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Antimicrobial and Antioxidant Effect of selected Essential Oils in Refrigerated Beef Burger

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ABSTRACT

Microbial growth and oxidation are considered to be the major causes of beef burger deterioration.Synthetic antimicrobial and antioxidants are therfore used to prevent or delay microbial growth and oxidation process. Unfortunately, these substances are not safe and may cause harmful impacts on human health, which arose the need to search for natural alternatives in the food industry. In this study, essential oils of clove, thyme, and rosemary serve as natural preservatives to increase the shelf life of refrigerated beef burger up to 7 days without using synthetic antimicrobial and antioxidant agents. Essential oils were added to beef burger at two different concentrations (1 and 1.5% v/w). In addition, two positive control samples for antimicrobial and antioxidant agent were treated with 2% potassium lactate (PL) and 100 ppm Butylated hydroxytoluene (BHT) respectively. All samples were kept at 4°C for 7 days (study period). Measurement of the antimicrobial activity of essential oils was performed by testing total plate count, total coliforms, and *Staphylococcus aureus.* The oxidation stability test was carried out by thiobarbituric acid reactive substances (TBARS) test, and expressed as mg of malonaldehyde/ kg beef burger, in addition to that, the pH of each sample was examined. Essential oils added to beef burger showed strong antimicrobial effects against the examined microbes. The obtained results revealed that, clove essential oil (CEO) added at concentration 1.5% reduced the microbial activity of *Staphylococcus aureus* by 54% compared with negative control sample, also, there is a significant difference between it and all treated samples. In addition, results indicated that CEO at both concentrations 1% and 1.5% had the most effect on reducing microbial growth of total coliform (TC) compared with other stored samples. Total plate count results showed that essential oils at all different concentrations used in this study led to reduction in microbial growth in the burger samples without any significant differences between these essential oils (EOs). Regardingthe chemical indicators, CEO at concentrations of 1% and 1.5% were the most effective and retard oxidation by 24% and 35%, respectively compared with negative control sample. Moreover, Thyme essential oil (TEO) at a concentration of 1% and 1.5%, reduced the percentage of oxidation by 14% and 31%, respectively compared with the negative control sample.

Keywords: *Meat burger, Essential oils, Antimicrobial agent, Antioxidants, Synthetic preservative, TBARs*

1. INTRODUCTION

Food products, require protection from deterioration during production, storage, and distribution in order to maintain desired shelf-life(Rasooli, 2007). Nowadays, mostly all food products contain food preservatives. The trends are to retain the natural characteristics of food, extend its shelf life and prevent natural ageing and discoloration that might occurred duringfood preparation steps (Sharma, 2015). Food preservation refers to the process of keeping foods safe and undamaged (Amit et al., 2017). It is accomplished by creating an unfavorable environment for microbiological spoilage and retarding chemical oxidation (Vaclavik et al., 2021). It is well known that food products can be spoiled by chemicals, microbes, and enzymes found in the food itself. In addition, food deterioration can occur during food transportation, in terms of loss or decrease in morphological attraction and decrease in food nutritional value (Ahmad et al., 2021). Specific synthetic chemical substances were used as food preservatives. ThesePreservatives, considered primarily effective for an extended time span and pausing or postponing the increase of bacteria, quell chemical reactions when food exposed to either oxygen or heat (Zaccheo et al., 2016). However, Food preservatives also prohibit the loss of some major amino acids, vitaminsin addition topromotion of flavors and colors of food (Sharma, 2015).The ultimate strategy that used to stretch fresh meat lifetime is the use of antimicrobial and antioxidant additives of synthetic origin, which were being questioned due to an increasing consumer demand for natural, healthy and safe preservatives (Yu et al., 2021). It was reported that, essential oils (EOs) exhibit their antimicrobial activity against food-borne pathogens by interfering with and destabilizing the phospholipid bilayer of the cell membrane, the enzyme systems and the genetic material of bacteria thus inhibit microbial deterioration (Burt, 2004; Kim et al., 1995). Therefore, the use of natural preservation such as essential oils methods considered very important, in preserving beef burgers.

