds such as plus oleic, linoleic, palmatic and other fatty acids [38] that showed antimicrobial effects against *Salmonella, E. coli* and *Staphylococcus aureus* [37, 39 & 40].

In this study we investigated the possible antimicrobial and antioxidant effects of essential oils as natural preservatives in beef burger during storage at 4 °C. Chemical properties such as pH-value, TBA -value and TVN-value in addition to the inhibitory effects of those oils on the growth of bacterial pathogens such as *S. aureus, S. enterica, E. coli* and *P. aeruginosa* in raw beef burger were investigated during storage at 4±1 °C for 5 days.

MATERIALS AND METHODS

Extraction of Essential Oils: The extraction of thyme, cumin and parsley essential oils was achieved by the hydro-distillation method proposed by Busatta *et al.* [41]. The dried leaves and seeds of the plants (100 g) were hydro-distilled for 3 h using a Clevenger type apparatus. Then, the essential oil was dried with anhydrous sodium sulfate and kept in sealed vials at 4°C. The extracted oils from thyme, cumin and parsley were added to beef burger at three different concentrations; 0.3, 0.6 and 1.2 % (v/w).

Preparation of Beef Burger: About 21.8 Kg of beef burger (Minced meat 70 %, fat 15 %, starch 3.5 %, cold water 10 % and salt 1.5 %) are directly transported to the laboratory in an ice box. All burger pieces were divided to 5 different groups. One group was left without any bacterial inoculation while the other 4 groups were artificially inoculated with 4 different bacterial strains (*Staphylococcus aureus* ATCC 6538, *Salmonella enterica* ATCC 25566, *E. coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 10145). Each of those 5 groups was divided into treated and untreated groups (control). The treated groups were treated with 0.3, 0.6 and 1.2 % (v/w) concentrations of each essential oil (thyme, cumin and parsley). Each burger piece (~35 g) was formed and placed on plastic foam meat trays, wrapped with polyethylene film and stored for 4 days at 4°C.

Chemical Examination: Chemical analyses were performed according to standardized methods. Ten grams of beef samples were homogenized with 100 mL distilled water for 1 min. The mixture was used to measure the pH value at room temperature by using pH Suntex meter [42]. Total volatile nitrogen (TVN) was measured according to the technique recommended by FAO [43]. Thiobarbituric

acid (TBA)-value (mg malonaldehyde (mal)/ kg) was estimated by distillation technique using 2- thiobarbituric acid 0.02 M as described by Vyncke [44].

Bacteriological Examination: Samples were homogenized and serial dilutions (10^{-4} to 10^{-8}) were prepared following the recommendation of ICMSF [45]. Determination of Total Bacterial Count (TBC) was performed according to ICMSF [46]. Mannitol Salt Agar (MSA) medium was used to isolate *Staphylococcus aureus* (ATCC 6538) from inoculated samples. Plates were incubated for 18-24 h at 37 °C and colonies of *S. aureus* were identified as yellow colonies and may have a yellow halo around the colony [47]. Xylose Lysine Deoxycholate agar (XLD) was used as a selective medium to isolate *Salmonella enterica* (ATCC 25566). Inoculated plates were incubated at 37 °C for 18-24h and pink colonies with or without black center indicate the presence of *Salmonella* species [48]. Eosin Methylene Blue agar (EMP) was used for the identification of *E. coli* (ATCC 8739). Plates were incubated at 37 °C for 18-24 h and colonies with green metallic sheen indicate the presence of *Escherichia coli* [49]. *Pseudomonas* Cetrimide Agar (PCA) was used to detect the presence of *Pseudomonas aeruginosa* (ATCC 10145). Plates were incubated for 18-24 h at 37 °C and colonies with yellow- green to green pigmentation were identified as *Pseudomonas aeruginosa* [49].

Measurement of Minimal Inhibition Concentration (MIC): The MIC of the extracted oils was tested according to NCCLS method with slight modification [50]. Briefly serial dilution of each oil ranging from 0.3, 0.6, 1.2 to 10% (v:v) was prepared in Tryptic Soy Broth (TSB). Each bacterial strain was grown overnight in Tryptic Soy Broth (TSB, Oxoid, Basingstoke, UK) medium at 37 °C. The overnight culture was used to start a day culture and each culture was used to determine the MIC at around 10^6 colony forming units (CFU)/ ml. Bacterial lawn was made by spreading 100 µl of bacterial culture on the surface of TSA plate. Then, 10 µl of each dilution was spotted on the top of this bacterial lawn in triplicates and the plates were incubated at 37 °C for 18-24 h. Minimum inhibitory concentrations (MICs) were determined after 24 h, as the lowest concentration of extracted oils that inhibit the visible growth of each organism. The presence of one or two colonies was disregarded. All experiments were applied in triplicates [31].

Statistical Analysis: Statistical analyses were performed using SPSS V.15.0 for Windows. ANOVA was carried out on data of the chemical and microbiological evaluations. Data are expressed as mean + SE [51].

RESULTS AND DISCUSSION

The effect of essential oils on the chemical properties of burger after four days of cold storage was investigated. The pH, TBA and TVN values were determined in both treated and untreated (control) samples according to the previously described method. Burger samples treated with high concentration (1.2 %) of thyme, cumin and parsley oil showed that, the values of pH were significantly low ($P < 0.05$) compare to control samples. The pH value of control was 6.56 at the beginning of the experiment (Day 0) and has increased to 6.67 after 4 days of storage at refrigeration temperature.

The treated samples with 1.2 % of thyme, cumin and parsley oils reduced the value of pH to 6.58, 6.59 and 6.63 respectively compare to the control samples after 4 days of storage (Figure 1). The highest reduction rate was observed with both thyme and cumin oil treatments. The increased pH value of control beef samples might be due to the effect of microbial growth which may cause protein hydrolysis and release of nitrogenous compounds that increase the pH value of meat [52]. The low values of pH in samples treated with the 3 essential oils after four days of cold storage might be due to the antimicrobial effect of phenolic compound in herb oils. Similar results have been reported before by Salem *et al*. [23] who stated that, minced beef treated with 1.0 and 1.5 % of thyme, garlic and lemon grass oils showed lower pH values in comparison with untreated samples (control) after 6 days of cold storage. The reduction effect of essential oils such as clove oil on pH values in treated meat after storage at cold temperature have been reported by other researchers [53].

