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Original research

Analyzing *Rosmarinus officinalis* essential oil and its nanoemulsion in beef burgers shelf life at refrigerator temperature

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A B S T R A C T

Recently, plant essential oils and their nanoemulsions have received great attention since they help reduce the microbial load and improve the food products' antioxidant activity. In the present research, rosemary essential oil (REO) and nanoemulations of rosemary essential oil (NREO), were added to a hamburger at different levels, and the properties were checked at refrigerator temperature. The results showed that the most compounds of REO were α-pinene (12.32%), 1, 8-Cineole (12.02%), Camphene (9.21%), Limonene (8.11%), Camphor (7.13%), Bornyl acetate (6.18%), Borneol (6.11%) and Verbenone (5.21%). The mean diameter of inhibition zone against *Staphylococcus aureus* and *E. coli* for NREO was considerably more than the one for REO ($p \leq 0.05$). The phenol content and antioxidant activity of REO were significantly higher than that of NREO ($p \leq 0.05$). In all the studied days, except on the 1st day, the highest amount of peroxide value, thiobarbituric acid and microbial population (mesophilic bacteria, psychrophilic bacteria and coliform bacteria of the samples) belonged to sample 1 (control sample). And the lowest amount of the mentioned factors belonged to hamburger containing NREO ($p \leq 0.05$). The highest sensory scores (smell, taste, texture, and overall acceptance) were for hamburger which contained NREO ($p \leq 0.05$). The hamburger containing NREO, was selected as the one with the superior treatment due to its lower microbial load, and higher sensory evaluation score.

Keywords: Hamburger; Nanoemulsion; *Rosmarinus officinalis*; Shelf life

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1. Introduction

Meat and their products are considered to be a significant source of fundamental nutrients in our daily diet (Mehta et al., 2015). Meat products, including hamburgers, are still popular among all kinds of consumers, especially the young generation (Viana et al., 2014). Products known as burgers play a significant role in people's nutrition and food diversity. Meat, the main components of these products, is one of the essential components of human food. It has a high dietary value, and its consumption provides a large amount of the body needs for nutritional compounds. Contrary, the desire of consumers to use "healthier" products that are free of chemical preservatives, has caused the food industry to look for a solution that, besides increasing the products' shelf life, satisfies the demands of consumers (Zhang et al., 2021). Nowadays, using natural additives, besides increasing organoleptic properties, can add unique features to foods that increase their shelf life. Among these natural additives, are essential oils and extracts. Some essential oils have potent antioxidant and antibacterial properties and studied as potential alternatives to chemical antioxidants in the meat industry (Soltaninezhad et al., 2020). Alizadeh Behbahani et al. (2021) reported the effect of using an edible coating, based on *Lepidium perfoliatum* seed mucilage (LPSM) containing chicory essential oil (CEO), on the quality and shelf life of beef cuts during 7 days of storage at 4°C. According to them, beef cuts that had CEO-LPSM coating, showed a significant inhibitory effect on lipid oxidation and microbial growth. In addition, beef cuts coated with CEO-LPSM had higher sensory scores during storage. Heydari et al. (2020) conducted a study on the effect of edible coating based on the Qodume Shirazi seed mucilage that contained lavender essential oil (LO). The result of their study showed that, from the point of view of microbiology, the duration of cold storage for the control sample and the coated sample without essential oil, was only 3 days, while it was 3, 6 and 9 days for coated samples

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containing 0.5, 1, 1.5 and 2% essential oil, respectively. Coated ostrich meat containing 2% LO had good quality with long shelf life. Plant essential oils (EO) are obtained from various plant parts using physical or chemical techniques. EO are rich source of bioactive compounds (Farshi et al., 2017). It improves the smell and taste of foods (Hashmi et al., 2008) and has antibacterial, antioxidant and antifungal properties (Allahqadari et al., 2010). These materials have limitations in the presence of light, ambient and high temperature, as well as in the presence of other environmental factors (Bettaieb et al., 2010; Nadeem et al., 2012). Nanoencapsulation of essential oil seems to be an attractive new idea to overcome these limitations. Amiri et al. (2018) believe that it is useful to use carriers to encapsulate these compounds. Encapsulation of bioactive compounds is an effective way to protect EO against environmental conditions (Hashmi et al., 2009; Yousefi et al., 2019). The use of biodegradable carriers has been developed to nanoencapsulate bioactive compounds (Bochicchio et al., 2016; Mohammadi et al., 2020). During encapsulation, a bioactive compound such as essential oil is placed inside. Nanoemulsion-based delivery system is one of the methods of encapsulation (Jemaa et al., 2019). During the past decades, the use of nanoemulsions as antimicrobial agents, has been considered as a new and promising innovation because these compounds, have broad inhibitory effects against bacteria, enveloped viruses and fungi through disrupting the outer membrane. Nanoemulsions are thermodynamically suitable for fusing with lipid membranes. This effect is increased by electrostatic attraction between the cationic charge of the nanoemulsion and the anionic charge in the pathogens and it leads to cell lysis and the death of the pathogen because the performance of nanoemulsions is in the form of a non-specific disruption of the bacterial membrane. As a result, a resistant strain is not created and thus, they have been considered a promising antibacterial agent (Guerra-Rosas et al., 2016). Hashemi et al. (2023), investigating the effect of antimicrobial and antioxidant activity of nanoemulsion and free *Carum copticum* essential oil on the shelf life of rainbow trout burgers, stated that comparatively speaking, lipid oxidation, total volatile nitrogen production and microbial growth were lower in coated fish burger samples with alginate which were made rich with CEO nanoemulsion. The use of alginate coating with CEO nanoemulsion can be helpful to extend the fish burgers' shelf life reserved at 4°C. After investigating the antibacterial, antioxidant, and sensory features of *Ziziphora clinopodioides-Rosmarinus officinalis* essential oil nano encapsulated by applying sodium alginate in lamb meat patties during cold storage for 12 days, Karimifar et al. (2023) stated that the encapsulated nanoparticles can inhibit the growth of *Escherichia coli*. Inoculated *Staphylococcus aureus* and O157:H7 significantly reduced and delayed lipid oxidation of lamb patties in comparison with samples with REO and control samples. In addition, the nanoparticles effectively reduced the odor formation and discoloration in patties. Thus, NaAlg-Z-REO nanoparticles can minimize sensory deterioration or oxidative and bacterial growth while storing. Rosemary plant, scientifically known as *Rosmarinus officinalis*. L is a medicinal plant from the mint family. The antimicrobial and antioxidant features of rosemary plants have been known for about 30 years. During this time, many studies were conducted on this plant, all of which confirmed its antibacterial and antioxidant properties. The antimicrobial effect of rosemary plant on *Staphylococcus epidermidis, Staphylococcus aureus, Proteus vulgaris, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Escherichia coli, and Bacillus subtilis* has been proved in laboratory studies. About the antioxidant power, high anti-radical force and an inhibitory effect on the formation of hydroperoxides have been attributed to rosemary leaves. The most significant active ingredient in rosemary extract is carnosol. Also, other phenolic compounds, such as epi-rosmanol and iso-rosmanol, rosmarinic acid, and carnosic acid have been isolated from rosemary leaves. In the studies conducted on red meat, it has been proved that rosemary extract, not only prevents microbial spoilage and lipid oxidation but also prevents changes in the color of the meat during the storage period and increases the quality of the meat in terms of sensory factors (Hosseini et al., 2020; Alves et al., 2021; Mohsenabadi et al., 2018). In the present study, we analyzed rosemary essential oil (REO) and rosemary essential oil nanoemulsion (NREO) in Beef Burgers shelf life at refrigerator temperature.