Meat and meat products such as beef burgers can be damaged by microbes or by fat oxidation. Therefore, it is a significant thing to conserve meat products from spoilage by adding preservatives, especially natural preservatives, to prolong their shelf life and improve their properties (Alsaiqali et al., 2016; Aminzare et al., 2016). Lipid oxidation is considered to be the main cause of meat spoilage and meat products due to its high fat content. These damages lead to a loss of some nutritional value, repulsive taste and texture, and loss of water retention (Aminzare et al., 2019). Natural additives rather than synthetic additives, expected to improve meat quality without leaving any remains in the product or in the environment. Several plant-derived Essential oils can be effectively used in meat and meat products as natural alternatives to synthetic food additives, particularly as effective antimicrobial agents. Moreover, phenolic compounds, such as carvacrol, eugenol, and thymol, are mainly responsible for the antimicrobial activity of Essential oils to increase the permeability of cell membranes and leading to loss of cellular constituents (Liu et al., 2013). The main objectivesof this study is therfore to measure antimicrobial activity of essential oils of thyme, clove, and rosemary on most common bacteria found in beef burger, and todetermine the optimum concentration of essential oil that reduced microbial growth or retard lipid oxidation in refrigerated beef burger. Other aims of this study are to increase the shelf life of refrigerated beef burger and to compare the effect of natural and synthetic preservatives during the storage time of refrigerated beef burger.

2. MATERIALS AND METHODS

Preparation of beef burger samples

The packed vacuum frozen boneless beef was obtained from Siniora Food Industries Company (Jerusalem-Palestine). Meat was thawed until it reached - 5ºC degree at its core, then, plastic cover was removed, and the meat was minced into sizes of 4 mm. Fat ingredients, was minced by the aid of mincer machine into 4 mm and mixed in with the other ingredients. The mixture then was mixed and homogenized for about four minutes. After that, beef meat mixwas divided into nine Formulas (1.5 kg each):

Formula1- remained as negative control (without antimicrobial or antioxidant agents) and the rest eight were treated and prepared as follows:

Formula 2- added with antioxidant positive control (100 ppm Butylated hydroxytoluene).

Formula 3- added with antimicrobial positive control (2 % potassium lactate).

Formula 4- added with 1% rosemary essential oil (REO).

Formula 5- added with 1.5 % REO.

Formula 6- added with 1 % thyme essential oil (TEO).

Formula 7- added with 1.5 % TEO.

Formula 8- added with1 % clove essential oil (CEO).

Formula 9 -added with1.5 % CEO.

The samples then chilled to 0°C,shaped into patties (90g per patty), packed in plastic bags, and closed tightly before being stored in the refrigerator at 4°C for 7 days (study period).

Microbial analysis

The microbial analysis performed was total plate counts (TPC), total coliform (TC), and *Staphylococcus aureus*. Peptone water and Plate Count Agar were sterilized by autoclaving at 121°C for 15 min.Violet Red Bile Agar (VRBA) used for determination of coliform counts was sterilized by boiling. *S. aureus* analysis was performed using Baird-Parker Agar. The media used were prepared and sterilized according to the guidelines given by the manufacturers. All determination were performed according to the procedures illustrated in provirus literatures (Shahbazi & Shavisi, 2018; Adam & Abugroun, 2010; Firstenberg-Eden et al., 2004)

Chemical analysis

A thiobarbituric acid reactive substances (TBARS) assay was performed to determine the oxidative stability of refrigerated beef burgers as described by (Al-Rimawi et al., 2017). For analysis, 5 g of sample was placed in a beaker containing 50 ml of 0.38% TBA and 15% trichloroacetic acid prepared in 0.25N HCl solution. The sample was homogenized (IKA T-25 Ultra Turrax Homogenizer) at 10,000 rpm for 3 min., and three 15 ml aliquots from the homogenate were heated in a boiling water bath for 1 hour to develop a pink color and cooled in tap water. The boiled samples were then clarified by centrifugation (Labnet International C0226R) at 5000 rpm for 10 min., and absorbance was measured at 532 nm using a spectrophotometer (Jenway 74 Series Spectrophotometer). Higher oxidation of the beef burger sample indicates higher absorbance. An average of three absorbance values were used to determine the oxidative stability of the refrigerated samples. On the calibration curve, the absorbance of malondialdehyde (MDA) at various concentrations (1-15 ppm) was used to determine the amount of oxidation products (mg MDA / kg beef burger sample). The pH measurement of beef samples was determined using a pH Meter (Hanna HI 2210).