The results presented in Figure 2 showed that the means of TVN values of treated samples with 1.2 % thyme, cumin and parsley oils were significantly low in comparison to the untreated samples (control) after four days of cold storage. The highest reduction rate was observed with thyme and this significant decrease in TVN values in treated samples might be attributed to antimicrobial effect of those essential oils and to their ability to inhibit the activity of proteolytic enzymes [54].

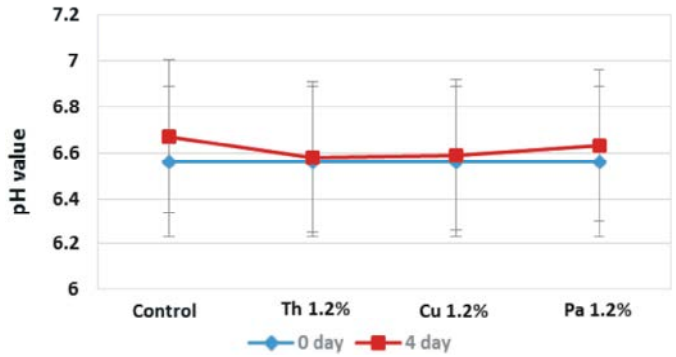

Fig. 1: pH values after treatment with high concentration (1.2 %) of essential oils in beef burger during storage (4±1°C) for 4 days

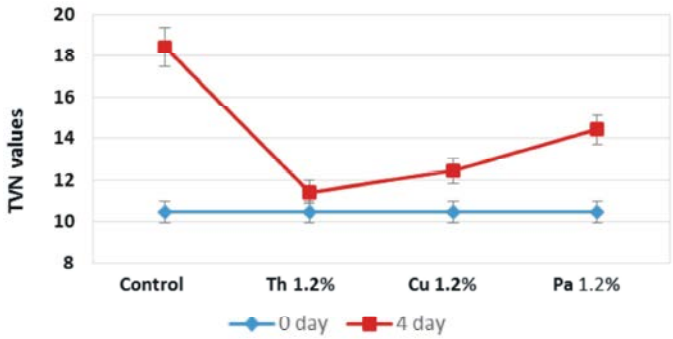

Fig. 2: TVN values after treatment with high concentration (1.2 %) of essential oils in beef burger during storage (4±1°C) for 4 days

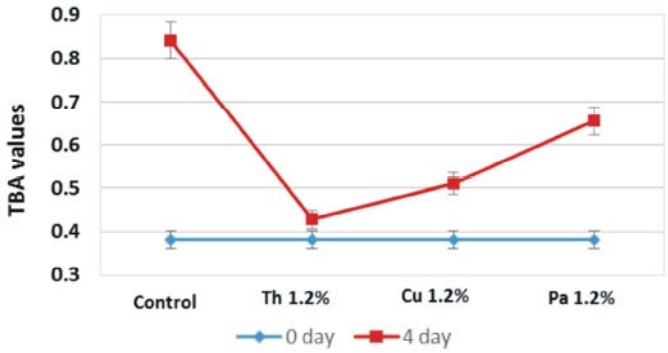

Fig. 3: TBA values after treatment with high concentration (1.2 %) of essential oils in beef burger during storage (4±1°C) for 4 days

Our findings are quite similar to the published results by Salem *et al*. [23] who stated that, the use of thyme, garlic and lemon grass oils successfully decreased the rate of TVN in treated beef in comparison to the control samples after six days of cold storage. Other studies have indicated that essential oils such as thyme, rosemary, marjoram oil and ginger significantly ($p < 0.05$) decreased the value of TVN in treated minced meat stored at cold temperature [55, 56].

The highest value of TBA was observed in control samples (0.842 mg malonaldehyde/ kg) after 4 days of storage at refrigeration temperature in comparison to the treated samples with essential oils. Thyme essential oil reduced the value of TBA significantly ($P < 0.05$) in comparison to control samples. The reduced rate of TBA values in treated samples might be due to the preservative effect of essential oils which slowed down or reduced the autolysis in meat muscles and accordingly reduced the

Table 1: Minimum inhibitory concentrations (MICs) of essential oils against several investigated bacterial strains (%).

	S. enterica	*E. coli*	*S. aureus*	*P. aeruginosa*
Thyme oil	< 0.6 %	> 0.3 %	> 1.2 %	> 1.2 %
Cumin oil	0.6 %	> 0.6 %	> 1.2 %	> 1.2 %
Parsley oil	0.6 %	0.3 %	> 1.2 %	> 1.2 %

