Received: 2 September 2017 | Revised: 3 December 2017 | Accepted: 31 December 2017

DOI: 10.1111/jfs.12443

ORIGINAL ARTICLE

WILEY Journal of Food Safety

Development of a novel edible coating made by Balangu seed mucilage and Feverfew essential oil and investigation of its effect on the shelf life of beef slices during refrigerated storage through intelligent modeling

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Abstract

In this study, multilayer perceptron (MLP) and radial basis function network (RBFN) were employed to predict the population of microbial pathogens, chemical changes and sensory attributes of the beef slices. The chemical composition of *Tanacetum parthenium* essential oil (TPEO) was determined through gas chromatography/mass spectrometry. Disk diffusion agar, well diffusion agar, pour plate, minimum inhibitory concentration, minimum bactericidal/fungicidal concentration were used to evaluated the antimicrobial effect of TPEO. The contents of phytochemical and total phenolic compounds as well as the antioxidant activity of TPEO were also measured. Camphor with a percentage of 44.2% was the major compound of TPEO. The total phenolic content and antioxidant power of TPEO were equal to 151.2 ± 2.10 μg/ml gallic acid equivalent and 57.25 ± 0.2 μg/ml, respectively. MLP and RBFN are both capable of fitting the data and predicting. However, RBFN, due to its lower mean squared error, had a better performance than MLP.

Practical applications

In recent years, given the concerns about the risks of the consumption of chemical and synthetic preservatives, there has been a considerable tendency towards replacing them with natural preservatives. *Lallemantia royleana* seed mucilage (LRSM) is a native Iranian hydrocolloid which can be utilized in producing edible coatings and in the formulations of food products. Feverfew is a valuable medicinal plant which is used in traditional medicine for the treatment of inflammation, pain, fever and infection. In the present study, LRSM and LRSM + 1% TPEO extended the shelf life of beef up to 3 days, whereas LRSM + 1.5% TPEO and LRSM + 2% TPEO resulted in a significant shelf life extension of the samples by 9 days, as compared with the control. These results suggested that LRSM coating combined with TPEO could be used as an effective natural alternative to improve the quality of beef during refrigerate storage.

1 | INTRODUCTION

Neural networks are suitable tools in functions approximation. A multilayer perceptron (MLP) is a feedforward artificial neural network that maps between numeric inputs and targets. A two-layer MLP with a sigmoid function in the hidden layer and a linear function in the output layer can fit multidimensional mapping problems provided that the data are consistent and there is sufficient number of neurons in its hidden layer. Levenberg–Marquardt (lm), Bayesian regularization (br), and scaled conjugate gradient (scg) are the three famous algorithms which are used to train MLP. lm usually needs more memory, but less time. In this algorithm, when generalization is no longer improved, training stops automatically, which is shown by a rise in the mean squared error (MSE) of the validation samples. br typically takes a longer time. At the same time, it can lead to an appropriate generalization for difficult, small, or noisy data sets. Training stops based on adaptive weight minimization (regularization). scg requires less memory. Training automatically stops when generalization stops improving, as demonstrated by an increase in the MSE of the validation samples (Haykin, 2001; MATLAB, 2016).

In mathematical modeling, a radial basis function network (RBFN) is an artificial neural network that employs radial basis functions as the transfer functions. A linear combination of the radial basis functions of the inputs and neuron parameters is the output of such networks. RBFN has many applications, including function approximation, time series prediction, classification, and system control (Schwenker, Kestler, & Palm, 2001; Tan, Wang, & Zurada, 2001).

Feverfew (*Tanacetum parthenium*), a member of Asteraceae, is a perennial herbaceous plant with sporadic fluff and a short straight root. It is widespread throughout Iran, including North, West, mountainous regions, center and East and observed in the provinces of Mazandaran, Gilan, Golestan, Kurdistan, Fars, Khorasan, Tehran, and Semnan. Feverfew originated from Kazakhstan, Middle Asia, and Mediterranean regions and is vastly spread throughout Europe, America, and Asia. It is a valuable medicinal plant which is used in traditional medicine for the treatment of inflammation, pain, fever, and infection (Akpulat, Tepe, Sokmen, Daferera, & Polissiou, 2005; Mohsenzadeh, Chehregani, & Amiri, 2011; Wu et al., 2007).

Lallemantia royleana is an oval seed about 3 mm in length and 0.7 mm in diameter. This native Iranian plant belongs to Lamiaceae family. When placed in water, the seed readily swells and releases a large volume of mucilage (soluble gum) which has many applications in traditional medicine. This plant grows widely in Asia and North Europe. *Lallemantia royleana* seed mucilage (LRSM) is a native Iranian hydrocolloid which can be utilized in producing edible coatings and in the formulations of food products (Amini & Razavi, 2012; Bahramparvar, Haddad Khodaparast, & Razavi, 2009).

In recent years, given the concerns about the risks of the consumption of chemical and synthetic preservatives, there has been a considerable tendency toward replacing them with natural preservatives. Research has shown that the long-term application of chemical preservatives leads to the outbreak of numerous complications including cancers. Therefore, utilization of such compounds has now decreased. Alternatively, application of medicinal plants has recently increased due to their less side effects. According to statistics, at least 30% of people suffer from foodborne diseases once a year in developed countries. Beef is one of the most perishable foodstuffs considering its pH, moisture content, nutrients, and fermentable carbohydrates. During storage, the oxidation of beef lipids and microbial growth could be retarded by antioxidants and antimicrobial compounds, increasing its shelf life and retaining its quality (Alizadeh Behbahani, Yazdi, Shahidi, Mortazavi, & Mohebbi, 2017; Huang et al., 2011; Kim, Cadwallader, Kido, & Watanabe, 2013; Zhou, Xu, & Liu, 2010).

There are numerous methods for the extension of beef shelf life, including the application of active modified atmosphere packaging, high temperatures (cooking), freezing, addition of organic acids and salts, fermentation, drying, smoking and more recently, utilization of the extracts and essential oils of medicinal plants (Perumalla & Hettiarachchy, 2011).

Microbial growth on the surface of food products is one of the major reasons behind spoilage and quality degradation. Lately, there has been an increasing trend toward the application of edible films and coatings loaded with natural substances (antimicrobial and antioxidant) in order to extend the shelf life of food products. An edible film or coating loaded with an antimicrobial agent inhibits microbial growth as soon as it is contacted with the food surface, thus extending its shelf life. Edible films or coatings control moisture transfer, oxygen diffusion, and aroma loss or absorption. Problems could be partially resolved using edible films or coatings, leading to the retention of the aroma and flavor of food products (Bourtoom, 2008; Debeaufort, Quezada-Gallo, & Voilley, 1998).

To the best of our knowledge, no study has been conducted toward LRSM edible coating loaded with TPEO. The purpose of this research was to identify the chemical substances and the antioxidant potential of TPEO together with its scavenging activity of free radicals and oxidation inhibition power. This study was also aimed at investigating the antibacterial and antifungal effects of TPEO oil and LRSM loaded with TPEO on the shelf life extension of beef slices during refrigerated storage (4°C) over a period of 18 days. In addition, we aimed to predict the population of pathogenic microorganisms, chemical changes, and sensory attributes of the beef slices using MLP and RBFN.

2 | MATERIALS AND METHODS

2.1 | Chemicals, reagents, and microbial media

All reagents and microbial media including: mueller hinton agar, mueller hinton broth, sabouraud dextrose agar, sabouraud dextrose broth, plate count agar, eosine methylene blue, and mannitol salt agar were supplied from Merck Co. Germany. The other chemicals were supplied as follows: peptone bacteriological (BDH Chemicals Ltd., England), tryptone soya broth and sodium chloride (East Anglia Chemicals, UK) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich).

2.2 | Collection and preparation of Feverfew

Feverfew was collected from Kurdistan province, Iran. After the confirmation of the plant scientific name (genus and species confirmed by herbarium, the herbal systematic laboratory of Ferdowsi University of Mashhad), it was rinsed with cold water, shadow-dried, and powdered using a lab grinder. Finally, the powder was screened with a lab sieve to uniform its particle size distribution and to enhance the essential oil extraction yield (Alizadeh Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017a).

2.3 | Essential oil preparation and determination of the extraction yield

About 50 g of the powdered Feverfew along with 750 ml distilled water were incorporated into a glass Clevenger apparatus (Adac Co.) which works based on hydrodistillation (dehydrate TPEO by Na_2So_4). Essential oil extraction was carried out for 3 hr with a rate of 1 ml/min. In order to quantify the extraction yield, the essential oil storage vial was weighed; then, the essential oil was poured into it and it was weighed again. The mass difference between the two measurements

was considered to be the extraction yield (Alizadeh Behbahani, Yazdi, et al., 2017).

2.4 | Identification of the essential oil chemical composition

Identification of the essential oil substances was performed by injecting 0.2 μl TPEO into a gas chromatograph and coupled to a mass spectrometer. Helium was employed as the carrier gas with a rate of 1.1 ml/min and an ionization energy of 70 eV. The essential oil chemical composition was identified using the normal spectrum of alkanes and calculating their retention index (Kovats retention index) and referring to the natural compounds library (Alizadeh Behbahani, Yazdi, et al., 2017).

2.5 | Estimation of total phenolic content and phytochemical constituents

The method of Folin–Ciocalteu is generally applied for the determination of total phenolic content (TPC). The result was expressed as mg of Gallic Acid Equivalents/g of the essential oil (GAEs). Phytochemical components (alkaloids, tannins, saponins, flavone, and glycosides) were measured according to qualitative methods (Alizadeh Behbahani et al., 2017a; Njoku & Obi, 2009).