Statistical analysis

The data were expressed as mean ± SD, statistical analysis was performed with one-way analysis of variance (ANOVA), followed by LSD's fisher test and letters of mean separation. A p value of less than 0.05 ($p < 0.05$) was considered statistically significant. The statistical analysis was performed using the Sigma Stat software (version 2.03).

3. RESULTS AND DISCUSSIONS

Results

Antimicrobial Activities of essential oils on Beef burger patties

Effect of essential oils on total plate count of beef burger patties

All beef burger samples that were kept for 7 days at 4°C showed an increase in bacterial growth from the first day until the seventh day. However, control sample showed developments of bacterial growth compared to treated samples which showed a very clear decrease in microbial growth during the preservation period. Similar effect of each of essential oils against microbial growth (Table 1) was observed as follows, TEO at a concentration of 1% and 1.5% led to a reduction in microbial growth by 69% and 71%, respectively. As for REO, at a concentration of 1% and 1.5%, it reduced microbial growth by 70% and 65%, respectively. In consequence, CEO at a concentration of 1% and 1.5% had its effect on reducing microbial growth by 69% and 63%, respectively, compared to the negative control, and the results also showed that the burger sample with potassium lactate had the lowest microbial growth at seven days. The results (Table. 1) summarized that all essential oils used in this study led to a reduction in microbial growth in the burger samples, without any significant differences between the concentrations of these oils and without any significant difference according to the potassium lactate.

Table 1Microbial analysis of total plate count content (cfu/gm) in beef burger during 7 days at different essential oil concentrations.

Treatments	0 day	4 days	7days
Negative control	1.6E+04±7.8E+02 e	7.1E+04±3.6E+03 b	24E+04 ±2.5E+04 a
P. L 2%	1.6E+04±7.8E+02 e	1.7E+04±1.1E+03 e	5.3E+04±4.9E+03c,b
TEO 1%	1.6E+04±7.8E+02 e	2.3E+04±1.5E+03 e	7.1E+04±1.1E+04 b
TEO 1.5%	1.6E+04±7.8E+02 e	1.8E+04±1.7E+03 e	8.5E+04±1.1E+04 b
REO 1%	1.6E+04±7.8E+02 e	4.9E+04±3.6E+03c,b	7.3E+04±7.0E+03 b
REO 1.5%	1.6E+04±7.8E+02 e	3.5E+04±2.0E+03 d	7.1E+04±3.2E+03 b
CEO 1%	1.6E+04±7.8E+02 e	1.3E+04±1.2E+03 e	9.0E+04±5.6E+03 b
CEO 1.5%	1.6E+04±7.8E+02 e	0.9E+04±4.0E+02 e	7.3E+04±2.5E+03 b

- Small letters (a-e) indicates significant differences in number of total bacterial count among samples during storage time, LSDs= 21860

Effect of essential oil on total coliform count of beef burger patties

Microbial growth of coliform bacteria in all treated and untreated burger samples, which were kept for seven days at a temperature of 4 °C, increased with the progress of storage up to the seventh day.The highest value appeared in the burger sample containing 1% TEO, while the lowest value was determined in the burger sample containing potassium lactate (Table 2).

The results showed that CEO had a clear effect on reduction of microbial growth in both concentrations (1 and1.5%). Nevertheless, treatment of beef burger with 1.5% CEO showed greater inhibitory action compared with that obtained for beef burger treated with 1% CEO (Table 2). A concentration of 1% and 1.5% CEO reduced microbial growth by 80% and 95% respectively compared with the negative control sample. Moreover, 1% and 1.5% REO, had an effect on reducing the microbial count by 10% and 14%, respectively, compared to the negative control sample. Whereas, thyme essential oil, at a concentration of 1.5%, reducing the total number of coliform bacteria by 13%, compared to the negative control (Table. 2).