2. Material and Methods

2.1. Extraction and purification of REO

The Rosemary plant was collected from the northwest region of Tehran in August. Its scientific name was confirmed at the Medicinal Plants Research Center, and at room temperature, in a dark place, its leaves and flowering branches were dried, and after grinding, the water essential oil was boiled. The grinded plant was extracted with water in a Clevenger device in the laboratory at a ratio of 1 to 10 for one hour. Then the obtained essential oil was dried under a vacuum rotary evaporator to reach the desired percentage. The obtained essential oil was stored in closed containers protected from light at 4°C (Hoseini et al., 2015).

2.2. Identification of essential oil constituents

To analyze REO qualitatively and quantitatively, the prepared sample was injected into GC-MS i.e., a gas chromatography with a mass spectrometer. The specifications and conditions of the GC-MS device are as follows: HEWLETT HP-6890: GC model, PACKARD, America, column type HP-5MS, column dimensions include 30 meters of length, 0.25 mm diameters and 0.32 microns of film thickness, column temperature programming, 60 – 220 ºC temperature of the injection site, 250 ºC helium carrier gas with a 1 milliliter flow rate every minute. Finally, according to the regular alkanes exit pattern, inhibition index, Coates index, and matching them with library patterns, the spectra related to each body were interpreted, and the constituent compounds of the essential oil were identified (Jafarzadeh et al., 2010).

2.3. Production of NREO

First, REO as an oil phase was mixed well with Tween 80 (Sigma Aldrich, Germany), a surfactant, applying a magnetic stirrer. Then the slightly acidified distilled water with citric acid (0.3%) made the aqueous phase and after that surfactant and oily phase mixture was gradually added to it on a magnetic stirrer with a rotational speed of 700 revolutions per minute to make an emulsion premix ready. Then, for 20 minutes, the prepared emulsion premix was subjected to ultrasonic waves in an ultrasound bath (frequency of 40 kHz and power of 100 watts) in order to break down the particles and produce nanoemulsion (Anuchapreeda et al., 2012).

2.4. REO and NREO tests

2.4.1. The average volume diameter of NREO drops

To gauge mean diameter of nanoemulsion droplets, initially, the nanoemulsion was diluted with a ratio of 1 to 50 to prevent multiple scattering. The size of the droplets was determined by Malvern DLS Ltd Instruments at a temperature of 25 ºC and an angle of 90 degrees (Ochtepe et al., 2016).

2.4.2. Investigating the antioxidant property of REO and NREO

In this technique, 0.2 ml of nanoemulsion was combined with 4 ml of 60 µM DPPH free radical methanolic solution and maintained for 1 hour at room temperature. After that, the solution's absorbance was read at a wavelength of 517 nm applying a spectrophotometer. Also, control sample was a sample with 0.2 milliliters methanol along with 4 milliliters DPPH, and with the help of equation number 1, the radical activity of scavenging was measured (Rajaei, 1390).

$$\text{Radical inhibition percentage} = \frac{\text{Sample absorption} - \text{Control absorption}}{\text{Control absorption}} \times 100 \quad (1)$$

2.4.3. Evaluation of total phenol content (TPC) of REO NREO

Phenolic compounds of REO were measured applying the Folin Ciocalteu method and gallic acid as standard. To achieve this, 10 milligrams of essential oil were added to 3 milliliters of distilled water, 2 milliliters of sodium carbonate solution (with a concentration of 75 grams per kilogram) and 0.25 milliliters of Folin Ciocalteu agent, and put in a hot water bath. It was placed in a greenhouse at 37 ºC for 30 minutes. Then, the sample's absorbance was read by a spectrophotometer at a wavelength of 765 nm. All the tests were performed in three repetitions, and the outcomes were stated regarding gallic acid (mg/g) as an index of phenolic compounds (Waterhouse et al., 2002).

2.4.4. Minimum bactericidal and inhibitory concentration of prepared REO and NREO

The minimum inhibitory concentration was gauged through the Broth Microdilution Method. The fresh overnight culture of the tested microorganisms was concentrated twice to prepare Mueller Hinton Broth (MHB) cell suspension for bacterial strains to get 103x1-2 cells/ml and 106 colony-forming units (CFU/ml). The studied essential oil was placed in 4% DMSO i.e., dimethyl sulfoxide and after that 100 µl of each dilution was placed in microwaves and separately inoculated using 100 µl bacterial suspension. After thorough mixing, the inoculated microplates were incubated at 37 °C for 24 hours. The lowest concentration to inhibit the significant growth of microorganisms was the minimum inhibitory concentration. To specify the minimum microbial concentration, 100 microliters of a portion of the broth was taken from each well, and then expanded on Mueller Hinton agar culture medium, incubated at 37 °C for 24 hours for bacteria incubation. The lowest concentration at which the incubated microorganisms were ultimately killed (99.9% reduction in CFU/ml in comparison with the control), defined as MBC or MMC. The positive antimicrobial control in this study was Gentamicin (Dra et al., 2017; El Hamdaoui et al., 2018).

2.4.5. The agar well diffusion test of REO and NREO

Bacterial strains and fungi tested as standard, including Gram-positive *Staphylococcus aureus* PTCC1112 (ATCC 6735) and Gram-negative *Escherichia coli* PTCC1330 (ATCC 8739) were obtained in lyophilized form the Scientific and Industrial Research Organization of Iran. Microbial samples were recovered based on standard methods. From a new microbial culture, a few colonies were moved to a sterile Mueller Hinton broth culture medium so that the turbidity of the resulting cultures was visually similar to the turbidity of a 0.5 McFarland tube (1.5×108 bacteria per milliliter) after vortexing. Then, the swap added to the microbial suspension was uniformly spread on the plate with the Mueller Hinton Agar culture medium. Next, pits or wells were made on the surface of the container by a sterile Pasteur pipette having 5 mm diameter and 2 cm distance from each other. We used different dilutions of essential oil/nano essential oil to fill each well. For 30 minutes, every examined plate was placed at room temperature and after that for one day, they were put in a 37 °C incubator. Then, the bacterial culture was measured in milliliters in terms of the formation or non-formation of inhibition zone, and their average was calculated (Dra et al., 2017; El Hamdaoui et al., 2018).