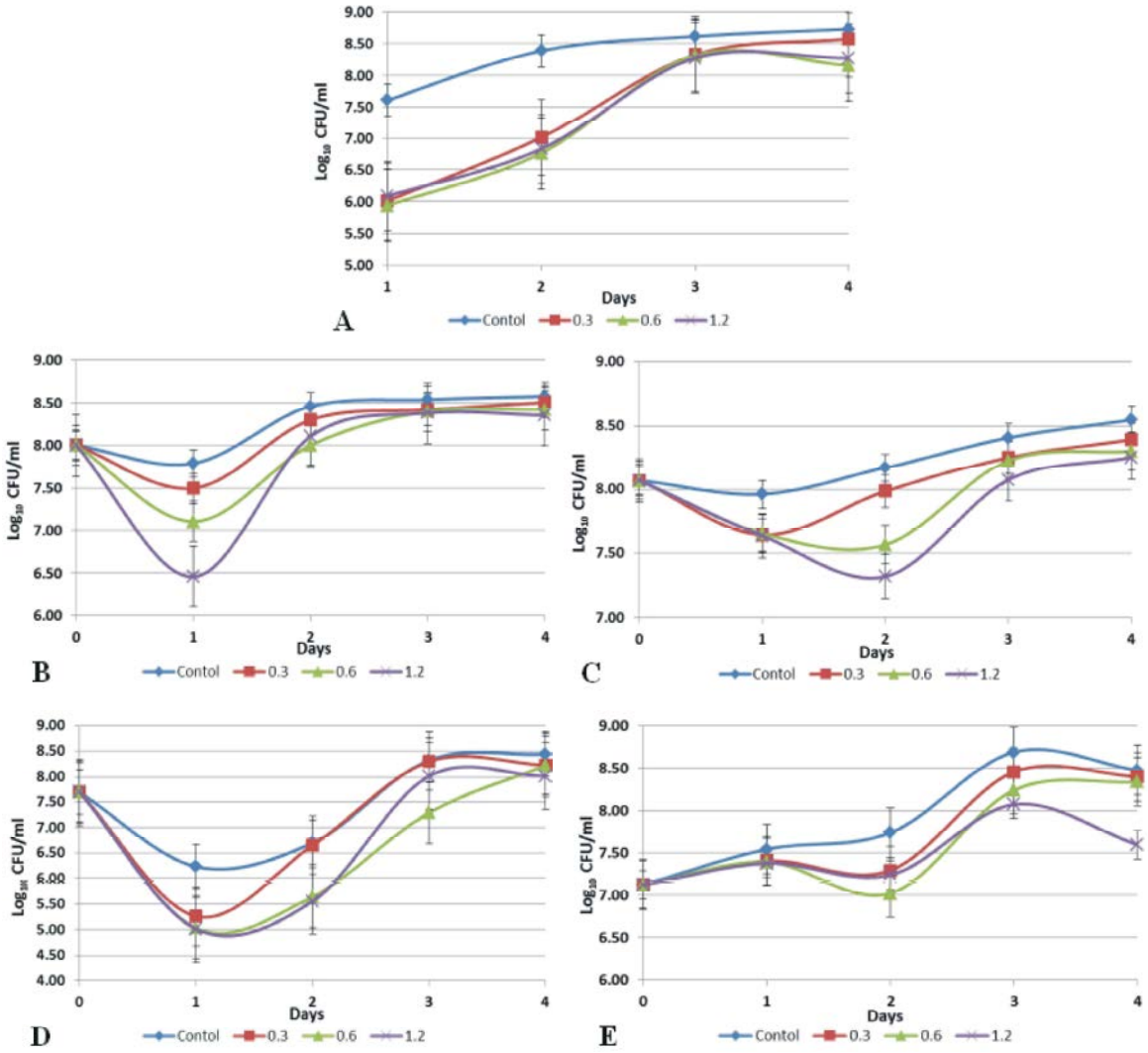

Fig. 4: Effect of different concentrations of parsley oil on A. TBC, B. *S. enterica*, C. *E. coli*, D. *S. aureus* and E. *P.aeruginosa* growth in beef burger during storage at refrigeration temperature (4±1 °C) for days 4 days.

breakdown of protein. The activity of used essential oils is due to their content of phenolic and flavonoid compounds [57]. The high value of TBA in control samples may be attributed to the auto oxidation of meat lipids in addition to the bacteriological and oxidative rancidity [23]. Our results are similar to those published by Ashour *et al.* [58] and Kassem *et al*. [59] who reported low values of TBA in treated meat with 0.05 % thyme oil after six days of storage at 4 °C. In another study, Sharafati-Chaleshtori *et al.* [60] did not observe any significant reduction in TBA values after adding different concentrations of basil essential oil to raw beef burger during storage 4 °C for 12 days.

Table 1 summarizes the values of MIC of the tested essential oils against the selected bacterial strains. The results demonstrated that all three essential oils displayed an antimicrobial activity with low and minimum concentration against both *S. enteric* and *E. coli* while higher concentrations are needed (> 1.2 %) to inhibit the growth of both *S. aureus* and *P. aeruginosa*.

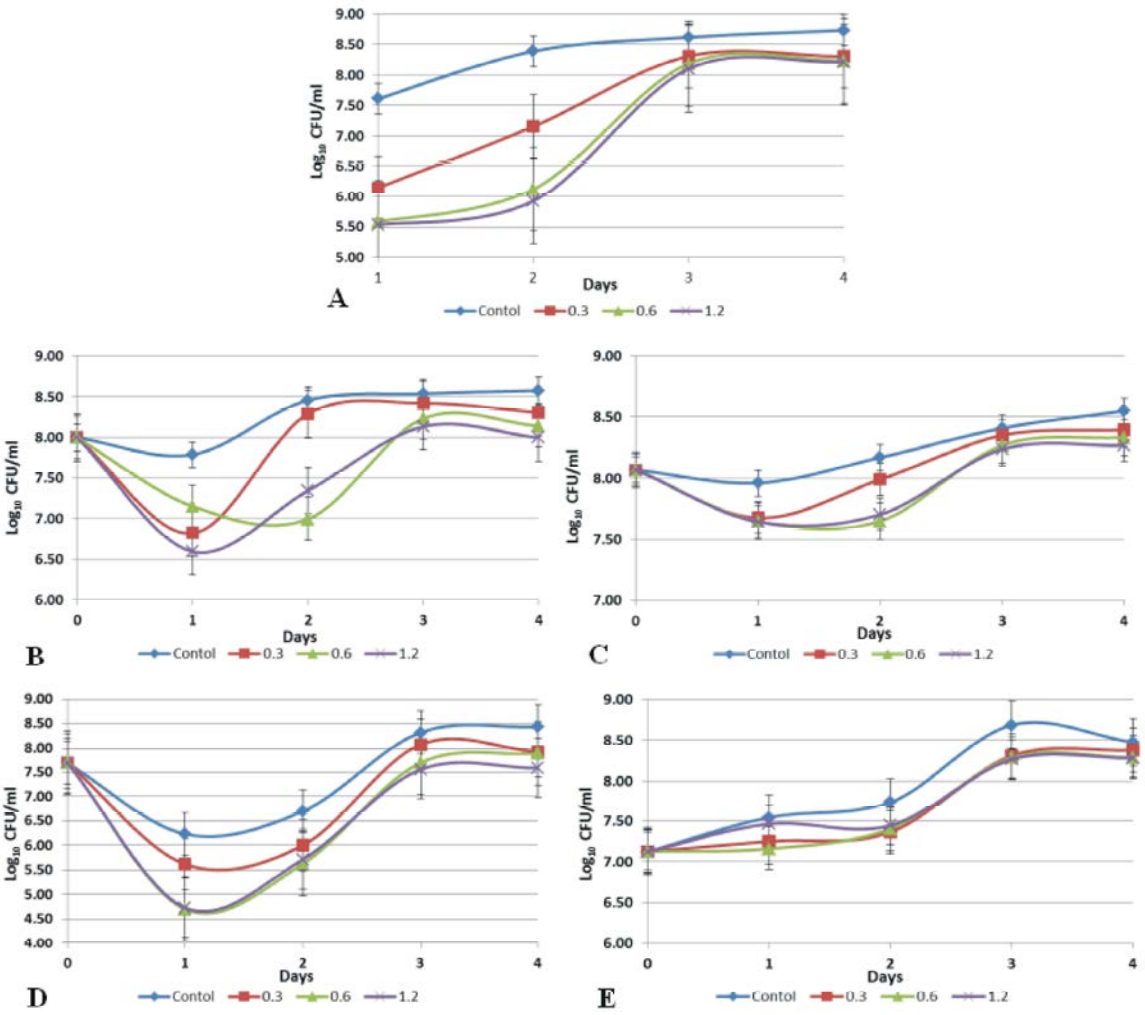

Fig. 5: Effect of different concentrations of cumin oil on A. TBC, B. *S. enterica*, C. *E. coli*, D. *S. aureus* and E. *P. aeruginosa* growth in beef burger during storage at refrigeration temperature (4±1 °C) for days 4 days