2.6 | Antioxidant potential

The antioxidant potential of TPEO was estimated according to the method previously described by Alizadeh Behbahani et al. (2017a), involving the method of DPPH. The radical scavenging activity percentage of DPPH was computed using the following equation:

$$\%\ \text{scavenging activity} = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100 \quad (1)$$

where "Abs control" denotes the absorbance value of DPPH together with ethanol and "Abs sample" represents the absorbance value of DPPH along with the sample.

2.7 | Extraction of LRSM

Lallemantia royleana was purchased from a local market in Mashhad, Iran. LRSM was extracted according to Salehi, Kashaninejad, and Behshad (2014). In this method, the conditions include the temperature of 50°C (control by Thermometer), water to seed ratio of 20:1 and pH value of 7. After extraction, LRSM was packaged in sealed bags and stored in a dry and cool place for further experiments.

2.8 | Preparation of the microbial strains

The strains utilized in this study included *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 23857, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, and *Candida albicans* PTCC 5027. All American type culture collections were obtained from the department of food science and technology of Ferdowsi University of Mashhad (FUM), Mashhad, Iran.

2.9 | Suspension preparation

Freshly cultured *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 23857, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, and *Candida albicans* ATCC 5027 colonies were suspended in 10 ml of normal saline. The suspension was agitated with a vortex mixer for 20 s. OD_{625} (absorbance 0.08–0.1) nm of each sample was adjusted using spectrophotometer. Subsequently, its concentration was adjusted to 1.5×10^8 cfu (colony forming unit)/ml based on a standard 0.5 McFarland (A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride, with 9.95 ml of 1% sulfuric acid) (McFarland, 1907).

2.10 | Antimicrobial susceptibility test

2.10.1 | Disk diffusion agar

In this method, blank paper disks with a diameter of 6.2 mm (Padtanteb Co.) were placed on the culture medium at certain distances from each other and the plate edge. They were immersed with 20 μl of 0.25, 0.5, 1, 2, 4, and 8% TPEO. In order to eliminate any microbial contamination, the essential oil had been sterilized with a syringe filter with a pore diameter of 0.45 μm before it was added to the disks. The disks of gentamycin, vancomycin, and amphotericin B were also used at a concentration of 10 μg/ml. after that, the culture media containing bacteria and fungi were incubated at 37 and 27°C for 24 and 72 hr, respectively. Eventually, the inhibition zone diameters formed around the disks were measured using a ruler and recorded (Alizadeh Behbahani, Yazdi, et al., 2017; Benkeblia, 2004).

2.10.2 | Well diffusion agar

In this method, 0.5 McFarland suspension was poured onto MHA and SDA at three points for bacteria and fungi and spread using a L-shaped spreader. Next, five wells with diameters of 6 mm were created on the surface of the culture media using the bottom of the Pasteur pipette. The bottom of the wells was blocked with molten agar to prevent the essential oil from diffusing into the bottom of the plate. About 20 μl of 0.25, 0.5, 1, 2, 4, and 8% TPEO was poured into four of the wells. The fifth well was regarded as the blank. After incubating the culture media containing bacteria and fungi at 37 and 27°C for 24 and 72 hr, given each well diameter, the diameter of the inhibition zone surrounding the well was quantified using a ruler and expressed in mm (Alizadeh Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017b; Tepe et al., 2004).

2.10.3 | Pour plate method

Pour plate method (PPM) is a qualitative method and its results are expressed as sensitive, semisensitive, and resistant. In this method, 0.2 g LRSM was transferred to a sterile 10-ml test tube followed by the addition of 5 ml distilled water. One milliliter of this solution was incorporated into petri-dishes of MHA and SDA culture medium (19 ml). The

final concentration of the extract would be 2 mg/ml (Alizadeh Behbahani et al., 2017a; Tabatabaei, Alizadeh, & Mortazavi, 2014).

2.10.4 | Determination of minimum inhibitory concentration through micro dilution broth

In this method, a stock solution with concentration of 512 mg/ml was prepared from TPEO. To that end, 5.12 g essential oil was mixed with 9.5 ml MHB (for bacteria), SDB (for fungi), and 0.5 ml dimethyl sulfoxide and sterilized with a syringe filter with a pore size of 0.45 μm. Five milliliters of this filtered solution were blended with 5 ml of the MHB and SDB culture medium in a sterile vial. This was continued to halve the concentrations and prepare sequential concentrations of 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 mg/ml. In order to estimate minimum inhibitory concentration (MIC) through micro dilution broth, a 96-well plate was employed. Two hundred microliter of each prepared dilution and eventually 20 μl of the microbial suspension (0.5 McFarland equivalent) were added to each well. The wells containing the culture medium and the microbial suspension without the essential oil were regarded as the positive control and the wells containing the essential oil and the culture medium were considered the negative control. After incubation at 37°C for 24 hr for bacteria and 27°C for 72 hr for fungi, 20 μl Triphenyltetrazolium chloride 5% was added to each well followed be incubation for 30 min for bacteria and 24 hr for fungi. A dark red or amethystine color is created in the wells in which microbial growth has occurred. The first concentration at which microbial growth did not take place and the red color was not observed, was considered to be MIC (Alizadeh Behbahani et al., 2017a; Tabatabaei, Alizadeh, Vasiee, Mortazavi, & Tabatabaie, 2015).

2.10.5 | Minimum bactericidal concentration or minimum fungicidal concentration

Given the results obtained from the MIC test, 100 μl was removed from the wells free of the red color, and cultured (pour plate) on MHA for bacteria and SDA for fungi. Subsequently, the culture media were incubated. The concentrations at which microbial growth did not happen, were regarded as the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of TPEO for the indicator microorganism (Alizadeh Behbahani, Yazdi, et al., 2017; Moreira, Ponce, Del Valle, & Roura, 2005).

2.11 | Beef sample preparation

Beef samples were purchased from a local market in Mashhad and immediately (in less than 10 min) transferred to the microbiology laboratory under refrigerated sanitized conditions while being packaged in sterile plastic bags. For coating with LRSM, the meat samples were first cut into suitable pieces with approximately the same sizes and coating was carried out subsequently (Alizadeh Behbahani et al., 2017b).

2.12 | Estimation of the beef chemical composition

The moisture, fat, and protein contents of the meat were quantified through AOAC (1990) methods. The pH values of the samples were measured with digital pH-meter (HI 221, Hanna Instruments, Woonsocket, RI) (AOAC, 1990).

2.13 | Preparation of LRSM coating and coating of the beef samples

Five grams of extracted LRSM was blended with 1.75 g Tween 80 (35% of the LRSM dry weight), made to 100 ml with distilled water, heated and agitated using a magnetic stirrer. Natural TPEO was added to the LRSM solution as a natural antimicrobial agent at 0, 1, 1.5, and 2% v/v. After that, one group out of the five groups of the samples was coated by being immersed in the LRSM solution for 1 min and the other groups were immersed in the LRSM solutions loaded with different concentrations of TPEO for the same duration. A group remained uncoated as the control (Alizadeh Behbahani et al., 2017b; Jouki, Mortazavi, Yazdi, Koocheki, & Khazaei, 2014).

2.14 | Microbiological analysis

The microbiological count of the control and the samples loaded with different TPEO concentrations was done according to Jouki et al. (2014) and Alizadeh Behbahani et al. (2017b). The microbiological count of the control and the samples loaded with different TPEO concentrations was done according to Alizadeh Behbahani et al. (2017b). Total viable count (TVC) was performed through PPM in PCA at 37°C for 48 hr. Psychrotrophic count (PTC) was conducted with the same culture medium at 7°C for 10 days. *E. coli* and *S. aureus* counts were carried out using EMB and MSA at 37°C for 24 hr. Fungi (molds and yeasts) count were done using SDA at 27°C for 72 hr. All of the above-mentioned microbiological counts were done on the 0th, 3rd, 6th, 9th, 12th, 15th, and 18th day.

2.15 | Chemical analyses

2.15.1 | Determination of 2-thiobarbituric acid

In brief, 2 g of the meat sample was mixed with 5 ml trichloroacetic acid 20% for 2 min. The mixture was then filtered using a filter paper and 5 ml of the filtrate was blended with 5 ml thiobarbituric acid (TBA) 0.01 M in a test tube which was placed in a water bath at 100°C until the color was created. The absorbance value of the color was measured at 532 nm (AOAC, 1999; Jouki et al., 2014).

2.15.2 | Estimation of peroxide value

In order to determine the peroxide value (PV) of the beef samples, their lipid was first extracted in a 250-ml Erlenmeyer flask to which 25 ml of acetic acid–chloroform solution was further added. After that, 0.5 ml saturated potassium iodide, 30 ml distilled water and 0.5 ml starch solution 1% were added to the flask. The released iodine was titrated with thiosulfate 0.01 N. The results were expressed in meq oxygen/kg lipid (Alizadeh Behbahani et al., 2017b; Jouki et al., 2014).

2.15.3 | pH measurement

The pH values of the meat samples were measured using a pH-meter (HI 221, Hanna Instruments, Woonsocket, RI) on the 0th, 3rd, 6th, 9th,

12th, 15th, and 18th day (Alizadeh Behbahani et al., 2017b; Sallam & Samejima, 2004).

2.16 | Sensory evaluation

The sensory properties of the beef samples were evaluated by 10 trained panelists working in the laboratory. Four meat samples treated differently, were separately presented to each of the panelists in small covered porcelain plates. The panelists did not know the experimental procedure and the samples were blind-coded with 3-digit random numbers. The judges scored the sensory attributes including color, odor, and overall acceptability through a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely). The samples receiving the overall scores of more than 4 were considered to be acceptable (Alizadeh Behbahani, Yazdi, et al., 2017; Hansen, Gill, & Hussa, 1995).