2.5. Preparing enriched hamburgers

For the preparation of hamburger samples, an equal amount of beef, obtained from the Qalugah and Sardast areas, was used. At first, excess fat and tallow, tendons and remaining pieces of bone were separated from the meat as much as possible, and then divided into pieces of about 250 grams. Then the prepared meat parts were transferred to the meat grinder and ground. Then along with other raw materials such as spices (black pepper, white mustard and ginger), salt, breadcrumbs, garlic, and onion, and after measuring the properties of nanoemulsions, 0.1% REO and 0.5% NREO were added to the hamburger material, and transferred to the cutter. Next, the produced dough was transferred to the hamburger compartment, and by a special mechanical device, samples weighing 100 grams were prepared, and placed between wax papers in polyethylene packaging for 21 days at 4 °C.

2.6. Hamburger tests

2.6.1. pH

After mixing 15 grams of burger sample with 150 milliliters of deionized water for 2 minutes, we gauged the pH on the obtained suspension using a pH meter. It should be noted that the pH meter was calibrated with 0.4 and 0.10 buffer solutions before use (Fernández-López et al., 2006).

2.6.2. Peroxide value

We solved fifty grams of hamburger in 150 milliliters of methanol and chloroform solution (in a ratio of 2:1) in a blender. After filtering the mixture, we added 50 ml of potassium chloride solution. 20 minutes past decanting and we collected the lower phase i.e., the organic phase. Then, we added 100 milliliters of methanol/potassium chloride solution (1:1 ratio) to it. Decanting was done for the second time, and the lower phase was separated. The solvent was evaporated using Ben-Marie at a temperature of 35 °C, and finally, we used the obtained oil for testing the peroxide number (Heydarian et al., 2014).

2.6.3. Lipid oxidation (TBARS)

Fat oxidation was evaluated based on the thiobarbituric acid test (TBARS). For this purpose, we mixed 20 grams of hamburger meat with 50 ml of 20% trichloroacetic acid for 2 minutes. The contents of the mixer were washed and mixed with 50 ml of water and after that filtered applying Whatman No. 1 filter paper. Then, 5 ml of the extract was mixed with 0.01 M 2-thiobarbituric acid and kept at 100 °C for one hour. Then we measured the absorbance of the pink color solution applying an ultraviolet-visible spectrophotometer at 532 nm. TBARS was reported as milligrams of malonaldehyde per kilogram of burger sample (Soltanizadeh & Ghiasi, 2015).

2.6.4. Microbial characteristics

10-gram samples of raw burgers were weighed aseptically, then homogenized with 90 ml of 0.1% sterile peptone water in a Stomaker or pulser for 1-2 minutes at room temperature. We prepared decimal serial dilutions for every sample in 0.1% peptone solution and 1 ml or 0.1 ml of the samples in appropriate dilution during two repetitions, mixed culture or surface, respectively to perform the total microbes count test. Based on the national standard 5272 and selected agar plates. The counting total live mesophyll microbes and psychrotrophs in the Kant agar plate culture medium were determined. The plates were placed in a greenhouse (or incubated) at 30 °C for 48 hours to count the total organisms and at 7 °C for ten days to count the psychrophilic organisms. The total number of forms was specified in violet red bile glucose agar culture medium after it was incubated for one day at 37 °C. We counted both the plates that had 30-300 colonies and salmonella based on Iranian standard No. 1810, Staphylococcus aureus using Brad Parker agar culture medium according to Iranian standard No. 8606-1, mold and yeast using yeast extract-dextrose-caramphenicol agar culture medium and incubation for 3-5 days at 25 °C based on Iranian standard No. 997 and Escherichia coli using the maximum possible number (MPN) according to Iranian standard No. 2946. *Salmonella*, coliforms, *Staphylococcus aureus*, *Escherichia coli*, yeast, and mold, were counted only on Burger's control samples to determine their health status (Fernández-López et al., 2006).

2.6.5. Sensory evaluation by the 5-point hedonic method

The sensory assessment of various cheese treatments was specified applying the hedonic test (the level of liking or enjoyment) on a five-point scale based on the parameters of taste, color, texture, and smell of the samples. The samples were given to the evaluators (30 untrained assessors between the ages of 20 and 35 from both genders) with a blind three-digit code (a table with a random number) in a complete block design. The samples were presented to the assessors with a specific evaluation questioner. The assessed sensory characteristics characterized color change (5, no color change, 1, severe color change); smell (5, very good; 1, very unacceptable or bad smell) and taste (5, very tasty; 1, no good taste), texture (5, hard; 1, very soft) and the average of the scores as Overall acceptance (5, very favorable, 1, very unacceptable) was determined (Bazargani-Gilani et al., 2015; Ojagh et al., 2010).

Table 1. The results of the amount and type of REO compounds.

Composition (µg/g.dw)	Concentration (%)	Rt (min)
3-Octanone	0.99	9.06
Tricyclene	0.66	7.87
α-Pinene	12.32	8.15
Camphene	9.21	8.16
Verbenone	0.56	8.59
Octen-3-ol	2.59	8.85
Borneol	6.11	13.67
Myrcene	0.92	9.17
α-Phellandrene	1.88	9.66
α-Terpinene	0.48	9.93
β-Cymene	1.03	١٩/١٤
Limonene	8.11	19.81
(E)-β-Ocimene	0.76	10.86
Linalool	1.05	11.67
Camphore	7.11	13.09
Verbenone	5.21	14.54
Terpinen-4-ol	1.32	14.71
α-Terpineol	3.72	13.94
α-Humulene	1.16	19.46
Linalyl acetate	2.75	15.14
Bornyl acetate	6.18	15
(E)-Caryophyllene	2.94	18.82
Neryl acetate	0.22	18.93
4-terpineol	2.88	19.14
1,8-Cineole	12.02	10.37

2.7. Statistical analysis method

The outcomes of the experiments for the experimental data were represented as the mean ± standard deviation of the measurements with 3 repetitions. Colony forming units (CFUs), in all experiments were converted to their logarithmic values before statistical analysis. The experimental data were compared with one-way ANOVA. Statistically significant differences between mean values (in cases where the overall effect of treatments is significant) were determined using Duncan's multi-range follow-up test. Statistical tests of the obtained results were performed using SPSS version 26 software. A critical level of $p \leq 0.05$ was considered for all data comparisons.