Different concentrations (0.3, 0.6 and 1.2 %) of the essential oils have been used to study their effect to control or inhibit the growth of different pathogenic bacteria in beef burger. Figure 4 presents the effect of different concentrations of parsley oil on the growth rate of TBC, *S. enterica*, *E. coli*, *S. aureus* and *P. aeruginosa* during storage at refrigeration temperature for 4 days. The results indicated that, parsley oil had a great reduction effect on the numbers of TBC, *S. enterica*, *E. coli*, *S. aureus* and *P. aeruginosa* during the first 2 days of the experiment. However, this inhibitory effect has been decreased by day 2 except with *P. aeruginosa*. Up to 2.5 $\log_{10}$ CFU/ml reduction rate has been observed with *E. coli* with the high concentration treatment (1.2 %) after 1 day of the experiment while the lowest reduction rate was observed with *P. aeruginosa* after 1 day of treatment. The other 2 lower concentrations (0.3 and 0.6 %) had less inhibitory effect in comparison to the high concentration. After 2 days of refrigeration storage, we noticed less effect and sometimes no effect of 0.3 % of parsley concentration on the growth of most bacteria especially *S. aureus*. The primary observed reduction of bacterial counts may be attributed to the sensitivity of pathogens to the cold storage temperature in addition to the antimicrobial effect of different compounds in parsley. Similar results have been reported by other researchers who observed an a ntimicrobial effect of parsley oil on *E. coli* [37, 38], *E. coli* O157:H7 and Micrococcus in a model food system [61] and *E. coli* and *Klebsiella* [39].

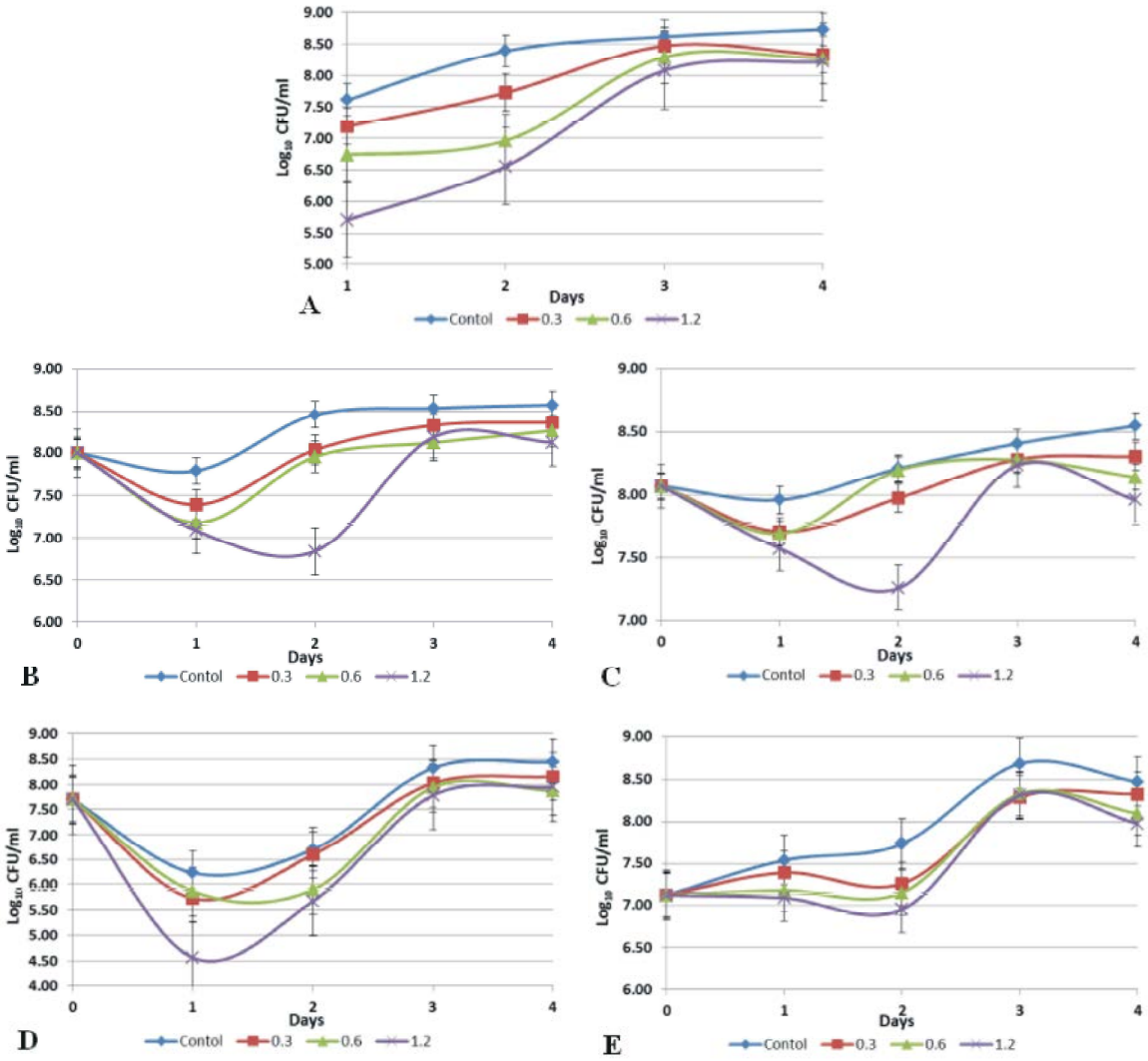

Fig. 6: Effect of different concentrations of thyme oil on A. TBC, B. *S. enterica*, C. *E. coli*, D. *S. aureus* and E. *P. aeruginosa* growth in beef burger during storage at refrigeration temperature (4±1 °C) for days 4 days

The antimicrobial effect of parsley oil when used with high concentration also has reported against *Pseudomonas* spp. [37, 40]. Some other essential oils such as oregano showed high reduction rate against *Pseudomonas* in meat samples during refrigerated storage [62]. Green tea was also reported to have a significant antibacterial activity against *Pseudomonas aeruginosa* [63]. The inhibitory effect of parsley may be attributed to the presence of phenolic compounds as indicated before [40, 64].