2.17 | Statistical analysis

Microsoft Windows Excel 2016 and SPSS (Version18.0, SPSS Inc., Chicago, IL) were used to analyze the obtained data. The data were initially assessed by analysis of variance (ANOVA), and the Duncan's multiple range test was subsequently applied to detect significant ($p < .05$) differences. All experiments were triplicated.

2.18 | Data analysis through MLP and RBFN

The data were analyzed though MLP and RBFN using MATLAB (2016). The network inputs included the number of storage days (0–18), LRSM (0 and 1, with 0 representing the absence of LRSM and 1 denoting the presence of LRSM) and TPEO (0–2%). The network targets included total bacterial count, Psychrotrophic count, total fungi count, *E. coli* count, *S. aureus* count, pH, PV, TBA, color, odor, and total acceptability. In MLP, a hidden layer and three training algorithms of lm, br, and scg were used. Tansig and purelin were employed as the transfer functions of the hidden and output layers, respectively. In order to determine the optimum number of neurons of the hidden layer, their number was first considered to be in the range of 1–35 and the data were analyzed using lm with 500 replicates. The MSE related to the test data was regarded as the performance function. After calculating the mean and median of the 500 obtained MSEs for each number of neurons, it was observed that the lowest MSE was obtained when there were six neurons in the hidden layer. Subsequently, data analysis was conducted using the three afore-mentioned algorithms with 500 replicates and eventually, the networks having the lowest MSEs were compared with each other.

In the analysis of the data using RBFN, radbas, and purelin were chosen as the transfer functions of the hidden and output layers. In this network, the maximum number of the hidden layer neurons is equal to the number of input vectors. In this research, given that the total number of the input were equal to 35 and 70% of them, namely 25 vectors were used for network training, the maximum number of the hidden layer neurons was considered to be 25. Preliminary analyses showed that this number of neurons led to the best results in terms of MSE and correlation coefficient (R) (data not shown). The spread of the radbas function was taken into account in the range of 1–100 and the data were analyzed with 100 replicates. By computing the median and mean of the MSEs pertaining to the test data, the spread ranging from 2 to 100 gave approximately similar results. Therefore, this parameter was considered to be equal to 20. It should also be noted that the spread values less than 1 and more than 100 were investigated whose results were not sustainable (data not shown).

3 | RESULTS AND DISCUSSION

3.1 | *Tanacetum parthenium* essential oil composition

The results of TPEO chemical composition analysis through gas chromatography/mass spectrometry (GC–MS) showed that a total of 27 compounds were identified which constituted 96.8% of the essential oil. Camphor (44.2%) was the major compound followed by camphene (12.45%), α-pinene (7.35%), chrysanthenyl acetate (6.14%), β-chrysanthenyl acetate (2.7%), and limonene (2.7%). These six components account for 75.54% of TPEO. The calculation of TPEO extraction yield indicated that 0.3 ml essential oil was obtained from 50 g Feverfew according to which the extraction yield (v/w) was equal to 0.6%. TPEO color was yellowish.

Izadi, Esna-Ashari, Piri, and Davoodi (2010), examined different parts of the Feverfew collected from the cities of Tehran and Hamedan. Their findings revealed that camphor was the major constituent in the whole oils (10.3–53.3%) followed by chrysanthenyl acetate (4.3–22.5%) and camphene (4.1–10.4%). Nevertheless, bornyl acetate, α-pinene, and p-cymene were only found in the plant samples from Hamedan (Izadi et al., 2010). Polatoglu, Demirci, Demirci, Gören, and Baser (2010), investigated Feverfew chemical composition. Their results demonstrated that camphor (49%), trans-chrysanthenyl acetate (22.1%), and p-cymene (5.2%) were the major constituents of TPEO (Polatoglu et al., 2010). Akpulat et al. (2005), verified the chemical composition of Feverfew in Turkey and concluded that camphor (56.9%), camphene (12.7%), and pcymene (5.2%) were the major components of TPEO. Furthermore, TPEO extraction yield was 0.43% (Akpulat et al., 2005).

Our findings conformed to those of the others to a large extent. The chemical compositions of essential oils differ from one another considering the variety, climate, growth stage, collection time, and growth location (Daferera, Ziogas, & Polissiou, 2000). This is important, because the compounds of the plants with various origins are different from each other. In all afore-mentioned studies, camphor was the major compound of TPEO. It is a disinfectant which has efficient antibiotic effects (Ernst & Pittler, 2000).

3.2 | Phytochemical analysis, antioxidant activity, and total phenolic content

The results concerning the identification of TPEO phytochemical compounds revealed that TPEO contains tannins, alkaloids, saponins, flavone, and glycosides and the performed tests confirmed the presence of these substances in TPEO. The TPC of TPEO was equal to 151.2 ±

2.10 μg/ml GAE. TPC was calculated on the standard curve through the Folin–Ciocalteu method. The antioxidant activity of TPEO was equal to 57.25 ± 0.2 μg/ml.

Many studies have proven the direct correlation between TPC and antioxidant activity (Alizadeh Behbahani et al., 2017a; Tepe et al., 2004). Tepe & Sokmen (2007), investigated the antioxidant and TPC of *Tanacetum densum* (Lab.) Schultz Bip. subsp. sivasicum Hub-Mor and Grierson, *Tanacetum densum* (Lab.) Schultz Bip. subsp. eginense Heywood and *Tanacetum densum* (Lab.) Schultz Bip. subsp. amani Heywood and declared that all of the studied plants had antioxidant activity. In addition, their results exhibited that there was a positive correlation between the antioxidant activity and TPC (Tepe & Sokmen, 2007). The results of the present study are consistent with theirs. Polatoglu et al. (2010), reported that Feverfew had antioxidant activity (Polatoglu et al., 2010). Cao and Prior (1998) and Alizadeh Behbahani et al. (2017b) reported that the differences in the TPC and antioxidant properties of different plants are influenced by a variety of factors such as climatic conditions (climate, soil, and altitude). They also stated that the differences between species, drying as well as the different methods of measuring TPC and antioxidant activity are the other reasons behind the difference in the results of various studies.

3.3 | Antimicrobial activity *in vitro*

3.3.1 | Well diffusion agar and disk diffusion agar

The antimicrobial effect of TPEO was investigated through disk diffusion agar (DDA) or Kirby-Bauer method. The results (Table 1) presented that TPEO had the most detrimental effect on *B. subtilis* at 8 mg/ml. The smallest inhibition zone diameter against different TPEO concentrations belonged to Gram-negative bacteria (*P. aeruginosa* and *E. coli*). The results also indicated that no inhibition zone was observed for *P. aeruginosa* and *E. coli* at the concentrations of 0.25 and 0.5 mg/ml. Inhibition zone was observed at all concentrations for all Gram-positive bacteria except *S. aureus* and *B. cereus*. No inhibition zone was observed for *S. aureus* and *B. cereus* at 0.25 mg/ml. *Candida albicans* was sensitive to TPEO at all concentrations. The results demonstrated that the investigated bacteria were significantly different ($p < .05$) in terms of susceptibility to TPEO through the Kirby-Bauer method. The pairwise comparison between the effects of TPEO concentrations on the bacteria revealed that there were significant differences between the concentrations and as TPEO concentration increased, the inhibition zone diameter increased, too. However, as shown in Table 1, no significant ($p < .05$) difference was observed between the concentrations of 4 and 8 mg/ml, and between 2 and 4 mg/ml in the case of *S. aureus* and *S. pyogenes*, respectively. The mean comparison of the inhibition zone diameters for Gram-negative bacteria revealed that TPEO was significantly ($p < .05$) effective on *P. aeruginosa* at all concentrations except 4 and 8 mg/ml (Table 1).

The results of the antimicrobial effects of TPEO through well diffusion agar (WDA) are summarized in Table 1. They showed that inhibition zone was observed for all Gram-positive bacteria at all concentrations. No inhibition zone was observed for *P. aeruginosa* at

TABLE 1 Antimicrobial effect of TPEO concentrations for some pathogenic bacteria and fungi "in vitro"

Microrganism	DDA (mm)		WDA (mm)							MIC	MBC/MFC
	0.25	0.5	0.25	0.5	1	2	4	8			
B. subtilis	8.80 ± 0.50	10.60 ± 0.50	9.60 ± 0.54	11.70 ± 0.50	13.20 ± 0.57	15.00 ± 0.28	16.50 ± 0.50	18.00 ± 0.57	0.25	0. 5
S. pyogenes	8.20 ± 0.50	9.90 ± 0.57	8.80 ± 0.50	10.60 ± 0.57	12.30 ± 0.28	14.00 ± 0.54	15.60 ± 0.57	17.20 ± 0.50	0. 5	1
S. aureus	-	7.70 ± 0.50	7.80 ± 0.57	9.50 ± 0.50	11.20 ± 0.50	12.70 ± 0.54	14.30 ± 0.57	15.50 ± 0.28	1	2
B. cereus	-	7.50 ± 0.57	7.30 ± 0.54	9.20 ± 0.28	10.80 ± 0.54	12.30 ± 0.57	14.00 ± 0.28	15.50 ± 0.54	1	4
E. coli	-	-	7.00 ± 0.50	8.60 ± 0.50	10.10 ± 0.50	12.00 ± 0.54	13.50 ± 0.54	15.00 ± 0.54	2	4
P. aeruginosa	-	-	-	7.30 ± 0.50	8.90 ± 0.50	10.40 ± 0.28	12.10 ± 0.50	13.00 ± 0.54	4	8
C. albicans	7.20 ± 0.54	9.00 ± 0.57	8.10 ± 0.50	9.90 ± 0.50	11.80 ± 0.28	13.50 ± 0.50	15.00 ± 0.57	16.60 ± 0.28	0. 5	1

[a]Values are expressed as mean ± *SD*.
[b]$N = 3$.