3. Results and Discussion

3.1. Analysis results of the phenolic compounds of REO

Based on the obtained results (Table 1), 25 compounds were recognized in REO, and the most compounds in order of abundance were α-pinene (12.32%), 1,8-Cineole (12.02%), Camphene (9.21%), limonene (8.11%), Camphor (7.13%), Bornyl acetate

(6.18%), Borneol (6.11%) and Verbenone (5.21%). Malkotian et al. (2012), analyzing the chemical composition of REO, acknowledged that 20 compounds were identified, and 82.09% of these compounds included 1-8 cineol, Borneo, alpha-pinene, camphor, linalool, camphene, limonene, and verbenone. Salek Mearaji et al. (2018) analyzed the constituents of REO. They stated that the most parts of REO are alpha-pinene at 25.4%, 1% and 8-cineole at 22.6%, and borneol at 12.4%, respectively.

3.2. *The results of measuring the particle size and zeta potential of REO by DLS*

The results showed that the particle sizes were 87.95% (33.81 nm), 6.85% (37.64 nm), 3.45% (54.75 nm), 1.05% (58.77 nm) respectively), 0.52% (33.37 nm), 0.18% (65.16 nm) (Fig. 1). The smallness of the droplet size and the unique properties of nanoemulsions have made advantages for their use in many applied technologies and lead to a more extended period of their physical equilibrium, they also sometimes have thermodynamic stability and are considered to be mature. The tiny size of the nanoemulsion droplets also prevents the phenomenon of creaming and sediment formation during storage since their small size prevents them from joining together and making surface flocculation. Also, due to the very fineness of the droplets, nanoemulsions have a large specific surface area. For this reason, they have a very high permeability, which has turned them into an effective transfer system.

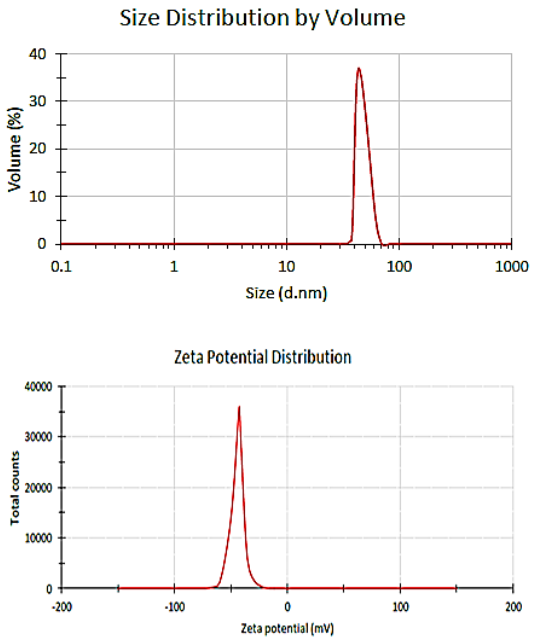

Fig. 1. DLS peaks and zeta potential of the samples.

3.3. *Comparison of the inhibition zone diameter, minimum inhibitory and bactericidal concentration of REO and NREO against Staphylococcus aureus, and E. coli*

The outcome of this research revealed that the minimum inhibitory, and bactericidal concentration against *E. coli* and *Staphylococcus aureus* for REO was significantly lower and the inhibition zone was greater than that of NREO ($p \leq 0.05$). On the other hand, for both cases (REO and NREO), the MIC and MBC against *Staphylococcus aureus* were less and the inhibition zone was greater than *E. coli* ($p \leq 0.05$). The MIC levels of REO and NREO were 0.4 ± 0.16 and 0.8 ± 0.33 mg/ml against *Staphylococcus aureus* and 6±0.25 and 16 ± 0.66 against *E. coli*, respectively. Also, the amount of MBC of REO and NREO was determined as 8 ± 0.33 and 8 ± 0.33 mg/ml against *Staphylococcus aureus* and 10 ± 0.41 and 16 ± 0.66 against *E. coli*, respectively. The result can also be because of the variation in cell wall composition between gram-positive and gram-negative bacteria. Hence, gram-negative bacteria possess a thick lipopolysaccharide layer in their outer membrane that covers the cell wall. In comparison to gram-positive bacteria that have only one layer of peptidoglycan in their design, the structure of gram-negative bacteria, makes the mentioned bacteria more resistant to essential oils. MBC, the minimum bactericidal concentration, is the least concentration of an antimicrobial substance that causes the death of a microorganism, and the minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial substance that has an inhibitory effect on the growth of a specific microorganism. This means that the microorganism exists in the environment, but it cannot multiply. The decrease in the number of microorganisms in these conditions is not due to the bactericidal effect of the extract. Still, it is due to the microorganism reaching the death phase, when it no longer multiplies, and its number decreases (Anonmous, 2009). Essential oils and plant extracts are widely used in the food industry and have antimicrobial properties against various microorganisms (Burt, 2004). Different opinions exist about the mechanism of essential oils, and plant extracts on microorganisms. Considering the numerous chemical groups in the components of essential oils and plant extracts, their antimicrobial activity is not due to a certain mechanism and these substances affect multiple targets in the cell (Bagamboula et al., 2003). Celiktas et al. (2007) have reported the minimum concentration inhibiting the growth of *E. coli* as 40. Pintore et al. (2007) reported it as 1, and Zizovic et al. (2009) reported it to be 12.8 to 32 mg/ml for different rosemary species. The outcomes revealed that, in a specific concentration of REO, the diameter of the non-growth zone was 19 ± 0.6 mm. NREO showed a non-growth diameter of 11 ± 0.5 mm against *Staphylococcus aureus*. Also, in the specific concentration of the REO, the diameter showed a lack of growth equal to 0.6 ± 16 mm in the NREO and a lack of growth equal to 6 ± 0.03 mm against *E. coli*. REO against *E. coli* and *Staphylococcus aureus* shows that during the study of Gachkar et al. (2007), the diameter of the inhibition zone was found to be 16 ± 0.7 mm, which is in line with the outcomes of the present research.

3.4. *Comparison of total phenol and antioxidant activity of REO and NREO*

The total phenol content and antioxidant activity of REO were significantly higher than those of NREO ($p \leq 0.05$). The inhibition percentage of essential oil (IC_{50}) is a concentration of essential oil that can trap or inhibit 50% of free radicals; its lower values indicate higher antioxidant activity of the extract. In all plants, antioxidant activity is directly linked to the amount of flavonoid and phenolic compounds. DPPH stable base species, which are stable free bases soluble in methanol, widely used to estimate and

investigate the oxidation capacity of various compounds. The DPPH free radical has a purple color and a strong and distinct color at 517 nm, and it can react with antioxidant compounds without oxygen and produce a sturdy yellow DPPH-H molecule by absorbing a hydrogen atom, which can be easily detected with a UV spectrophotometer and investigated. The property of free radical scavenging is one of the known mechanisms in preventing lipid oxidation by antioxidants (Netzel et al., 2007). Plants have many compounds that are of various structures. The compounds' extraction depends on various factors, the most significant of them are the solvent and extraction method. Phenolic compounds in vegetables and fruits fascinated many scholars because they have high potential for antioxidant activity. Phenolic compounds barricade free radical activity by donating hydrogen atoms. The total phenolic compounds in REO and NREO were 11.434 (mg/g) and 2.054 (mg/g), respectively.