The inhibitory effect of cumin oil on TBC, *S. enterica*, *E. coli*, *S. aureus* and *P. aeruginosa* in beef burger is presented in Figure 5. Cumin oil had a similar inhibitory effect like parsley and the highest reduction rate (2.5 $\log_{10}$ CFU/ ml) was observed with TBC followed by *S. enterica* (2.3 $\log_{10}$ CFU/ ml) after 1 day of treatment. The lowest inhibitory effect has been observed with both *E. coli* and *P. aeruginosa* (Up to 1 $\log_{10}$ CFU/ ml reduction with *E. coli*). Generally, all cumin oil concentrations showed a reduction effect on bacteria. However, there is no significant difference between 0.6 % and 1.2 % concentrations with most bacteria. The reduction rate observed with cumin oil with both TBC and *S. enterica* was greater than that observed with parsley oil during the first 2 days of treatment. Our results are quite similar to the results reported by Rasool [65] and Wanner *et al.* [66] who recorded antimicrobial properties of four different spices including cumin on *E. coli* in meat products.

The antimicrobial effect of cumin oil has observed before against *S. aureus* [28 & 67] and against *P. aeruginosa* [68]. Different concentrations of cumin showed inhibition effect against *S. aureus* in beef burger during cold storage [60] and its effect as a preservative is well known before due to its antioxidant properties [69 & 70] in addition to its ability to enhance the flavor of foods [71].

Data presented in Figure 6 showed that the high concentration (1.2 %) of thyme oil had a significant ($p < 0.05$) reduction on TBC, *S. enterica* and *E. coli* after 2 days of treatment and storage at refrigeration temperature. The maximum reduction rate (2.5 $\log_{10}$ CFU/ml) was observed with *S. enterica*. The high concentration (1.2 %) of the thyme oil had the highest inhibitory effect in comparison to the other two concentrations (0.3 and 0.6 %). The antimicrobial effect of thyme oil has been observed in a previous study. Solomakos *et al.* [33] reported that different concentrations (0.3, 0.6 or 0.9 %) of thyme oils were very effective in reducing the numbers of *E. coli* O157:H7 in treated minced beef. In addition, the addition of thyme and marjoram oils decreased the numbers of *E. coli* in minced pork after 24 h of storage at 5 °C [72]. In another study, the addition of 0.5 % thyme oil to chicken breast meat reduced the numbers of *E. coli* O157:H7 significantly [73].

The lowest inhibitory effect has observed with *P. aeruginosa*. However, previous results indicated that thyme oil was able to reduce the numbers of *Pseudomonas* in lamb meat and beef burger during cold storage [34, 74]. Other previous studies showed the effectiveness of different essential oil to reduce the numbers of *Pseudomonas* in different product including chicken breast meat samples [27, 75, 76]. The observed high effect of thyme to reduce the numbers of *S. aureus* has been reported before in beef burger [59] and minced beef during storage at 4°C [23,33]. Previous works showed similar results about the effect of herb oils such as oregano to reduce the numbers of *S. enterica* and *E. coli* O157:H7 in chicken breast meat after storage at 4 °C for 72 h [77] and Listeria in meat pieces [78].

In general, essential oils can be used to reduce the use of chemical preservatives and to produce safe food due to their antioxidant, antifungal and antibacterial activity. Essential oils can be used to prevent foodborne diseases and pathogenic bacteria to improve the quality of meat characteristics due to their aroma and also to extend shelf-life of its products. It has been observed that beef treated with parsley and thyme oil showed a better reduction effect than cumin on the bacterial count during storage at refrigeration temperature. The high concentrations of essential oils (0.6 and 1.2 %) had the highest inhibitory effect in comparison to the low concentration (0.3 %) with most bacteria during the first period of cold storage. Similar results were obtained [19, 73, 79, 80] and Viuda-Martos*et al.* [57] who reported that thyme, parsley, rosemary and clove and other essential oils had a significant inhibitory effect against several pathogenic and food-spoiling bacteria. For example, Muhammad and Ali [32], Husni *et al.* [40] and Patil *et al.* [81] reported that cumin, thyme and parsley oils had a significant inhibitory effect against *Salmonella* and also against *Shigella* [60] during cold storage for 9 days. Other essential oils like oregano, thyme and cinnamon [77, 82, 83] showed a good inhibitory effect against *Salmonella* in chicken breast meat and minced sheep meat.

Antimicrobial activity of different essential oils is directly linked to the presence of phenolic constituents such as eugenol cumin aldehyde, carvone, limonene, linalool and thymol [25, 84, 85]. Most of those active phenolic compounds are able to inhibit the growth of bacteria by targeting the amino acid decarboxylase for example [86]. For example, carvone, carvacrol, cinnamaldehyde and eugenol showed a significant inhibitory effect against *S. aureus* [87, 88]. The refrigeration temperature may also contribute to the initial reduction rate of the bacterial numbers due to their sensitivity to the cold chock. Those active compounds of essential oils may interact with the bacterial cell membrane, causing the leakage of its cellular components and accordingly cause the cell death [89]. They also can inhibit the activity of enzymes involved in the energy regulation and metabolism [31]. The reduced inhibitory activity of essential oils added to meat may be attributed to the presence of fats and other components in meat [31].

CONCLUSIONS

In conclusion, some essential oil tested in this study could be used as natural preservatives to extend the shelf-life of beef burger and to minimize lipid oxidation. Some oils showed minimum to moderate antimicrobial effect, thus they rather could be used with other essential oils to control bacterial infection in meat products. Further studies are required to study the active compounds of these essential oils.