0.25 mg/ml, whereas it was observed for *E. coli* at all concentrations. In WDA, due to the direct contact between the essential oil and the microorganisms on the surface of the culture medium, the inhibition zone diameter was larger than that of DDA, because in DDA, it was necessary that TPEO diffuse into the surface of the culture medium through a paper disk. As can be seen in Table 1, TPEO concentrations of 4 and 8 mg/ml did not significantly ($p < .05$) differ in the case of *P. aeruginosa*. Significant differences were observed for other microorganisms at all concentrations.

The results of WAD and DDA demonstrated that Gram-positive bacteria were more sensitive to TPEO than the Gram-negative ones. In general, the sensitivity profile of the microorganisms studied in this research is as follows:

> *Pseudomonas aeruginosa*> *Escherichia coli*> *Bacillus cereus*> *Staphylococcus aureus*> *Candida albicans*> *Streptococcus pyogenes*> *Bacillus subtilis*.

The higher resistance of Gram-negative bacteria to the essential oils of medicinal plants could be attributed to the more complex structure of the cell membrane of these bacteria compared with the single-layer structure of the Gram-positive ones. Gram-positive bacteria have mucopeptide in their cell walls, while lipopolysaccharides constitute the majority of the cell walls of Gram-negative bacteria like *P. aeruginosa and E. coli*. Conversely, it seems that the resistance of bacterial cells depends on the solubility rate and extent of antimicrobial compounds (TPEO) in the lipid moiety of the microorganism cell membrane. At the same time, this could not be compelling reason for the higher susceptibility of Gram-positive bacteria than the Gram-negative ones. Moreover, the difference in the hydrophobicity of the cell membrane surfaces can also be mentioned as an influential factor (Holley & Patel, 2005).

Similar studies concerning the antimicrobial effects of the extracts and essential oils of medicinal plants which have been carried out on a variety of Gram-positive and Gram-negative bacteria, confirm the above sentences (Jouki et al., 2014; Sureshjani, Yazdi, Mortazavi, Behbahani, & Shahidi, 2014). Tassorelli et al. (2005), declared that Feverfew contain compounds such as camphor and α-pinene (Tassorelli et al., 2005). According to the findings of the present study, camphor is the major constituent of TPEO. Ernst and Pittler (2000) and Marino, Bersani, and Comi (2001), stated that camphor acts as a strong antibiotic and has a disinfecting effect. As a result, it could be claimed that camphor is one of the principle antimicrobial components of Feverfew. Izadi et al. (2010) examined the antimicrobial effect of Feverfew through DDA on Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus*, and *Staphylococcus aureus*), Gram-negative bacteria (*Yersinia enterocolitica*, *Klebsiella oxytoca*, *Serratia marcescens*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and a yeast (*Candida albicans*). They concluded that Feverfew had a more pronounced antimicrobial effect on the Gram-positive bacteria (Izadi et al., 2010). Their results are consistent with ours.

3.3.2 | Pour plate method

The results of investigating the antimicrobial effect of LRSM through the screening antimicrobial activity method presented that LRSM screening antimicrobial activity method at 2 mg/ml, inhibited *B. subtilis* and *S. pyogenes* from growing. However, 2 mg/ml LRSM, had no significant effect on *B. cereus*, *E. coli*, and *P. aeruginosa*. It is not able to prevent the growth of these bacteria on culture.

To the best of our knowledge, no study has been performed yet on the antimicrobial effect of LRSM. However, some research has been conducted on the antimicrobial effect of *Lallemantia royleana*. Sharifi-Rad, Hoseini-Alfatemi, Sharifi-Rad, and Setzer (2015) examined the chemical composition in addition to the antifungal and antimicrobial activities of *Lallemantia royleana* essential oil (LREO) and identified 37 constituents in it. These researchers reported that LREO possess antifungal and antibacterial effects (Sharifi-Rad et al., 2015). Mahmood et al. (2013) investigated the antibacterial activity of *Lallemantia royleana* and declared that this herb has antimicrobial effect (Mahmood et al., 2013).

3.3.3 | Minimum inhibitory concentration

The results of MIC are exhibited in Table 1. The MIC of TPEO ranged from 025 to 4 mg/ml depending on the type of microorganism. The results revealed that *P. aeruginosa* with a MIC of 4 mg/ml was the most resistant and *B. subtilis* with a MIC of 0.25 mg/ml was the most sensitive to TPEO. As mentioned earlier, the higher resistance of Gram-negative bacteria than the Gram-positive ones is attributed to the differences in their cell walls.

Polatoglu et al. (2010) verified the antimicrobial effect of TPEO through microdilution broth on 5 Gram-negative and 5 Gram-positive species. They maintained that *B. cereus* and *S. aureus* were the most sensitive bacteria to TPEO while the Gram-negative ones were the most resistant ones (Polatoglu et al., 2010). The findings of the present study conform to theirs.

3.3.4 | Minimum bactericidal concentration and minimum fungicidal concentration

The results of MBC and MFC of TPEO are presented in Table 1. MBC and MFC are complementary tests for MIC. The results showed that MBC and MFC were equal to or more than MIC for all of the microorganisms. The MBC and MFC of TPEO varied between 0.5 and 8 mg/ml depending on the type of microorganism. The results indicated that *P. aeruginosa* with a MBC of 8 mg/ml was the most resistant bacterium to TPEO. MFC was equal to 1 mg/ml for *C. albicans*.

The mechanism of the effect of the extracts and essential oils on fungi has not clearly been understood; although it is ascribed to the morphological changes in most reports (Alizadeh Behbahani, Yazdi, et al., 2017; Inouye, Takizawa, & Yamaguchi, 2001).

3.4 | Chemical analyses of beef

The chemical composition of beef was quantified through AOAC (1990) standard methods. The beef had 65.27% moisture, 13.69% fat, and 19.45% protein. The percentage of each ingredient was similar to those previously reported by Alizadeh Behbahani et al. (2017b), Emir-oğlu, Yemiş, Coşkun, and Candoğan (2010), and Yin and Cheng (2003). The difference in the chemical composition of the beef of various

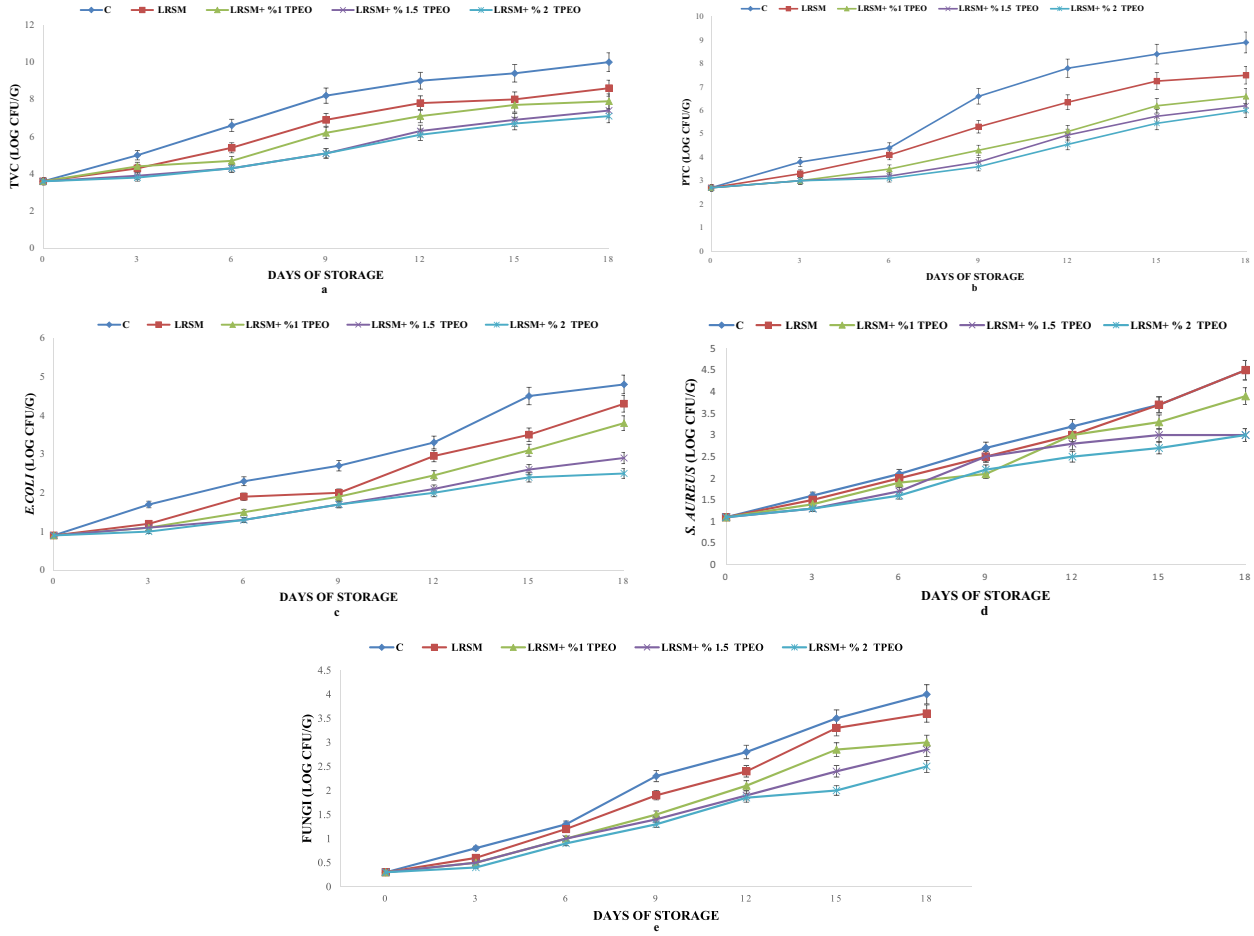

FIGURE 1 Total viable count (a), Psychrotrophic count (b), *Escherichia coli* count (c), *Staphylococcus aureus* count (d), and Fungi count (e) of the beef samples without and with LRSM during storage at 4°C for 18 days. Control: uncoated samples, LRSM: samples coated with LRSM, LRSM + TPEO: samples coated with LRSM loaded with TPEO. Bars represent the standard deviation ($n = 3$)

regions is associated with the nutrition, age, environment, slaughter time, and other environmental factors (Alizadeh Behbahani et al., 2017b).