3.5. *Test results of hamburger samples during the storage period*

3.5.1. *pH changes*

The pH of fresh meat after slaughter is determined according to the lactic acid produced from anaerobic glycogen, and the final pH in fresh meat is 5.5 (Sultani & Kadior, 2013). The pH of meat is considered to be one of the critical quality factors. Because the stability of meat color, smell, taste, crispness, and edible quality affects the degree of spoilage with microbial enzymes and also the growth of micro-organisms (Unal et al., 2014). The pH value is one of the critical influencing factors in the emulsion strength of meat products, and hamburgers. Any change in the pH of the product harms its functional characteristics, together with water retention capacity (Moradi et al., 2012). The results of the present research, revealed that the pH of the samples on days 1 and 3 ($p > 0.05$) was almost the same and on the other days under investigation, sample 1 (control sample) had the lowest pH value and the highest pH value belonged to sample 3 (REO nanoemulsion hamburger) ($p \leq 0.05$). From the 1st day to the 28th day, the pH of all samples abated significantly ($p \leq 0.05$) (Table 2). According to the report of Garrido et al. (2011), it was on pork hamburger pieces. The comparison of the mean values showed that the highest pH value in the essential oil treatment related to the NREO treatment was 5.79, and the lowest pH value was 5.72 in the control treatment, which showed a significant difference. Among the observation times, the highest value was received at zero time at the rate of 5.86, and the lowest at the 28th day at the rate of 5.64. The outcomes revealed that adding free and micro-coated essential oil to the hamburger formulation had a significant effect on preventing the decrease in the pH of the samples, which is due to the antimicrobial effects of REO and NREO in hamburger. Biswas et al. (2004), obtained similar results. They observed that the pH of the control samples was significantly higher than the pH of the other samples during storage time as well as during the whole study time, so the controlled release increases the functional activity of the control sample to an extended period. Takma et al. (2019) noticed similar outcomes for the effect of active film containing black cumin essential oil on the pH of chicken breast meat (Ozvural et al., 2019). The effect of eatable coating with green tea extract, on the pH of hamburger samples was consistent with the outcomes of the present study. Analogues outcomes were acquired by Vilela et al. (2016) regarding the effect of adding rosemary and bay leaf extract on minced veal meat. The results of the treatments containing finely coated essential oils, were better therefore on the 21st day. The lowest pH values were maintained in the hamburger containing NREO and the highest amounts were reported in the control treatment. The cause of this is the growth in the antibacterial property of NREO or maintaining the stability of the antibacterial features of the essential oil for a more extended time after encapsulation. Thus, REO and NREO barricade the creation and development of amine and nitrogenous compounds that cause spoilage in hamburger samples. With the passage pf time, the pH of all models decreased considerably ($p \leq 0.05$). Such a reduction in the pH value indicates the growth of LAB bacteria in the hamburger which is kept in the refrigerator. LAB bacteria are the most essential factor in reducing the pH in the hamburger samples kept in the fridge. The initial reduction in pH is mainly because of the growth of lactic acid bacteria and the accumulation of lactic acid (Sedaghat et al., 2015). In 1995, FAO announced in a report that the pH of food, can be a good indicator of the health and safety conditions of nutrition. According to the report of the Codex Organization in 2003, the pH of animal meat should be less than 7-7.5 for this food item to be safe for consumers (Ojagh et al., 2010). As the results of this research showed, pH changes during the storage period had a decreasing trend, mainly due to microbial and enzyme activities during the storage period. The trend of pH changes is directly related to the trend of microbial population changes in the target sample; The results obtained from pH changes were utterly consistent with the results of the total microbial population test; in this way, the samples containing REO and NREO had a slighter pH decrease than the control sample due to the lower microbial population.

3.5.2. *Peroxide value*

On the first day, there was no noticeable statistical difference in the amount of Peroxide value in the samples ($p > 0.05$), and on the other days under investigation, sample 1 (control sample) had the highest amount of peroxide value and the lowest amount of Peroxide value belonged to Hamburger containing NREO ($p \leq 0.05$) (Table 2). From the 1st day to the 28th day, the amount of Peroxide value in all samples increased significantly ($p \leq 0.05$). Generally, the Peroxide value is milliequivalent grams of peroxide or active oxygen present in one kilogram of oil or fat sample. Peroxide value is an indicator to show the amount of oxidative damage in oils and fats and in oxidation, peroxide or active oxygen is produced in oils. The presence of peroxide in oil as a catalyst accelerates oxidation. The most common method of measuring the intensity of oxidation for the initial stages of oxidation is Peroxide value. Since peroxides are tasteless and odorless compounds, consumers are not able to recognize them. Still, the mentioned compounds motivate the organization of secondary compounds like aldehydes, ketones, and alcohols, which are more stable during the heat process and cause foul odor and bad taste (unpleasant sensory characteristics) in the product (Ozyurtr et al., 2011). During the storage period, oxidation of lipid in meat is a cause of decreasing the quality of meat. Free radicals in meat create aldehydes that expand and develop the taste of fat spoilage and change the color of meat. Lipid oxidation in meat has a complex mechanism. During this process, besides the adverse effects on color and taste, the protein solubility are reduced too, and finally, the nutritional value of the product decreases (Shahidi & Zhong, 2005). Also, the results showed that the highest Peroxide value is related to the control hamburger samples. The mentioned outcomes are in line with the

findings of Jalili et al. (2013). The team investigated the effects of modified atmosphere packaging and green tea aqueous extract on the shelf life of silver carp minced meat at fridge temperature. The outcomes showed that the fish shelf life is longer in samples containing essential oil. In this research, hamburger containing NREO produced less peroxide, and with nano emulsification of REO, the amount of peroxide value reduced dramatically. The findings are in line with the report of Heydarian et al. (2014). During the study of the effects of basil essential oil on the quality of chicken fillets while storing, they announced that the Peroxide value was always higher in the samples without essential oil. The phenolic compounds in the extracts, essential oils of plants and fruits, all have antioxidant properties. REO applied in the formula has prevented the oxidation of fat in samples containing extracts and this is due to the effect of phenolic compounds in REO (Maqsoudloo et al., 2015). According to Nasiri et al. (2013), the peroxide value increases with time, and the result obtained in this research is consistent with the results of the upcoming work. Ucak et al. (2011) studied the effect of REO on mackerel fish. They reported that changes in the Peroxide value were observed in all groups during the storage period. In the research results of Al-Belushi et al. (2005) in the study of *Argyrosomus heinie* fish burgers during three months of storage at -20 °C, it was stated that the amount of Peroxide value was associated with slight changes at the beginning of the first two weeks and after that until the second month, it increased. Then it showed slight modifications.