In comparison with the potassium lactate added burger sample, there is no significant difference between PL and CEO at a concentration of 1.5%. Also, there is no significant difference between TEO and REO at the same concentration, while 1% concentration of TEO had no effect on microbial growth. The results indicate that CEO at both concentrations had the most effect on reducing microbial growth compared to the negative control sample and the rest of the other samples.

Table 2 Effect of essential oils (thyme, rosemary, and clove) at concentrations (1%and 1.5%) on total coliform (cfu/gm) in refrigerated beef burger after 7 days of storage.

Treatment	**0 day**	**4 days**	**7 days**
Negative Control	1.5E+01±1 j	38E+02±265 e	5.5E+03±306 b
P. L 2%	1.5E+01±1 j	3.1E+02±31 j	0.09E+03±10 j
TEO 1%	1.5E+01±1 j	8.8E+02±125 h,i	8.0E+03±0.0 a
TEO 1.5%	1.5E+01±1 j	5.3E+02±61 i,j	4.8E+03±322 d
REO 1%	1.5E+01±1 j	36E+02±153 e	5.0E+03±400 c
REO 1.5%	1.5E+01±1 j	21E+02±252 f	4.8E+03±100 d
CEO 1%	1.5E+01±1 j	3.0E+02±29 j	1.1E+03±58 g,h
CEO 1.5%	1.5E+01±1 j	2.1E+02±21 j	0.27E+03±26 j

- Small letters (a-j) indicates significant differences in number of total bacterial count among samples during storage time, LSDs= 348

Effect of essential oil on *Staphylococcus aureus* count of beef burger patties

The effect of different essential oil on the *Staphylococcus aureus* during 7 days of storage of refrigerated beef burger at 4 ^{O}C was shown in (Table 3). The results indicated that, the growth of *Staphylococcus aureus*in all samples increased significantly during the period of storage. However, the highest value was obtained from the beef patties of control sample, and the lowest value was in beef patties treated with 1.5% CEO at seventh days.It was found that, CEO at a concentration 1% and 1.5% significantly reduced the number of colonies by 15% & 54% respectively after 7 days of storage compared with the negative control sample (Table 3). Also, TEO at concentration 1% and 1.5% were significantly reduced the number of colonies by 22% & 35% respectively after 7 days of storage compared with the negative control sample. In addition, the clove at 1.5% concentration significantly reduced the number of colonies by 36% compared with potassium lactate after 7 days of storage. Thyme essential oil at concentrations 1% and 1.5% didn't significantly affect the *S. aureus* growth compared with potassium lactate. However, REO didn't significantly affect the microbial activity at both concentrations compared with negative control. The results revealed that CEO at concentration 1.5% reduced the microbial activity of *S. aureus* by 54% compared of negative control after 7 days of storage, also, there is significant difference between it and all treated samples. Clove and thyme EOs are both natural substances that are not harmful when consumed in medicine and food products.

Table 3 Effect of essential oils of medicinal plants (thyme, rosemary, and clove) at concentrations 1% and 1.5% on total count of *Staphylococcus aureus*(cfu /gm) in beef burger after 7 days of storage.

Treatments	0 day	4 days	7 days
Negative Control	1.9E+02±21 f	3.5E+02±32 d,e	6.4E+02±40 a
P. L 2%	1.9E+02±21 f	3.2E+02±30 d,e	4.6E+02±23 c,d
TEO 1%	1.9E+02±21 f	2.8E+02±21 e	5.0E+02±30 b,c
TEO1.5%	1.9E+02±21 f	2.7E+02±25 e	4.2E+02±25 c,d
REO 1%	1.9E+02±21 f	3.1E+02±30 d,e	6.2E+02±105 a,b
REO 1.5%	1.9E+02±21 f	3.8E+02±65 d	5.9E+02±47 a,b
CEO 1%	1.9E+02±21 f	3.1E+02±31 d,e	5.4E+02±57 b,c
CEO 1.5%	1.9E+02±21 f	2.3E+02±31 e,f	2.9E+02±25 e

- Small letters (a-f) indicates significant differences in number of total bacterial count among samples during storage time, LSDs=83