3.5 | Microbiological analyses

3.5.1 | Total viable count

The results of the microbial analyses are illustrated in Figure 1a. The results demonstrated that the TVC of the control varied from 3.6 Log cfu/g to 10 Log cfu/g during the entire storage. According to the International Commission of Microbiological Specifications for Foods (ICMSF), the maximum permitted TVC of fresh beef is equal to 7 Log cfu/g or 10^7 cfu/g (ICMSF, 1986). As a result, the TVC of the control (6.6 Log cfu/g) was acceptable on the 6th day. It reached 8.2 Log cfu/g on the 9th day which exceeded the standard permitted limit. Consequently, the shelf life of the control was at most 6 days. The results also showed that the TVC of the beef samples coated only with LRSM exceeded the standard permitted limit on the 12th day. The shelf life of such samples was at most 9 days. In general, the TVC of the control and the treated meat samples followed an increasing trend during the 18 days of storage. The increase in the TVC of the samples coated with LRSM + TPEO was less pronounced than those of the control and the ones coated with LRSM. The slope of the increase in the TVC of the sample coated with LRSM + 2% TPEO was more gentle than those of the other treatments. The TVC of the sample coated with LRSM + 2% TPEO was equal to 7.1 Log cfu/g on the 18th day, which was slightly higher than the standard permitted limit. Based on ICMSF, the shelf life of this sample was equal to 15 days. According to the obtained results, the shelf life of the sample coated with LRSM + 1.5% TPEO was also equal to 15 days; nevertheless, the TVC of this sample was higher than that of the former. It was also observed that coating the samples with LRSM, LRSM + 1% TPEO, LRSM + 1.5% TPEO and LRSM + 2% TPEO increased their shelf lives up to in order 3, 3, 9, and 9 days compared with the control. Quality loss of a food product during storage occurs due to microbial contaminations which can result in spoilage and poisoning. This is more pronounced in the case of some foodstuffs like meat and not only threaten the consumers' health, but also do they cause economical damages.

Shavisi, Khanjari, Basti, Misaghi, and Shahbazi (2017) studied the polylactic acid film containing propolis ethanolic extract, cellulose nanoparticle, and *Ziziphora clinopodioides* essential oil on the chemical, microbial, and sensory properties of minced beef during refrigerated

storage for 11 days. Their results revealed that the beef shelf life increased under the afore-mentioned treatments (Shavisi et al., 2017). Smaoui et al. (2016) investigated the effect of *Mentha piperita* essential oil on minced beef and claimed that this essential oil well prevented pathogenic microorganisms from growing (Smaoui et al., 2016). Xi, Liu, and Su (2012) examined the effect of green tea extract on oyster meat. They concluded that the addition of this extract brought about an increase in the meat shelf life (Xi et al., 2012).

3.5.2 | Psychrotrophic count

PTC is the most common bacteria on the surface of meat and meat products at refrigerator temperature. PTC variations in the control and the treated samples are depicted in Figure 1b. The results of ANOVA and mean comparison of PTC demonstrated that there were significant differences between the treatments at 5% significance level. The PTC of the control varied from 2.7 to 8.9 Log cfu/g during the whole storage period. The major and minor changes in PTC belonged to the control and the sample coated with LRSM + 2% TPEO, respectively. The results also indicated that there were no significant differences between the samples coated with LRSM, LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO. At the same time, the increase rate of PTC was lower for the sample coated with LRSM + 2% TPEO than for the other ones.

Pseudomonas species are extremely aerobic and are not able to survive in the absence of oxygen (Alghooneh, Alizadeh Behbahani, Noorbakhsh, & Yazdi, 2015). LRSM coating, as a barrier to oxygen, inhibits the growth of Pseudomonas which are of the most important PTC. Alizadeh Behbahani et al. (2017) investigated the effect of plantain seed mucilage loaded with tarragon essential oil on beef shelf life. They came to conclusion that plantain seed mucilage prevented oxygen from diffusing into the meat surface, thus reducing the growth of PTC (Alizadeh Behbahani, Yazdi, et al., 2017). Their results conform to the findings of the present study. Sarker et al. (2010) reported that the lipase and protease produced by PTC were heat-resistant, thus giving rise to the meat lipolysis and proteolysis and caused the meat putrefaction and off-flavor by decomposing triacylglycerol and proteins (Sarker et al., 2010).

3.5.3 | *Escherichia coli* count

The results of *E. coli* count in the control and the treated samples during the 18 days of storage are described in Figure 1c. They presented that the rise in the *E. coli* count of the sample coated with LRSM + 2% TPEO significantly ($p < .05$) differed from that of the other samples except the one coated with LRSM + 1.5% TPEO. During storage (18 days), *E. coli* count of all the samples was raised; however, the increase rate was lower in the samples coated with TPEO compared with the other ones. The highest increase in *E. coli* count was associated with the control. The *E. coli* count of the control was equal to 4.8 Log cfu/g on the 18th day of storage.

These results for the inhibition of *E. coli* growth in beef are in a very good agreement with the previous *in vitro* ones as well as with the MIC values. Djenane et al. (2012) examined the antibacterial effect of Lavandula and Mentha essential oils on minced beef. They cited that these essential oils affected *E. coli* and extended the meat shelf life (Djenane et al., 2012). Our results are consistent with theirs.

3.5.4 | *Staphylococcus aureus* count

The results of *S. aureus* count during storage are illustrated in Figure 1d. The *S. aureus* count of all of the samples followed an increasing trend. The results showed that the effects of the different concentrations of TPEO (1, 1.5, and 2%) on *S. aureus* count were not significantly ($p > .05$) different from one another. Nonetheless, the increase rate of *S. aureus* count was lower for the sample coated with LRSM + 2% TPEO than for the other ones.

Bozorgi and Vazirian (2016) investigated the antioxidant activity and TPC of *Lallemantia royleana* and declared that this herb possesses antioxidant activity and attributed it to the presence of phenolic compounds (Bozorgi & Vazirian, 2016). Research has shown that the phenolic compounds present in essential oils and edible coatings can be an effective factor in controlling the growth of spoilage bacteria.

3.5.5 | Total yeast and mold (fungi) count

The total yeast and mold (fungi) count of the beef samples stored at 4°C during storage (18 days) is exhibited in Figure 1e. The results demonstrated that the total fungi count of the samples was 0.3 log cfu/g on the 0th day. Since fungi are aerobic and grow on meat surface and LRSM, as a barrier, decreased the oxygen concentration on the sample surfaces, the fungi were properly inhibited from growing in all of the coated samples.

The findings of the present study conform to the those of the other researchers (Alizadeh Behbahani et al., 2017b; Alizadeh Behbahani, Yazdi, et al., 2017). The results showed that the total fungi count of the sample coated with LRSM + 2% TPEO was lower than that of the other ones. The total fungi count of the control was equal to 4 Log cfu/g on the 18th day, which was significantly ($p < .05$) different from the treatment of LRSM + 2% TPEO.

3.6 | Chemical analyses

3.6.1 | Thiobarbituric acid

The variations in the TBA of the beef samples during refrigerated storage are depicted in Figure 2a. The TBA of the control lied in the range of 0.08–1.1 mg malondialdehyde (MDA)/kg during the entire storage period. On the 18th day of storage, the TBA of the samples coated with LRSM + 1% TPEO, LRSM + 1.5% TPEO and LRSM + 2% TPEO was equal to 0.52, 0.47, and 0.31 mg MA/kg, respectively. Although the TBA changes followed an increasing trend during storage, the increase rate of TBA in the samples comprising TPEO was lower than that of the control and the ones coated with LRSM.