3.5.3. Thiobarbituric acid

The results of this research showed that on the first day, there was no important statistical difference in the amount of thiobarbituric acid in the samples ($p > 0.05$), and on the other days under investigation, sample 1 (control sample) had the highest amount of thiobarbituric acid, and the lowest amount of thiobarbituric acid belonged to hamburger containing REO ($p \leq 0.05$) (Table 2). The amount of thiobarbituric acid (TBA) is extremely applied to show the point of lipid oxidation, and TBA active compounds indicate the second stage of oxidation, when peroxides are oxidized to aldehydes, ketones, and alcohols. Along with Malonaldehyde, which is able to produce red pigment with TBA, there are other aldehydes which may enter in the reaction too. Thus, the amount of secondary oxidation products is specified as thiobarbituric acid reactive compounds (TBARS) (milligram equivalent of malonaldehyde per kilogram of the sample) (Raeisi et al., 2015). The determination of TBA shows the amount of secondary oxidation products that is a sensory characteristic and significantly affects the product (Hu et al., 2005). So that the threshold of perception of pungency was caused by oxidation by consumers is 0.5 mg of malonaldehyde per kilogram of meat product (Sheard et al., 2000). In the current research, the increase in the antioxidant activity of essential oils is probably due to the increase in the ability to distribute and spread in the fat phase and the protective effects of nanocoating on REO in hamburgers. The micro coating of REO increased the antioxidant activity of the essential oil. Also, it maintained the effects of the essential oil in preventing the secondary reactions of fat oxidation until the 28th day of storage. Djenane and Roncalés (2018) conducted an observation in the storage of minced meat during storage at a temperature of 9 °C which showed that the amount of TBA on the last day of storage was 1 and 2.5 mg per kilogram and 3.6 in the control sample. Zengin et al. (2014), in studying the effect of clove and thyme essential oil, observed that the formation of secondary oxidation products in minced meat significantly reduced in comparison with the control sample, which was because of the high anti-radical and revitalizing activity of this essential oil. Tajik et al. (2015), in the study of thyme essential oil on buffalo meat burger, obtained similar results too. Research has proven that the phenolic compounds in plants have antioxidant properties and lead to postponing the fat oxidation and thus fat reduction through inhibiting the formation of malondialdehyde. Dezhban et al. (2014), while investigating the changes in TBA number in silver carp fish samples containing REO, announced that with increasing the concentration of REO, the number of thiobarbituric acids decreased, and the samples without essential oil were statistically very different. The compounds in essential oils are good electron and proton givers, and their close radicals are really stable because electron moves in the benzene ring and does not have a site sensitive to oxygen attack. The REO compounds include carnosol, carnosic acid, rosmannone, rosemary null and rosemary moninone in the essential oil. They neutralize free radicals and can also prevent metal ions like Fe^{+2}, therefore the speed of active oxygen molecules building, decreases (Mohamed et al., 2012).

Table 2. pH, pV, TBA changes of hamburger samples during the storage period.

		Control	Burger with REO	Burger with NREO
pH	Day 1	5.88± 0.00 ^{aA}	5.86± 0.01 ^{aB}	5.85± 0.00 ^{aA}
	Day 3	5.80± 0.02 ^{aB}	5.81± 0.01 ^{aB}	5.82± 0.01 ^{aB}
	Day 7	5.75± 0.05 ^{bC}	5.79± 0.01 ^{aC}	5.80± 0.01 ^{aC}
	Day 14	5.63± 0.01 ^{cD}	5.74± 0.01 ^{bD}	5.76± 0.01 ^{aD}
	Day 28	5.53± 0.00 ^{cE}	5.68± 0.01 ^{bE}	5.72± 0.00 ^{aE}
pV	Day 1	0.49± 0.01 ^{aE}	0.48± 0.01 ^{abE}	0.46± 0.01 ^{bE}
	Day 3	0.75± 0.01 ^{aD}	0.60± 0.01 ^{bD}	0.51± 0.02 ^{cD}
	Day 7	1.02± 0.01 ^{aC}	0.87± 0.01 ^{bB}	0.62± 0.01 ^{cC}
	Day 14	1.47± 0.02 ^{aB}	1.07± 0.02 ^{bB}	0.84± 0.04 ^{cB}
	Day 28	1.73± 0.02 ^{aA}	1.37± 0.02 ^{bA}	1.07± 0.02 ^{cA}
TBA	Day 1	0.14± 0.01 ^{aA}	0.15± 0.02 ^{aA}	0.14± 0.01 ^{aA}
	Day 3	0.55± 0.01 ^{aA}	0.41± 0.01 ^{bA}	0.37± 0.01 ^{cA}
	Day 7	0.98± 0.01 ^{aA}	0.75± 0.01 ^{bA}	0.57± 0.00 ^{cA}
	Day 14	1.16± 0.02 ^{aA}	0.97± 0.02 ^{bA}	0.78± 0.01 ^{cA}
	Day 28	1.51± 0.05 ^{aA}	1.37± 0.02 ^{bA}	`1.17± 0.02 ^{cA}

Various capital letters show an important difference in the column, and other small letters show an essential difference in the row ($p \leq 0.05$).

Table 3. Changes in the microbial population in hamburger samples during the storage period (Log CFU/g).