Antioxidant activity of different essential oils on beef burger patties

TBA test has been widely used to measure lipid oxidation in meat and meat products (Hu et al., 2010). Essential oils of thyme, rosemary, and clove were added to refrigerated beef burger as natural antioxidant in two concentrations (1% and 1.5%). The oxidative stability of beef patties was examined by conducting thiobarbituric acid reactive substances (TBARS) assay. Absorbance obtained from these assays at 532 nm, were then used to calculate amount of oxidation by TBARS. Results are given in Table 4 as mg of MDA/ kg beef burger. The initial TBARS value of refrigerated beef burger was determined as 3.27 mg MDA/kg beef burger. Although the level of lipid oxidation was increased in all samples during the study period it was noticed that treatment with essential oils reduced the lipid oxidation of beef burger by different percentages, the best antioxidant activity was obtained from beef sample treated with 1.5 % CEO. The results of the oxidation test for beef burger samples kept at a temperature of 4°C for a period of 7 days, in which the amount of MDA/kg was measured using the TBARs method (Table 4), showed that, all samples had the oxidation developed, and this was evident by the increase in the amount of MDA compound produced during the study period. However, some essential oils used in this study led to reduce oxidation compared with the negative control sample, whereas, clove essential oil at a concentration of 1% and 1.5% was the most effective in oxidation reduction by 24% and 35%, respectively compared to the negative control sample. Thyme essential oil, at a concentration 1% and 1.5%, reduced the percentage of oxidation by 14% and 31%, respectively compared with the negative control sample. Compared with BHT, CEO and REO at a concentration of 1.5% were better than BHTby 13% and 7%, respectively. Also, there is no significant difference between BHT and CEO at a concentration of 1%. Results showed that REO at both concentrations did not show any effect on reducing oxidation.

Table 4 Oxidation products of refrigerated beef burger samples treated with different concentrations of essential oils (1%, and 1.5%) determined by TBARS (mg MDA/ kg) method.

Treatments	0 day	4 days	7 days
Negative control	3.27±0.19 j	12.78±0.15 g	26.34±0.51 b
BHT 100 ppm	3.27±0.19 j	5.37±0.51 i	19.64±1.26 d,e
TEO 1%	3.27±0.19 j	11.82±0.51 g	22.78±1.09 c
TEO 1.5%	3.27±0.19 j	11.40±0.32 g	18.26±0.95 e,f
REO 1%	3.27±0.19 j	12.53±0.63 g	28.52±1.19 a
REO 1.5%	3.27±0.19 j	11.94±0.32 g	28.69±1.12 a
CEO 1%	3.27±0.19 j	6.92±0.44 h	19.98±1.02 d

CEO 1.5%	3.27±0.19 j	5.87±0.19 i	17.17±0.07 f

Small letters (a-j) indicate differences in the amounts of oxidation products for control samples and treated samples (1%, and 1.5%) at each storage time, LSDs=1.58

Changes in pH of beef burger patties.

Storage time affected all the treatments by changing the pH values during the storage period with a slight effect. Lowest pH was found for the sample with potassium lactate with a pH of 5.86. It was shown in (Table 5, and Figure 1), that pH of P.L sample changed more on day 7 than other stored samples. Similar results were obtained for BHT treated sample showing a decrease in pH value (5.98) after 6 days of storage. The treated samples with essential oils didn't showed significant difference compared with negative control sample.

4. DISCUSSION

Microbial activity

Total plate count, total coliform, and *Staphylococcus aureus* count of the three different essential oils (TEO, REO, and CEO) are given in Tables (1-3) respectively. Plenty of spices and herbs exert antibacterial influences due to their essential oil fractions. Studies revealed that, essential oils from plants likethyme, sage, rosemary, clove, garlic, and onion show antimicrobial activity against both bacteria and molds (Fu et al., 2007; Sahalie et al., 2018). The antimicrobial activity of the plants varies depending on the composition, structure and functional groups of the oils (Bhavaniramya et al., 2019). Phenolic compounds present in essential oils are responsible for their antimicrobial activity (Medina et al., 2006; Silva et al., 2012). The mechanism of action of phenolic compounds on bacterial cell has been partially attributed to interact with protein membranes, causing deformation in their structure and interfering with their functionality (Álvarez-Martínez et al., 2020). In addition, Nieto (2020)reported that carvacrol and thymol are capable of inactivating essential enzymes, reacting with cell membranes and altering genetic material.