Owing to consisting of various fatty acids, beef oxidation is of great importance and is regarded as one of the main reasons behind the meat off-flavor and off-odor (Kim et al., 2013). According to the obtained results, TPEO (in all the samples) had an appropriate inhibitory effect on lipid oxidation in beef during storage. This could be associated with the presence of phenolic compounds in TPEO. The permitted limit for TBA in meat is equal to 1 mg MDA/kg. The results revealed that the TBA of

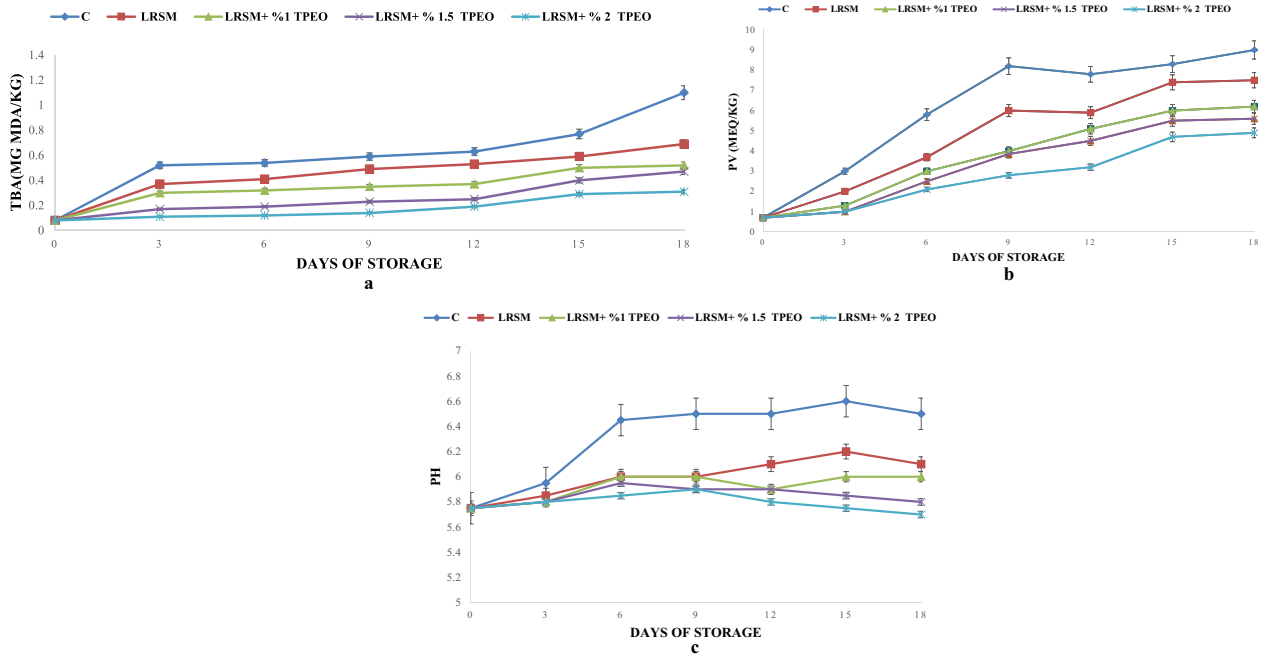

FIGURE 2 Changes in TBA values (a), Peroxide values (b) and pH (c) of the beef samples during storage at 4°C for 18 days

the control was slightly higher than this limit at the end of storage. Conversely, this value was lower than 1 for the samples containing TPEO on the 18th day, even though the TBA of all the samples rose during storage. Smeti, Atti, Mahouachi, and Munoz (2013) reported that the TBA of the light lamb meat samples (control and the ones containing rosemary essential oil) was raised during storage (Smeti et al., 2013). The findings of the present study indicated that the incorporation of TPEO into beef protected it against lipid oxidation. Phenolic compounds are known to prevent the formation of free radicals and the propagation of their reactions through the complexation of transition metal ions including iron (McBride, Hogan, & Kerry, 2007). Hence, the powerful *in vitro* antioxidant activity of TPEO also had a protective effect on real beef. Kim et al. (2013) examined the antioxidant and antimicrobial effects of leafy green vegetable extracts on the shelf life of meat products. They ascribed the control of lipid oxidation to the phenolic compounds and the antioxidant activity of leafy green vegetable extracts and their results were in agreement with those of the present study.

3.6.2 | Peroxide value

Figure 2b, depicts the PV changes of the control and the treated beef samples during the refrigerated storage (4°C). The PV of the control (meq oxygen/kg) in the samples ranged from 0.7 to 9 during storage. An increase was observed in the PV of all the samples during storage. Likewise, the results showed that the PV of all of the treatments was elevated during the 18 days of storage. The increase in the PV of the samples coated with LRSM had a steeper slope than that of the samples coated with LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO.

At the same time, according to the findings of the present study, it was realized that TPEO contained phenolic compounds (151/2 ± 2.10 μg/ml GAE) and its antioxidant activity was equal to 57.25 ± 0.20 μg/ml. Consequently, the delay in the lipid oxidation of the beef slices during storage could be ascribed to the phenolic compounds and antioxidant activity of TPEO which was partially capable of preventing the meat spoilage. Alizadeh Behbahani et al. (2017b), suggested the antioxidant activity of plantain seed mucilage as the principle reason for the delay in the putrefaction of the beef slices during refrigerated storage.

The permitted limit of meat PV is equal to 7 meq oxygen/kg (Alizadeh Behbahani et al., 2017b). Based on the accomplished results, the PV of the control was higher than this limit on the 9th day. Accordingly, the shelf life of the control was equal to 6 days in terms of PV. The PV of the sample coated with LRSM exceeded the permitted limit on the 15th day, whereas the PV the ones coated with LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO never exceeded this limit during storage.

3.6.3 | pH

The results of pH changes in the meat samples during refrigerated storage are exhibited in Figure 2c. The pH value of beef can be different after slaughter and influenced by various factors including age, sex, nutrition, and slaughter conditions). During storage, pH is affected by the variation in nitrogen compounds and the activity of bacteria. The dissociation of nitrogen compounds (proteins) leads to an increase in pH during storage. Proteolytic bacteria play an important role though. Bacteria consume carbohydrates in the first place, which are followed by amino acids. Ammonia is produced due to the consumption of amino acids by bacteria, resulting in an increase in pH (Hulankova, Borilova, & Steinhauserova, 2013; Michalczyk, Macura, Tesarowicz, & Banaś, 2012; Tajkarimi, Ibrahim, & Cliver, 2010).

The initial pH value of the beef samples was equal to 5.75. ANOVA demonstrated that the pH values of the samples coated with LRSM, LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO

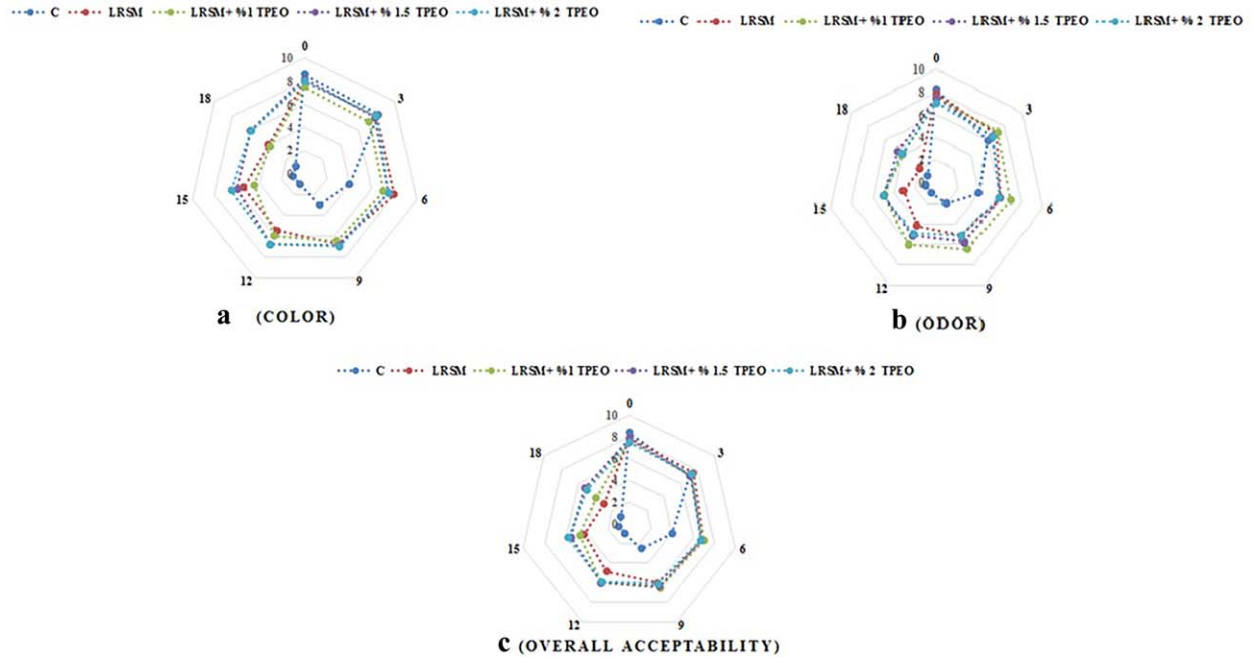

FIGURE 3 Sensory evaluation [color (a), odor (b), and overall acceptability (c)] of beef during storage at 4°C for 18 days. Bars represent the standard deviation ($n = 3$)

were lower than that of the control during storage (18 days). The presence of TPEO in LRSM seems to have reduced the diffusion of carbon dioxide, thus raising its concentration accompanied by a decrease in pH which in turn can be effective on reducing the beef microflora. These results are consistent with those of Jouki et al. (2014).

3.7 | Sensory evaluation

The results of the sensory evaluation (color, odor, and color) of the beef samples through the 9-point hedonic scale are presented in Figure 3a–c. The major obstacle to the application of essential oils in food products is their intense scent which diminishes their use as preservatives. Utilization of essential oils in combination with edible films and coatings is one of the solutions for resolving this issue. In the present study, TPEO did not have a negative effect on the sensory properties on the beef samples from the panelists' point of view ($p < .05$). A comparison between the scores obtained from sensory evaluation, showed that there were significant differences between the control and the samples coated with LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO ($p < .05$). Based on the method previously described by Jouki et al. (2014) and Fan, Chi, and Zhang (2008), the scores lower than 4 are not acceptable. Accordingly, the control obtained a score of 4 on the 6th day, while the samples coated with LRSM + 1.5% TPEO and LRSM + 2% TPEO achieved the scores of 4 and 5.9, respectively on the 18th day. According to the scores of total acceptability, the maximum shelf lives of the control and the samples coated with LRSM, LRSM + 1% TPEO, LRSM + 1.5% TPEO, and LRSM + 2% TPEO were equal to 6, 15, 15, 18, and 18 days, respectively. It was also observed that sensory evaluation had the highest correlation with TVC in the case of the control as the maximum shelf life was equal to 6 days based on both of the tests. There were also differences between the results of sensory evaluation and TVC in the case of the other samples, conforming to the findings of other researchers.