		Control	Burger with REO	Burger with NREO
Mesophile	Day 1	3.22± 0.02 ^{aD}	3.12± 0.00 ^{bD}	3.13± 0.02 ^{bD}
	Day 3	5.38± 0.02 ^{aC}	4.38± 0.00 ^{bC}	4.14± 0.02 ^{cC}
	Day 7	7.28± 0.02 ^{aB}	6.23± 0.00 ^{bB}	5.78± 0.07 ^{cB}
	Day 14	9.76± 0.06 ^{aA}	8.46± 0.10 ^{bA}	8.45± 0.06 ^{bA}
	Day 28	10.24± 0.02 ^{aA}	8.91± 0.03 ^{bA}	8.45± 0.04 ^{cA}
Psychophile	Day 1	2.68± 0.03 ^{aA}	2.41± 0.05 ^{bA}	2.46± 0.04 ^{cA}
	Day 3	4.32± 0.02 ^{aA}	3.23± 0.03 ^{bA}	3.11± 0.02 ^{cA}
	Day 7	5.92± 0.02 ^{aA}	4.88± 0.02 ^{bA}	4.37± 0.06 ^{cA}
	Day 14	7.64± 0.07 ^{aB}	4.88± 0.02 ^{aB}	7.30± 0.09 ^{bB}
	Day 28	9.27± 0.01 ^{aA}	8.14± 0.01 ^{bA}	7.84± 0.01 ^{cA}
Coliform	Day 1	2.52± 0.02 ^{aA}	2.37± 0.07 ^{bA}	2.41± 0.03 ^{abA}
	Day 3	3.83± 0.02 ^{aA}	2.94± 0.00 ^{bA}	2.81± 0.02 ^{cA}
	Day 7	5.61± 0.04 ^{aA}	4.83± 0.05 ^{bA}	4.49± 0.05 ^{cA}
	Day 14	9.17± 0.00 ^{aA}	7.92± 0.03 ^{bA}	7.69± 0.01 ^{cA}
	Day 28	7.64± 0.07 ^{aA}	7.56± 0.05 ^{aA}	7.30± 0.09 ^{bA}
Salmonella	Day 1	Negative	negative	negative
	Day 3	Negative	negative	negative
	Day 7	Negative	negative	negative
	Day 14	Positive	negative	negative
	Day 28	Positive	negative	negative

Various capital letters show an important difference in the column, and other small letters show an essential difference in the row ($p \leq 0.05$).

Table 4. Changes in sensory evaluation in hamburger samples during the storage period.

		control	Burger with REO	Burger with NREO
Odor	Day 1	5± 0.00 ^{aA}	5± 0.00 ^{bA}	5± 0.02 ^{aA}
	Day 3	3± 0.00 ^{cA}	3.75± 0.00 ^{bA}	4.70± 0.57 ^{aA}
	Day 7	2± 0.00 ^{cA}	2.75± 0.00 ^{bA}	3.33± 0.57 ^{aA}
	Day 14	2± 0.00 ^{cA}	2.75± 0.10 ^{bA}	2.33± 0.57 ^{aA}
	Day 28	1± 0.00 ^{cA}	1.70± 0.03 ^{bA}	2± 0.57 ^{aA}
Taste	Day 1	5± 0.00 ^{aA}	5± 0.00 ^{bA}	5± 0.02 ^{aA}
	Day 3	3± 0.00 ^{cA}	3.75± 0.00 ^{bA}	4.60± 0.57 ^{aA}
	Day 7	2± 0.00 ^{cA}	2.75± 0.00 ^{bA}	3.33± 0.57 ^{aA}
	Day 14	2± 0.00 ^{cA}	2.75± 0.10 ^{bA}	2.53± 0.57 ^{aA}
	Day 28	1± 0.00 ^{cA}	1.70± 0.03 ^{bA}	2± 0.57 ^{aA}
Texture	Day 1	5± 0.00 ^{aA}	5± 0.00 ^{bA}	5± 0.02 ^{aA}
	Day 3	3± 0.00 ^{cA}	3.75± 0.00 ^{bA}	4.60± 0.57 ^{aA}
	Day 7	2± 0.00 ^{cA}	2.75± 0.00 ^{bA}	3.33± 0.57 ^{aA}
	Day 14	2± 0.00 ^{cA}	2.75± 0.10 ^{bA}	2.50± 0.57 ^{aA}
	Day 28	1± 0.00 ^{cA}	1.70± 0.03 ^{bA}	2± 0.57 ^{aA}
Overal acceptability	Day 1	5± 0.00 ^{aA}	5± 0.00 ^{bA}	5± 0.02 ^{aA}
	Day 3	3± 0.00 ^{cA}	3.75± 0.00 ^{bA}	4.50± 0.57 ^{aA}
	Day 7	2± 0.00 ^{cA}	2.75± 0.00 ^{bA}	3.33± 0.57 ^{aA}
	Day 14	2± 0.00 ^{cA}	2.75± 0.10 ^{bA}	2.33± 0.57 ^{aA}
	Day 28	1± 0.00 ^{cA}	1.70± 0.03 ^{bA}	2± 0.57 ^{aA}

Various capital letters show an important difference in the column, and other small letters show an essential difference in the row ($p \leq 0.05$).

Therefore, comparing to the control, in the treatments containing REO, REO with antioxidant compounds, led to a reduction in the oxidation of fats which is in line with the outcomes of this research on the effect of REO in inhibiting fat oxidation reactions. By increasing the storage time from 0 to 28 days, the oxidation of the hamburger samples increased significantly, and this increase was significantly lower in the samples containing NREO, and in the antioxidant activity of NREO compared to similar levels and the free essential oil was more. These results were attributed to the destruction of free essential oil during sample storage and the protective effects of NREO in hamburgers, as well as the controlled release of flavonoid and phenolic compounds of the essential oil during the 28-day storage period (Taghvae et al., 2014). It can be said that the NREO expands its antioxidant property, and prolongs its effectiveness in the storage period. The outcomes of the present research are consistent with the outcomes of Rashidaie Abandansari et al. (2019) considering the effect of free and micro-encapsulated REO on the shelf life and the quality characteristics of meat while storing.