Essential oils of clove,thyme, and rosemary were shown to possess strong antibacterial activity against *Salmonellatyphi*, *Staphylococcusaureus*, and *Pseudomonasaeruginosa*(Conner, 1993). Furthermore, clove and rosemary essential oils showed antibacterial effect against meat spoilage pathogenic bacteria (Gonelimali et al., 2018; Ouattara et al., 1997; Sharafati Chaleshtori et al., 2015). Our results agree with the findings ofXu et al. (2016), who showed that clove essential oil exhibited strong antibacterial activity against S*taphylococcus aureus*. These results might be attributed to the presenceof phenolic compoundssuch as carvacrol, eugenol and thymol that are able to inhibit bacterial growth (Kim & Wei, 1995; Ojagh et al., 2011). Radünz et al. (2019) reported that, clove essential oil showed inhibitory effect up to the concentration of 0.304 mg/ml against *S. aureus*, *E. coli*, *L. monocytogenes* and *S. typhimurium* bacteria. Thus, this effect was independent of the characteristics of the microorganism membranes. The action of clove essential oil is ascribed to the presence of eugenol which promotes the rupture of the bacterial cytoplasmic membrane, so increasing its permeability and allowing the extravasation of the ions and the loss of intracellular proteins,causing bacterial death (Devi et al., 2010; Radünz et al., 2019; Sharafati Chaleshtori et al., 2015).Thyme essential oils exhibit antimicrobial activities against a wide range of pathogenic microorganisms (Cai et al., 2019). Many studies have demonstrated that, carvacrol and thymol possess potent antibacterial agents against both Gram-positive and Gram-negative bacteria (Kachur & Suntres, 2020). Ghaderi-Ghahfarokhi et al. (2016) studied the antimicrobial activity of thyme essential oil and they showed that thyme essential oil had an effect on reducing the microbial growth of *Staphylococcus aureus* bacteria in refrigerated beef burgers. Treatment of minced beef with 1%TEO inhibited *E. coli* O157:H7 growth during storage at 7°C (Selim, 2011).Similar action of TEO against *E. coli* O157:H7 was previously confirmed in vitro at temperatures higher than that of refrigeration (Burt & Reinders, 2003; Imelouane et al., 2009).It was found that, the major phenolic compound of thyme essential oil (ie, thymol, and carvacrol) are primarily responsible for their significant antimicrobial activity against bacterial growth (Kachur & Suntres, 2020). Thyme essential oil effectively inhibited the growth of *Escherichia coli* and lactic acid bacteria in chicken breast meat stored at 4°C. This prevents the chicken meat spoilage and extends the shelf life of the fresh product (Fratianni et al., 2010). Thymol is the major component ofTEO which is structurally similar to carvacrol having hydroxyl group at a distinct position on the phenolic ring (Mousavian et al., 2021). It is evident that the interaction of thymol affects membrane permeability which in turn causes loss of membrane potential, leakage of K^+ ions, and ATP and carboxy fluorescein (Jafri et al., 2019; Zhou et al., 2019). In addition to this, thymol also interacts with periplasmic proteins by hydrophilic and hydrophobic interactions (Jafri et al., 2019). The rosemary essential oil inhibitory effects on total coliform during the study period shown in (Table 4.2). The inhibitory activity of rosemary essential oil was significantly lower than clove essential oil. Our results agree with the finding of Canillac and Mourey (2001) who reported that rosemary essential oil rich in monoterpenes, arepermissive for the growth of *E. coli* and *E. aerogenes*. However, these bacteria are susceptible to essential oils containing large amounts of phenolic and oxygenated compounds.