3.8 | Artificial neural networks

3.8.1 | Multilayer perceptron

The results revealed that the MSEs of the training, validation, and testing stages of the network trained with lm, decreased consistently. This is one of the indicators of a network appropriateness. Furthermore, the results revealed that at all stages, especially at testing which indicates the prediction ability of a network, the targets (empirical results) and outputs (theoretical results) were close to each other and the correlation coefficient was always higher than 0.99 except for the validation stage whose correlation coefficient was slightly lower than this value. Moreover, it was observed that the error (the difference between the targets and outputs) mean, MSE, and RMSE (root mean squared error) of the network were always low, especially at the testing stage which in turn confirms the high prediction ability of the network. The low standard deviation also demonstrated that the network error data were so close to the error mean and considering the proximity of the mean to zero, it could be concluded that most of the error data were near zero. This means that there was good affinity between the targets and outputs and the network is highly capable of predicting new observations (Figures 4 and 5).

The results showed that the MSEs of the training and testing stages of the network trained with br, both decreased with the same trend, showing the network adequacy. It should also be noted that br algorithm do not have the validation stage. As illustrated in Figures 6 and 7, the networks targets and outputs have a good affinity and the

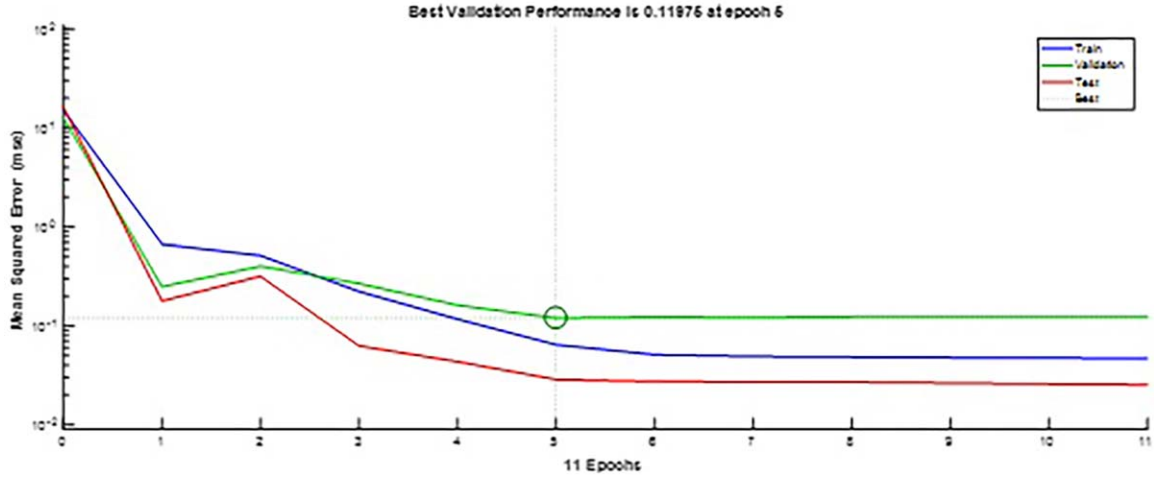

FIGURE 4 The performance plot of the MLP trained using the lm algorithm

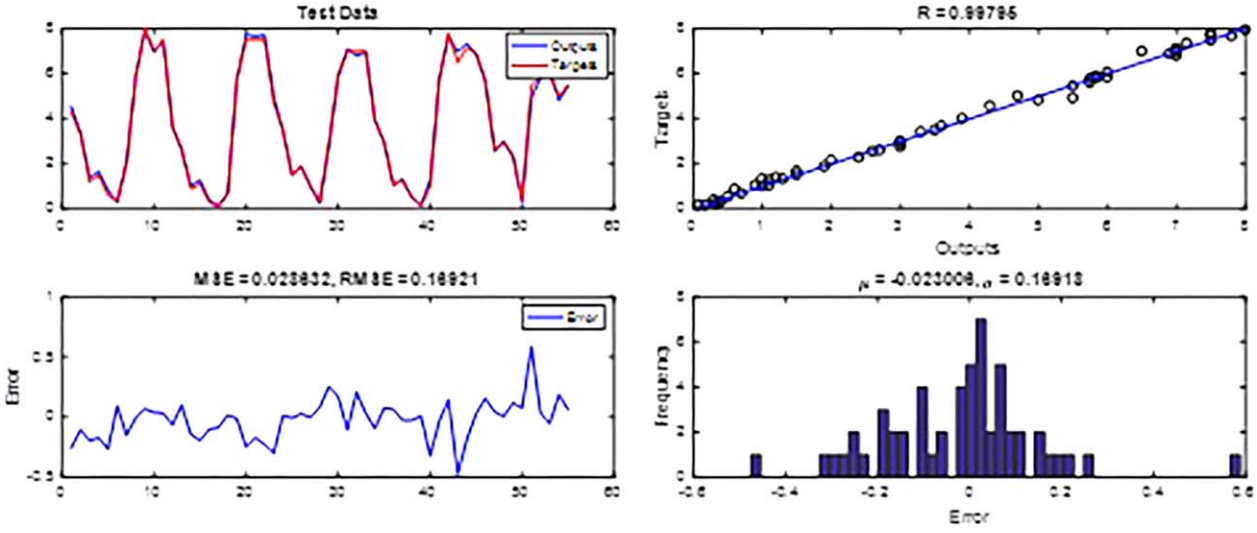

FIGURE 5 The data and error plots of the testing stage of the MLP trained using the lm algorithm

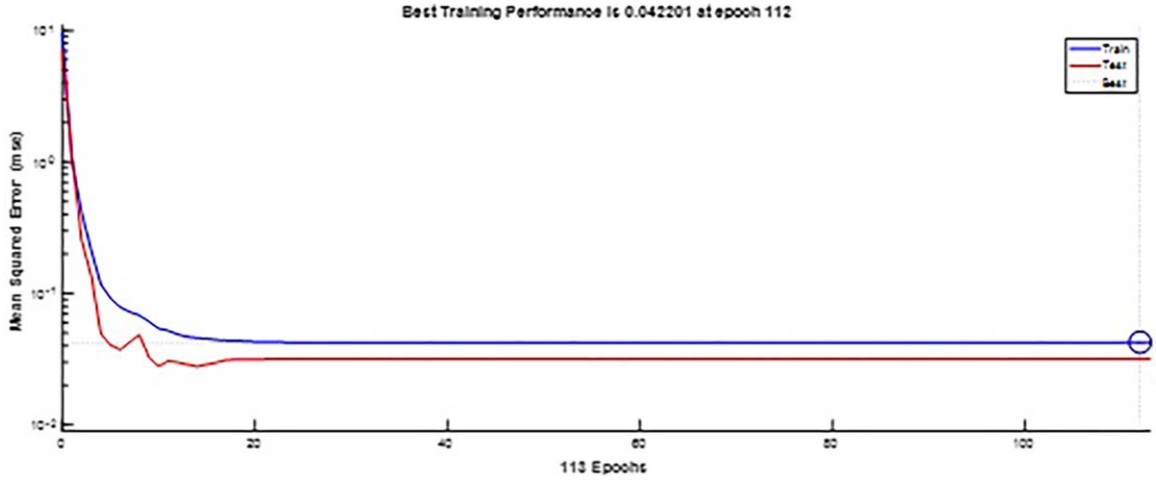

FIGURE 6 The performance plot of the MLP trained using the br algorithm

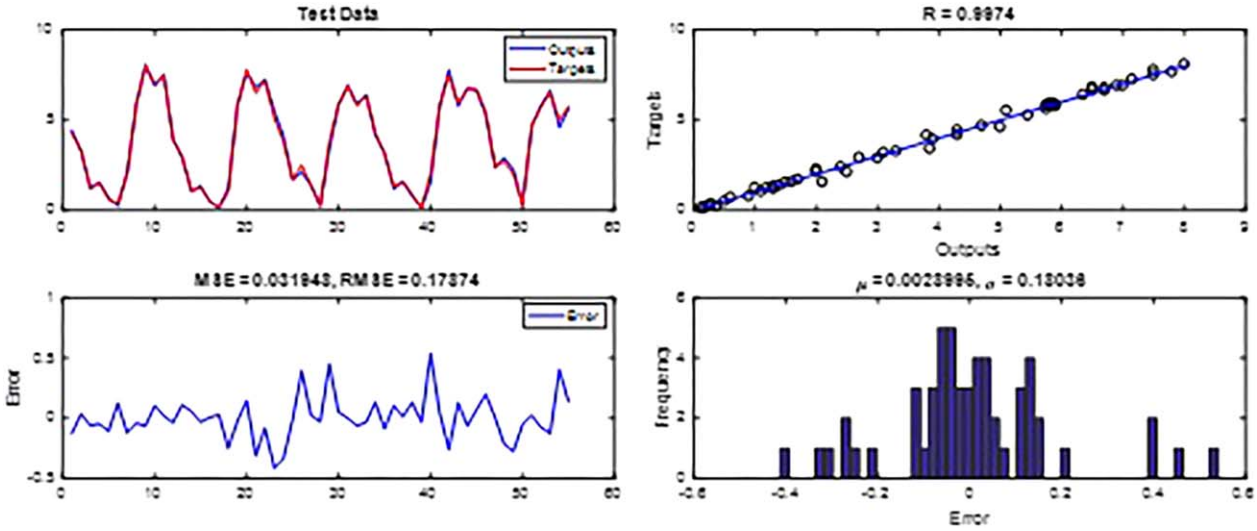

FIGURE 7 The data and error plots of the testing stage of the MLP trained using the br algorithm

correlation coefficient is always more than 0.99. In addition, the error mean, MSE, and RMSE of the network were always low, especially at the testing stage. This also confirms the high ability of the network to predict new conditions. The standard deviation of the testing stage also proved that the network error data were so close to the error mean and given the nearness of the mean to zero, it could be concluded that most of the error data were close to zero. This means that there was an acceptable affinity between the targets and outputs and the network can predict new observations properly.