3.5.4. Results of microbial tests

3.5.4.1. Mesophilic bacteria

According to the standard, the maximum number of live mesophilic bacteria in each gram of hamburger can be 1x106 CFU/g (National Standard of Iran, No. 2304, 2015). In this research, the number of live mesophilic bacteria in all samples and all-time intervals were within the standard range. The results of this research showed that on the first day, there was no important statistical difference in the population of mesophilic bacteria in the samples ($p > 0.05$), and on the other days under investigation, the highest population of mesophilic bacteria belonged to control and the hamburger containing REO ($p \leq 0.05$) has the lowest population of mesophilic bacteria. With the passage of time, from the 1st day to the 28th day, the population of mesophyll bacteria in all samples grew considerably ($p \leq 0.05$) (Table 3). The outcomes of the studies revealed that essential oils have high antimicrobial power against the population of bacteria and spoilage agents. The NREO and REO, were influential in reducing the total quantity of bacteria during the storage time in comparison with the control one. In all the samples, the number of living mesophilic bacteria increased with rising the time of storage. However, the rate of increase in the number of living mesophilic bacteria was not the same for all samples. Regarding the difference between the samples, the samples which were treated with nano essential oil had a lower number of live mesophilic bacteria, and the number of bacteria was higher in the control sample on all the tested days. The control sample had no antimicrobial activity and when REO was added, it showed good antimicrobial activity. REO has antimicrobial properties, and acts as a source of antimicrobial substances against pathogenic pathogens. This trend indicates the increase of antimicrobial effects of NREO and also the controlled release of REO. The cause of this is the growth in the antimicrobial features of the essential oil after nanoencapsulation (Shan et al., 2007). Other researchers mentioned the growth of antimicrobial features of plant essential oils after nano-coating by various carriers (Mazandaran, 2016; Rashidi, 2019). Considering the tiny amounts of essential oils used to inhibit the growth of bacteria, it is highly likely that there will not be noticeable adverse effects on the organoleptic properties of foods (Shehina & Khaksar, 2011). In this regard, Geissman (1954) observed that oxidized phenols have a more substantial effect. The possible mechanism of these compounds, such as flavonoids and flavonols, is enzyme inhibition through responses with sulfhydryl groups or non-specific reactions with microbial proteins, like extracellular proteins, and organizing a complex with the cell wall or causing disruption in the microorganisms' cell membrane. Choulitoudi et al. (2016) investigated the antioxidant and antimicrobial activity of thymbra in the edible coating of goldfish. The researchers stated that the essential oil of thyme alone revealed moderate antimicrobial and antioxidant protection. Bukvicki et al. (2014), studying the effect of horvatii savory essential oil in laboratory conditions and local control of Listeria monocytogenes in pork meat, reported that the MIC i.e., the minimum inhibitory concentration for bacteria ranged from 0.03 to 0.57 mg/ml liter and for yeasts from 0.56 to 23.2 mg/ml, whereas the minimum concentration of bacteria/yeast MBC/MYC was from 0.07 to 1.15 and 1.11 to 57.5 mg/ml. The liter was different for bacteria and yeasts. This essential oil was more effective against bacteria than yeasts, and they generally stated that savory essential oil could be helpful for preserving and increasing the raw or processed meat products' shelf life.

3.5.4.2. Psychrophilic bacteria

The results of this research showed that on the 1st day, there was no significant statistical difference in the crowd of psychrophilic bacteria in the samples ($p > 0.05$). On the other days under investigation, the highest population of psychrophilic bacteria belonged to sample 1 (control sample) and the lowest population of psychrophilic bacteria belonged to hamburger containing NREO ($p \leq 0.05$). From the 1st day to the 28th day, the population of psychrophilic bacteria in all samples increased significantly ($p \leq 0.05$) (Table 3). The highest growth in the quantity of psychrophilic bacteria, was observed in the control sample, and the lowest increase in the number of psychrophilic bacteria was observed in the hamburger containing NREO. Also, REO and NREO showed different behavior in reducing the number of psychrophilic bacteria, which can be because of the fact that coated nano drone is more useful and also because of the better protection of bioactive compounds in coated nano drone type. Ibrahim Salam (2007) reported that psychrophilic bacteria are the most critical group of responsible microorganisms and that there is spoilage in products kept at low temperatures. The effect of essential oils such as thyme and marjoram, which contain high thymol and carvacrol, on the sum total of cold-prone bacteria in meat has also been reported (Ojagh et al., 2010).

3.5.4.3. Coliform

The present study results revealed that on the 1st day, no significant statistical difference in the coliform bacteria crowd of the samples was observed ($p > 0.05$). On the other days under investigation, the highest coliform bacteria population belonged to the sample. 1 (control sample), and the lowest coliform bacteria population belonged to hamburger containing NREO ($p \leq 0.05$) (Table 3). The coliform population in the hamburger containing REO decreased more than that of the hamburger containing NREO on the day of production. However, it was not statistically significant with the nanocoated sample. This can be attributed to the direct and unhindered contact of REO with microbes. From the 1st day to the 28th day, the coliform bacteria population of all samples increased significantly ($p \leq 0.05$). REO and NREO showed different behaviors in reducing the microbial population of this family. The reason for this can be attributed to the controlled and gradual release of effective compounds from the coated nanotubes over time. In contrast, the micro-covering sample showed a controlled and gradual release over time and reduced the bacteria population to a greater extent. The most common contaminants of meat and meat products are Enterobacteriaceae bacteria (coliforms), which produce biogenic amines due to their activity, and the formation of these products causes food poisoning and a decrease in product quality (Pasbani et al., 2016). One of the health indicators for evaluating meat and meat products is the presence of Enterobacteriaceae bacteria (coliforms). Gram-negative bacteria are generally considered resistant to natural antimicrobial compounds due to the unique feature of the outer membrane, nevertheless, the beneficial effects of adding REO, especially in the micro coated state, were very impressive in reducing the population of this group of bacteria.

3.5.4.4. *Salmonella*

The outcomes of this research revealed that in all the samples and on all the days of study, none of the samples had *Salmonella* bacteria negative. In the control sample on the 14th and 28th, *Salmonella* bacteria were evaluated as positive (Table 3).

3.5.5. *Sensory test results*

The results of this research showed that on the first day, statistically the score of smell, taste, texture and overall acceptance of the samples were almost the same ($p > 0.05$), and on the other days under investigation, the lowest score of the mentioned factors belonged to sample 1. Control sample), and the highest score belonged to hamburger containing NREO ($p \leq 0.05$). From the 1st day to the 28th day, the scores of the sensory factors of all the samples decreased significantly ($p \leq 0.05$) (Table 4). Oxidation of lipid in meat is one factor that leads to the decrease in meat quality during the storage period. Free radicals in meat cause the formation of aldehydes, that expand and develop the taste of fat spoilage and changes the color, taste and smell of meat. Lipid oxidation in meat has a complex mechanism. During this process, besides the adverse effects on color and taste, protein solubility is reduced too and finally, the nutritional value of the product decreases. In the current research, one reason for the reduction of the taste score with time can be attributed to the compounds created during the oxidation of lipids. Formanek et al. (2003) reported that rosemary extract, in addition to preventing lipid oxidation and microbial spoilage, prevents meat color changes during the storage period and increases meat quality regarding sensory factors. The reason for this was attributed to the structural compounds of the rosemary plant, its antimicrobial and antioxidant features, and the prevention of oxidative damage. Noori et al. (2018) in the study of the antioxidant and antimicrobial activity of edible coating based on nanoemulsion having ginger essential oil and its influence on the quality and safety of chicken breast fillets, stated that the highest overall acceptance score related to the fillet coated with 6% ginger essential oil nanoemulsion during storage.

4. Conclusion

This research showed that REO could delay fat oxidation in hamburger samples due to its phenolic compounds. In all the studied days, except on the 1st day, the highest amount of peroxide value, thiobarbituric acid and microbial population (mesophilic bacteria, psychrophilic bacteria and coliform bacteria of the samples) belonged to sample 1 (control sample). And the lowest amount of the mentioned factors belonged to hamburger containing NREO. The highest sensory scores (smell, taste, texture, and overall acceptance) were for hamburger which contained NREO. The hamburger containing NREO, was selected as the one with the superior treatment due to its lower microbial load, and higher sensory evaluation score.

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Not applicable.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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