Chemical analysis

Phenolic acids are found ubiquitously and well documented for other health protective effects like antimicrobial, anticancer, anti-inflammatory, and anti-mutagenic (Kumar & Goel, 2019). The major component of the clove oil includes eugenol, and eugenol acetate whereas the minor constituent includes vanillin, tannins, the constituents like methyl amyl ketone, and methyl salicylate add up to the characteristic pleasant odours(Kumar et al., 2021). Our findings (Table 4) is consistent with Kong et al. (2010), who observed thatclove extracts are highly effective antioxidants in cooked pork patties and stabilized red color (myoglobin) during refrigerated storage. The results indicate that an increasing of clove essential concentration from 1% to 1.5% leading to decrease the oxidation rate of beef burger. These results could be related to the amount of hydroxyl groups within the phenolic structures of constituents present in clove essential oil. In this investigation, the antioxidant activity of clove and thyme EOs at concentration of (1.5%) showed significantly inhibition compared to BHT which agreed with Jirovetz et al. (2006) who investigated thatthe antioxidant property of phenolic compounds surpass the activity of synthetic antioxidant such as BHT. Such findings may be attributed to the high antioxidant effect of thyme essential oil, which is related to the scavenger nature of its flavonoids and phenolic content as apigenin, luteolin, thymonin, carvacrol, 1,8-cineole,and thymol (Kassem et al., 2011a). Our results in (Table 4.4), also steady with Ghaderi-Ghahfarokhi et al. (2016) that showed a significant improvement in the reduction of thiobarbituric acid reactive substances (TBARS) amount for thyme essential oil treated burgers in comparison with control samples after 8 days of chilled storage. The role of the antioxidants is to neutralize the free radicals in biological cells, the free radicals having a negative impact on living organisms. A special role in neutralizing the effects of the oxidative stress related to the presence of free radicals is played by the enzyme called superoxide dismutase (Munteanu & Apetrei, 2021). The effect of natural and synthetic antioxidants on pH values of beef burgers stored at 4 ºC for 7 days is presented in (Table 5). At begining, the pH of the controls and all tested samples had the values (6.03). The pH values of all treatments with essential oils were similar therefore; it was not affected by essential oil addition. These results are in agreement with the results obtained by Mohamed and Mansour (2012) which reported that, addition of natural herbal extracts to beef patties did not significantly change the pH values of all formulas after preparation and during storage. However, after the storage period, the pH values of beef burger samples treated with potassium lactate and BHT decreased to 5.86 and 5.98, respectively.

Table 5 pH of refrigerated beef burger samples (controls and treated samples)

Treatments	0 day	4 days	7 days
Negative control	6.03±0.04 b,c	6.02±0.02 b,c	6.02±0.04 b,c
P.L 2%	6.03±0.04 b,c	5.83±0.03 e	5.86±0.04 d,e
BHT 100ppm	6.03±0.04 b,c	5.97±0.03 c	5.98±0.09 c
TEO 1%	6.03±0.04 b,c	5.94±0.04 c	6.05±0.05 b,c
TEO 1.5%	6.03±0.04 b,c	6.05±0.05 b,c	6.03±0.04 b,c
REO 1%	6.03±0.04 b,c	6.19±0.04 a	6.03±0.04 b,c
REO 1.5%	6.03±0.04 b,c	6.16±0.05 a	6.05±0.03 b,c
CEO 1%	6.03±0.04 b,c	5.98±0.14 c	6.02±0.03 b,c
CEO 1.5%	6.03±0.04 b,c	6.21±0.02 a	6.03±0.02 b,c

Small letters (a-e) indicate differences in the pH values of products for control sample and treated samples (1%, and 1.5%) at each storage time, LSDs=0.07