In the case of the network trained using scg, the results clarified that the MSEs of the training, validation, and testing stages decreased along with each other, revealing that the scg algorithm was also successful in training the network. As can be seen in Figures 8 and 9, the network targets and outputs had a strong proximity at all the stages, especially at testing and the correlation coefficient was always higher than 0.99. Additionally, the error mean, MSE, and RMSE of the network were always low, especially at the testing stage. This also indicates the high predictive ability of the network.

3.8.2 | Radial basis function network

As depicted in Figure 10, the targets and outputs of RBFN were near each other and the correlation coefficients were always acceptable. Furthermore, the error mean, MSE, and RMSE of the network were always close to zero, especially at the testing stage. Demonstrating the high capability of the network of predicting new observations. The low standard deviation—as mentioned earlier—reveals that the error data of the network were so close to the error mean and since the error mean, especially at the testing stage, was approximately equal to zero, it could be declared that the prediction error was very small and RBFN was well able to predict the new observations. In conclusion, we can claim

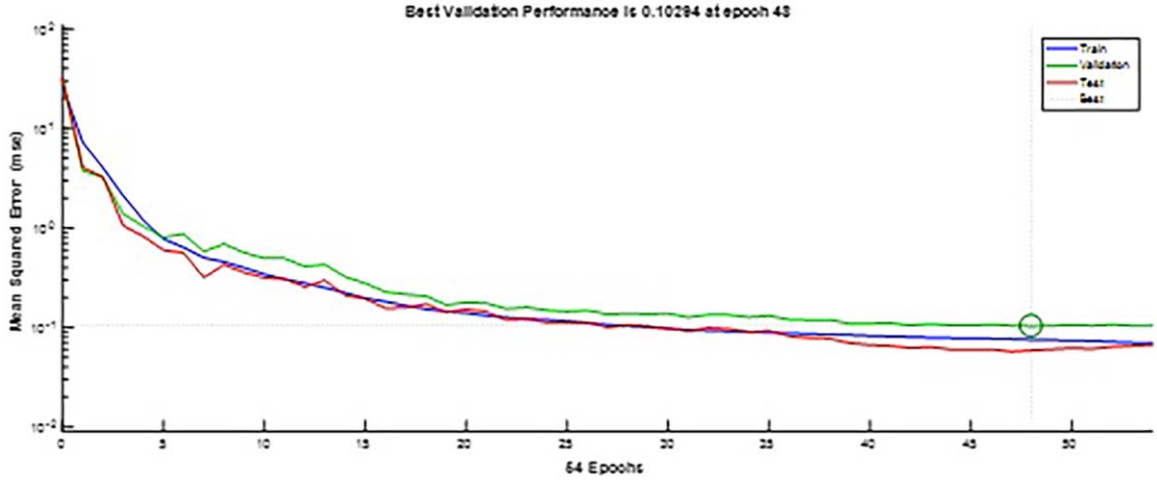

FIGURE 8 The performance plot of the MLP trained using the scg algorithm

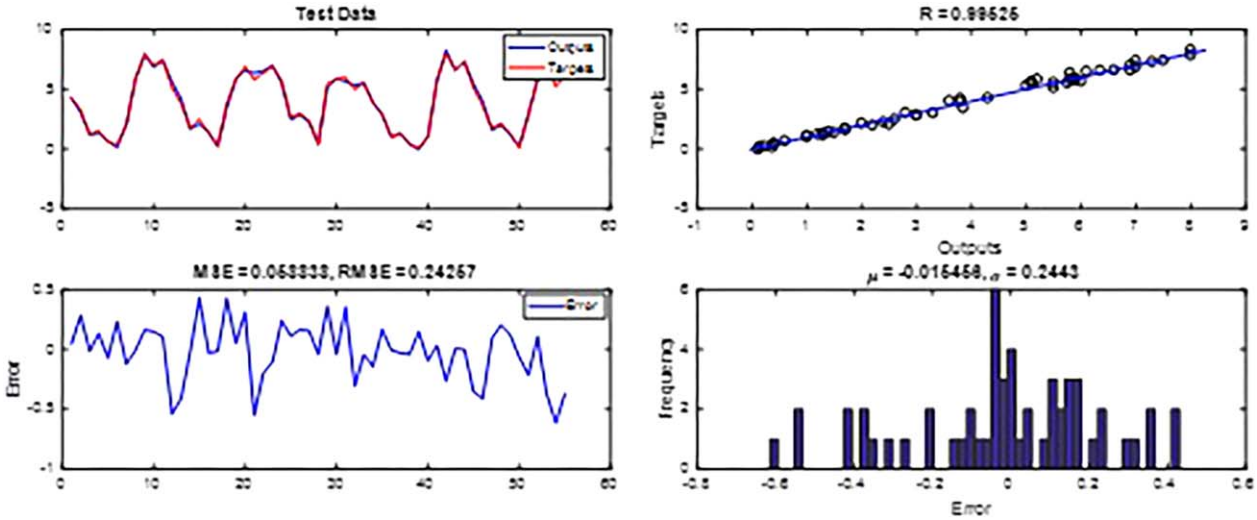

FIGURE 9 The data and error plots of the testing stage of the MLP trained using the scaled conjugate gradient (scg) algorithm

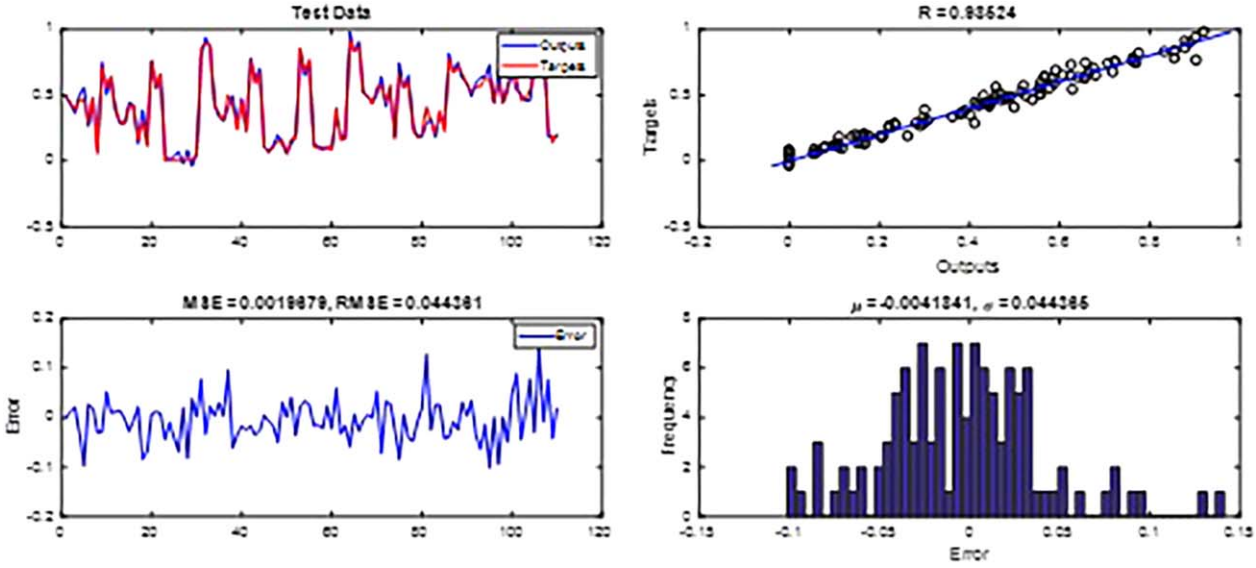

FIGURE 10 The data and error plots of the testing stage of the RBFN

that MLP and RBFN were both highly capable of fitting the data and predicting untested conditions. At the same time, RBFN seems to have performed better owing to its lower testing MSE.

4 | CONCLUSION

The results of the present study showed that TPEO had antioxidant and antimicrobial activities. Phytochemical analyses confirmed the presence of alkaloids, tannins, saponins, flavone, and glycosides. Camphor was the major constituent of TPEO. The results of the present study indicated that adding TPEO protected beef against lipid oxidation. Therefore, the strong *in vitro* antioxidant activity imparted by TPEO also had a protective effect on real beef. TBA and PV correlated properly with the microbiological data and sensory attributes. LRSM and LRSM + 1%TPEO extended the microbial shelf life of the beef samples up to 3 days. Conversely, LRSM + 1.5%TPEO and LRSM + 2%TPEO led to a significant shelf life extension of the beef by 9 days as compared with the control.

ACKNOWLEDGMENTS

The authors wish to express their profound gratitude sincerely to Applied Microbiology Research Center, Systems Biology, and Poisonings institute, Research Deputy of Baqiyatallah University of Medical Sciences.

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How to cite this article: Alizadeh Behbahani B, Imani Fooladi AA. Development of a novel edible coating made by Balangu seed mucilage and Feverfew essential oil and investigation of its effect on the shelf life of beef slices during refrigerated storage through intelligent modeling. *J Food Saf*. 2018;38:e12443. https://doi.org/10.1111/jfs.12443