REFERENCES

1. Judge, M.D., E.D. Aberle, J.C. Forest, M.B. Hedrick and R.A. Merkel, 1990. Principle of meat science, Kendall Hunt Publishing Company, Dubuque, Iowa, USA.
2. Morrissey, P.A., D.J. Buckley, P.J.A. Sheehy and F.J. Monahan, 1994. Vitamin E and meat quality. Proceed. Nutrit. Soc., 53: 289-295.
3. Altabari, G., 2009. Meat hygiene and safety. AL-Ahsa Municipality, pp: 311.
4. Mensah, P., D. Yeboah-Manu, K. Owusu-Darku and A. Ablordey, 2002. Street Food in Accra, Ghana: how safe are they?. Bull. W.H.O., 80: 546-556.
5. Komba, E.V.G., E.M. Mkupasi, A.O. Mbyuzi, S. Mshamu, D. Luwumbra, Z. Busagwe and A. Mzula, 2012. Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro municipality, Tanzania: implications for public health. Tanzania J. Health Res., 14: 1-12.
6. Borch, E., M.L. Kant-Muermans and Y. Blixt, 1996. Bacterial spoilage of meat and cured meat products. Int. J. Food Microbiol., 33: 103-120.
7. Altabari, G. and A.M. Al-Dughaym, 2002. The role of sanitary inspection of meat in relation of food poisoning. InThe Proceedings of the second annual scientific meeting for environment hygiene (Meat Hygiene), Riyadh, pp: 180-203.
8. Zhou, G.H., X.L. Xu and Y. Liu, 2010. Preservation technologies for fresh meat– a review. J. Meat Sci., 86(1): 119-128.
9. Lawrie, R.A. and D.A. Ledward, 2006. Lawrie's meat science. Seventh English, edition ed. Cambridge England: Wood head Publishing Limited.
10. USDA (United States Department of Agriculture), 1995. Processing Inspectors' Calculations Handbook (FSIS Directive 7620.3). Accessed Dec. 19.
11. Sebranek, J.G., 1979. Advances in the technology of nitrite use and consideration of alternatives. J. Food Technol., 33: 58-62.
12. Lhirondel, J.L., 1999. Are dietary nitrates a threat to human health? , In J. Morris and R. Bates (ed.), Fearing food: risk, health and environment, Oxford, UK, pp: 38-47.
13. Nychas, G.J.E., P.N. Skandamis, C.C.A. Tassou and K.P. Koutsoumanis, 2008. Meat spoilage during distribution. J. Meat Sci., 78: 77-89.
14. Stiles, M.E. and J.W. Hastings, 1991. Bacteriocins production by lactic acid bacteria: potential for use in meat preservation. Trends Food Sci. Technol., 2: 247-251.
15. Politeo, O., M. Jukic and M. Milos, 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimumbasilicum*.) compared with its essential oil. J. Food Chem., 101: 379-385.
16. Wangensteen, H., A.B. Samuelsson and K.E. Malterud, 2004. Antioxidant activity in extracts from coriander. J. Food Chem., 88: 293-297.
17. Raharjo, S. and J.N. Sofos, 1993. Methodology for measuring malonaldehyde as ad product of lipid peroxidation in muscle tissues. J. Meat Sci., 35: 145-169.
18. Ayala-Zavala, J.F., L.D. Toro-Sanchez, E. Alvarez-Parrilla, H. Soto-Valdez , O. Martin-Belloso, S. Ruiz-Cruz and G.A. Gonzalez-Aguilar, 2008. Natural antimicrobial agents incorporated in active packaging to preserve the quality of fresh fruits and vegetables. Stewart Postharvest Review, 4: 1-9.
19. Angioni, A., A. Barra, E. Cereti, D. Barile, J.D. Coïsson and M. Arlorio, 2004. Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil (*Rosmarinusofficinalis L.*). J. Agric. Food Chem., 52(11): 3530-3535.
20. Dzudie, T., C.P. Kouebou, J.J. Essia-Ngang and C.M.F. Mbofung, 2004. Lipid sources and essential oils effects on quality and stability of beef patties. J. Food Engine., 65: 67-72.
21. Mielnik, M.B., S. Sem, B. Egelandsdal and G. Skrede, 2008. By-products from herbs essential oil production as ingredient in marinade for turkey thighs. J. Food Sci. & Technol., 41(1): 93-100.
22. Ahn, J., I.U. Grun and A. Mustapha, 2004. Antimicrobial and antioxidant activities of natural extracts *in vitro* and in ground beef. J. Food Prot., 67: 148-155.
00. Salem, A.M., R.A. Amin and G.S.A. Afifi, 2010. Studies on antimicrobial and antioxidant efficiency of some essential oils in minced beef. J. Am. Sci., 6: 691-700.
23. Nalini, N., K. Sabitha and M.V.P. Viswanathanp, 1998. Spices and glycoprotein metabolism in experimental colon cancer rats. J. Med. Sci. Res., 26(11): 781.