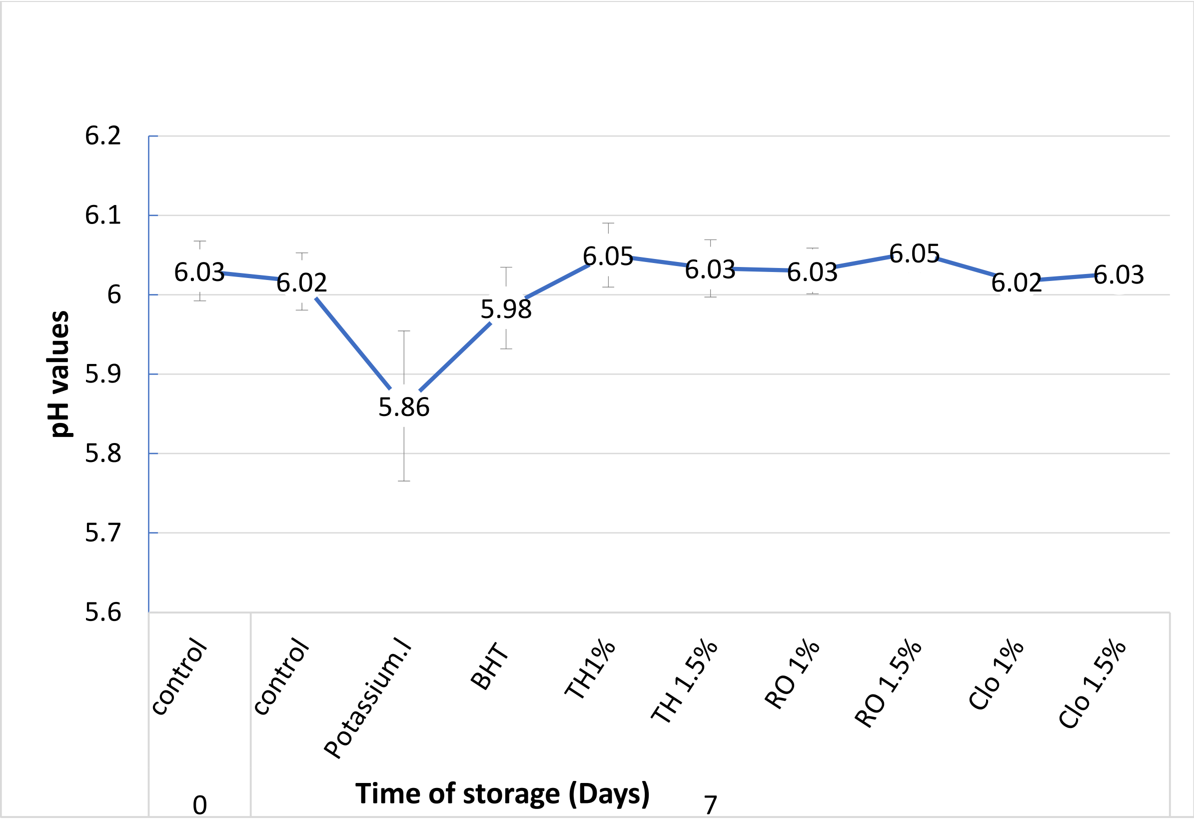

Figure 1pH values of refrigerated beef burger samples at zero and 7 days.

5. CONCLUSION

In the last few decades, consumers are demanding healthy safe food with least concentration of synthetic food additives and least heat treatment. There are many ongoing studies on the biological properties of essential oils for their possible use as alternatives to synthetic antioxidant such as BHT. Essential Oils which have a wide spectrum of antimicrobial activity are major source of polyphenols, which can be used in many types of food such as meat products as an alternative to chemical preservatives. In this study, three types of essential oils have been used for detection of their antibacterial activity against total plate count, total coliform, and *S.aureus*. Antioxidant activity of the EOs was also studied using TBARS test.

All Essential Oils in this study exhibit antibacterial activity, clove was amongst the strongest. These activities are mainly attributed to the presence of the active compounds in their compositions. An increase in the clove and thyme essential oils concentrations lead to a decrease in bacterial counts of *S.aureus* and total coliform. All essential oils, clove, thyme, and rosemary have good antibacterial properties on total plate count without significant difference between the two concentrations (1% and 1.5%). This means that there is no point in using a high concentration of oils, thus, the lower concentration (1%) can be used. Although the results clearly indicated that rosemary essential oils showed a reduction in total plate count and total coliform, it showed no effect on growth of *S. aureus*. The clove and thyme provided the best antioxidant protection against beef burgers deterioration during 7 days of storage at 4^{O}C. Moreover, clove at 1.5% was superior to BHT in inhibiting the lipid oxidation. However, rosemary essential oil demonstrated a decrease in their antioxidant capacity. In conclusion, the application of essential oils on beef burger reduces the microbial growth and delayed oxidative deterioration, thus can be used as alternatives to synthetic preservatives to increase the shelf life of refrigerated beef burger.

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