24. Derakhshan, S., M. Sattari and M. Bigdeli, 2008. Effect of sub-inhibitory concentrations of cumin (*Cuminumcyminum L.*) seed essential oil and alcoholic extract *on the morphology*, capsule expression and urease activity of *Klebsiellapneumoniae*. Int. J. Antimicrobial Agents, 32: 432-436.
25. Najda, A., J. Dyduch and N. Brzozowski, 2008. Flavonoid content and Antioxidant activity of Caraway roots (*Carumcarvi L.*). J. Vegetable Crops Res., 68:127-133.
26. Stefanini, M.B., R.O. Figueiredo, L.C. Ming and A.F. Junior, 2003. Antimicrobial activity of the essential oils of some spice herbs. Acta Horticulturae, 597: 215-216.
27. Fakoor, M.H. and I. Rasooli, 2008. Pathogen control by antioxidative characteristics of *Cuminumcyminum* and *Rosmarinus*officinalis essential oils. ISHS Acta Horticulturae, pp: 786.
28. Hosseini, M.H., S .H. Razavi, S.M.A. Mousavi, S.A.S. Yasaghi and A.G. Hasansaraei, 2008. Improving antibacterial activity of edible films based on chitosan by incorporating thyme and clove essential oils and EDTA. J. Appl. Sci., 8: 2895-2900.
29. Sadeghi, E., A.A. Basti, A. Misaghi, T.Z. Salehi and S.B. Osgoii, 2010. Evaluation of effects of *Cuminumcyminum* and *probiotic* on *Staphylococcus aureus* in feta cheese. J. Medic. Plants, 9(34): 131-141.
30. Burt, S., 2004. Essential oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol., 94(3): 222-253.
31. Mohammed, A.K.Z. and B. Ali, 2006. An investigation of thyme effect on Helicobacter pylori. Middle-East J. Sci. Res., 1: 54-57.
32. Solomakos, N., A. Govaris, P. Koidis and N. Botsoglou, 2008. The antimicrobial effect of thyme essential oil, nisin and their combination against Listeria monocytogenes in minced beef during refrigerated storage. J. Food Microbiol., 25(1): 120-127.
33. Emiroglu, Z.K., G.P. Yemis, B.K. CosKun and K. Candogan, 2010. Antimicrobial activity of soy edible films incorporated with thyme and oregano essential oils on fresh ground beef patties. J. Meat Sci., 86: 283-288.
34. Olaitan, A.O., U.S. Chukwudi and Y.A. Margaret, 2010. Antimicrobial potentials of some spices on beef sold in Gwagwalada market, FCT, Abuja. Department of Biological Sciences, University of Abuja, Nigeria, Academia Arena, 2(7): 15-17.
35. Soysal, Y., 2004. Microwave drying characteristics of parsley. J. Biosystems Engine., 89: 167-73.
36. Diez, J.G., 2015. Essential oils of herbs and spices in dry-cured meat products. Chemical characterization, antimicrobial properties, effect on foodborne pathogens and sensory acceptability in chouriço. Universidade de Tras-Os-Montes E Alto Douro Vila Real (PhD. Thesis).
37. Wong, P.Y. and D.D. Kitts, 2006. Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinumcrispum*) and cilantro (*Coriandrumsativum*) extracts. J. Food Chem., 97: 505-515.
38. Dostalova, L., L. Dostalova and L. Kalhotka, 2014. Antimicrobial Activity of Aqueous Herbal Extracts. J. Mendel Net., pp: 403-406.
39. Husni, F., E. Elbadrawy and A.A. Al-Atoom, 2015. Evaluation of antioxidant and antimicrobial activities of ethanolic extracts of Parsley (*Petroselinumerispum*) and Coriander (*Coriandrumsativum*) plants grown in Saudi Arabia. J. Advan. Res., 3(4): 1244-1255.
40. Busatta, C., R.S. Vidal, , A.S. Popiolski, A.J. Mossi, C. Dariva, M.R.A. Rodrigues, F.C. Corazza, M.L. Corazza, J. Vladimir Oliveira and R.L. Cansian, 2008. Application of *Origanummajorana L.* essential oil as an antimicrobial agent in sausage. J. Food Microbiol., 25: 207-211.
41. Hayes, J.W., J.R. Leathwick and S.M. Hanchet, 2010. Fish distribution patterns and their association with environmental factors in the Mokau River catchment, New Zealand. New Zealand J. Marine and Freshwater Res., 23: 171-180.
42. FAO (Food and Agriculture Organization of the United Nations), 1980. Manual of food quality control, 3- commodities. United Nations, Rome.
43. Vyncke, W., 1970. Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. FetteseifenAstrichmitted, 2: 1084-1094.
44. ICMSF (International Commission on Microbiological Specifications for Foods), 1978. Microorganism in food: Their significance and methods of enumeration. 2nd ed. Vol. 1. Univ. of Toronto, Presses, Toronto and Buffalo. Canada.
45. ICMSF (International commission on microbiological specifications for foods), 1996. Microorganisms in foods. 5 microbiological specifications of food pathogens. Clays Ltd, St Ives Plc, Bungay Suffolk, UK.

46. ISO (International Organization for Standards), 1999. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive *Staphylococci* (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird Parker agar medium, 1st ed., ISO6888-1:1999.
47. ISO (International Organization for Standards), 1993. Microbiology - General guidance on methods for the detection of *Salmonella.* ISO 6579: 1993(E) 3 rded.
48. FDA (Food and Drug Administration), 1998. Bacteriological Analytical Manual, 8th edition publication by FDA, U.S.
49. NCCLS, 2003. Performance standards for antimicrobial disk susceptibility tests. Approved standard, 8th ed. NCCLS document M2-A8. NCCLS, Wayne, Pa.
50. Gomez, K.A. and A.A. Gomez, 1984. Statistical procedures for agriculture research. John Wiley and Sons Editor Inc. USA (2ed edition), Chapter, 3: 129-184.
51. Benjakul, S., W. Visessanguan, C. Thongkaew and M. Tanaka, 2003. Comparative study on physicochemical changes of muscle proteins from some tropical fish during frozen storage. J. Food Res. Int., 36(8): 787-795.
52. Aureli, P., A. Costantini and S. Zolea, 1992. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. J. Food Prot., 55(5): 344-348.
53. Yassin, Nessrien, M.N., 2003. Effect of storage conditions on the quality parameters of differently treated fish. Ph. D. Thesis, Fac. Agric., Ain-Shams Univ. Cairo. Egypt.
54. El-Desouky, A.L., H.E.M. Bahlol and A.M.A. Sharoba, 2006. Effect of some essential oils and preservatives on the growth of *E. coli: O157:H*7 and quality of refrigerated minced meat. Ann. Agric. Sci. Moshtohor, 44(4): 75-1695.
55. Baker, I.A., A.D.O. Khalil and N.H. Khabat, 2015.Effect of thyme leaves extract on quality of lamb and chicken meat during storage. J. Univ. Zakho, 3(2): 198-204.
56. Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez- Alvarez, 2010. Effect of added citrus fibre and spice essential oils on quality characteristics and shelf-life of mortadella. J. Meat Sci., 85: 568-576.
57. Ashour, M.M.S., R.K. Moawad and G.F. Bareh, 2014. Quality enhancement and shelf-life extension of raw beef patties lactate/thyme essential oil during refrigerated storage. J. Appl. Sci. Res. Formulated With, 9(13): 6699-6709.
58. Kassem, G.M., O.A. Atta-Alla and F.H.M. Ali, 2011. Improving the quality of beef burger by adding thyme essential oil and Jojoba oil. Arch. Zootec., 60: 787-795.
59. Sharafati-Chaleshtori, R.S., N. Rokni, M. Rafieian-Kopaei, F. Drees and E. Salehi, 2015. Antioxidant and antibacterial activity of basil (*Ocimumbasilicum* L.) essential oil in beef burger. J. Agr. Sci. Tech., 17: 817-826.
60. Ulate-Rodriguez, J., H.W. Schafer, E.A. Zottola and P.M. Davidson, 1997. Inhibition of *Listeria monocytogenes, Escherichia coli O157:H7* and *Micrococcus luteus* by linear furanocoumarins in culture media. J. Food Prot., 60: 1046-1049.
61. Soultos, N., Z. Tzikas, E. Christaki, K. Papageorgiou and V. Steris, 2009. The effect of dietary oregano essential oil on microbial growth of rabbit carcasses during refrigerated storage. J. Meat Sci., 81: 474-478.
62. Lee, Y.L., T. Cesario, Y. Wang, E. Shanbrom and L. Thrupp, 2003. Antibacterial activity of vegetables and juices. J. Nutrit., 19(11-12): 994-6.
63. Wahba, N.M., S.A. Amany and Z.E. Zedan, 2010. Antimicrobial effects of pepper, parsley and dill and their roles in the microbiological quality enhancement of traditional Egyptian Kareish cheese. J. Foodborne Pathogens and Disease, 7(4): 411-418.
64. Rasool, M.H., 2013. Antimicrobial activity of plant essential oils against the growth of *Escherichia coli*. J. Pharmacy, 3(6): 01-06.
65. Wanner, J., S. Bail, L. Jirovetz, G. Buchbauer, E. Schmidt, V. Gochev, T. Girova, T. Atanasova and A. Stoyanova, 2010. Chemical composition and antimicrobial activity of cumin oil (*Cuminumcyminum, Apiaceae*). J. Nat. Prod. Common., 5(9): 1355-1358.
66. Akgül, A. and M. Kıvanç, 1989. Antibacterial effects of spices, sorbic acid and sodium chloride. Doğa. Turk. J. Agric. For., 13:1-9.
67. Agaoglu, S., N. Dostbil and S. Alemdar, 2007. Antimicrobial activity of some spices used in the meat industry. Bull Vet. Inst. Pulawy, 51: 53-57.
68. Aktug, S.E. and M. Karapinar, 1987. Inhibition of food borne pathogens by thymol, eugenol, menthole and anethole. Int. J. Food Microbiol., 4: 161-166.
69. Ristori, C.A., M.S. Pereira and D.S. Gelli, 2002. Oefeito da pimenta do reinomoídafrente a contaminação*in vitro* com *Salmonella* rubislaw. Revista do Instituto Adolfo Lutz, 61: 131-133.

70. Al-Jedah, J.H., M.Z. Ali and R.K. Robinson, 2000. The inhibitory action of spices against pathogens that might be capable of growth in a fish sauce (*mehiawah*) from the Middle East. Int. J. Food Microbiol., 57: 129-133.
71. Krisch, J., Z. Pardi, R. Tserennadmid, T. Papp and C. Vagvolgyi, 2010. Antimicrobial effects of commercial herbs, spices and essential oils in minced pork. Volume ActaBiologicaSzegediensis, 54(2): 131-134.
72. Fratianni, F., L.D. Martino, A. Melone, V.D. Feo, R. Coppola and F. Nazzaro, 2010. Preservation of chicken breast meat treated with thyme and balm essential oils. J. Food Sci., 75(8): 528-535.
73. Karabagias, I., A. Badeka and M.G. Kontominas, 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. J. Meat Sci., 88: 109-116.
74. Chouliara, E., A. Karatapanis, I.N. Savvaidis and M.G. Kontominas, 2007. Combined effect of oregano essential oil and modified atmosphere packaging on shelf life extension of fresh chicken breast meat, stored at 4°C. J. Food Microbiol., 24(6): 607-17.
75. Chaudhry, N.M.A. and P. Tariq, 2008. *In vitro* antibacterial activities of Kalonji, Cumin and Poppy seed. Pakistan J. Bot., 40: 461-467.
76. Ravishankar, S., L. Zhu, C.W. Olsen, T.H. McHugh and M. Friedman, 2009. Edible apple film wraps containing plant antimicrobials inactivate foodborne pathogens on meat and poultry products. J. Food Sci., 74(8): 440-445.
77. De Oliveira, M.M., D.F. Brugneram and R.H. Piccoli, 2013. Essential oils of thyme and Rosemary in the control of *Listeria monocytogenes* in raw beef.Brazilian J. Microbiol., 44(4): 1181-1188.
78. Conner, D.E., 1993. Naturally occurring compounds. In: Antimicrobials in foods. Eds., Davidison, P.M. and A.L. Branen. New York: Marcel Dekker, pp: 441-468.
79. Elgayyar, M., F.A. Draughon, D.A. Golden and J.R. Mount, 2001. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot., 64(7): 1091-1024.
80. Patil, S.D., P.P. Maknikar, S.J. Wankhade, C.S. Ukesh and M.K. Rai, 2016. Chemical composition, antimicrobial and antioxidant activity of essential oils from cumin and ajowan. J. Nusantara Bioscience, 8(1): 60-65.
81. Skandamis, P.N., E. Tsigarida and G.J. Nychas, 2000. Ecophysiological attributes of *Salmonella typhimurium* in liquid culture and within a gelatin gel with or without the addition of oregano essential oil. World J. Microbiol. Biot., 16: 31-35.
82. Bajpai, V.K., K.H. Baek and S.C. Kang, 2012. Control of *Salmonella* in foods by using essential oils: J. Food Res. Int., 45: 722-734.
83. Mahmoud, B.S.M., K. Yamazakia, K. Miyashitab, S. Shikc, C. Dong-Sukd and T. Suzuki, 2004. Bacterial microflora of carp (Cyprinuscarpio) and its shelf-life extension by essential oil compounds. J. Food Microbiol., 21: 657-666.
84. Barbosa, L.N., V.L.M. Rall, A.A.H. Fernandez, P.I. Ushimaru, I.S. Probst and A. Fernandez, 2009. Essential oils against foodborne pathogens and spoilage bacteria in minced meat. J. Foodborne Pathogens and Disease, 6(6): 725.
85. Ojagh, S.M., M.A. Sahari, M. Rezaei and S.V. Hosseini, 2011. Applicability of â-carotene and green tea polyphones as two natural antioxidants in preservation of common kilka (Clupeonella cultriventris Caspia) with ice. Int. J. Agric. Res., 1(4): 174-181.
86. Ouattara, B., R.E. Simard, R.A. Holley, G.J.P. Piette and A. Begin, 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol., 37: 155-162.
87. Rahman, M.S.A., S. Thangaraj, M.S. Salique, K. Feroz khan and S.E.Natheer, 2010. Antimicrobial and biochemical analysis of some spices extract against food spoilage pathogens. Int. J. Food Safety, 12: 71-75.
88. Sokovic´, M. and L.J.L.D. Van Griensensven, 2006. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom. Eur. J. Plant Pathol., 116(3): 211-224.