The Journal of Medicinal Plants and By-Products

Original Article

Investigating the Antioxidant Activity of two Medicinal Plants; *Thymus daenensis* Celak. And *Echinophora cinerea* Boiss. Essential Oils in Soybean Oil by Neuro-Fuzzy Modeling

Sajad Ghaderi[1], Vahid Karimian[2*] and Parvin Ramak[3]

[1] *Department of Nutrition, Faculty of Health and Nutrition Sciences, Yasuj University of Medical Science (YUMS), Iran*
[2] *Department of Forest, Range and Watershed Management, Faculty of Agriculture and Natural Resources, Yasouj University, Yasouj, Iran*
[3] *Research Division of Natural Resources, Lorestan Agricultural and Natural Resources Research and Education Center, AREEO, Khorramabad, Iran*

*Corresponding author: Email: V. Karimian@yu.ac.ir

Article History: Received: 15 November 2023/Accepted in revised form: 26 May 2024

ABSTRACT

The aim of this study was to evaluate the composition of essential oils from *Thymus daenensis* and *Echinophora cinerea* and to predict oil oxidation using Neuro-Fuzzy modeling. In the hydrodistillation (HD) method, the starting time and total time for essential oil accumulation were 24 and 235 minutes, respectively. In the microwave-assisted hydrodistillation (MACE) method, these times were 8 and 58 minutes, respectively. The results indicated that the extraction yields of *T.daenensis* and *E.cinerea* using the HD and MACE methods were 2.26%, 2.34%, 1.18%, and 1.37%, respectively. For E. cinerea, the results were as follows. *T. daenensis* at a dosage of 500 mg/kg and E. cinerea at a dosage of 1000 mg/kg exhibited the most effective antioxidant activity. GC-Mass analysis results showed that thymol (39.8%) and p-cymene (19.2%) are the major compounds of *T.daenensis*, while α-phellandrene (16.6%), α-pinene (16.5%), p-cymene (15.8%), and thymol (13.3%) are the major compounds of *E. cinerea's* essential oil. Prediction of antioxidant activity for *T. daenensis* and E. cinerea involved using a feedforward backpropagation network with a topology of 2-4-3 and 2-3-3 as the optimized models, respectively. Neuro-fuzzy modeling was a suitable approach for assessing soybean oil's oxidation rate and evaluating essential oils' antioxidant properties.

Keywords: Medicinal plant, *Thymus daenensis*, *Echinophora cinerea*, Essential oil, Neuro-Fuzzy

INTRODUCTION

Among the most common methods to prevent lipid oxidation in vegetable oils, using antioxidants has been a practice for decades. However, recent studies suggest synthetic antioxidant compounds could present potential hazards and carcinogenic effects. Furthermore, natural antioxidants such as tocopherols and their derivatives, which can be used as substitutes for BHA and BHT, show limited effectiveness in certain systems and raise manufacturing costs. Consequently, there is a need to identify alternative natural and safe sources of antioxidants for incorporation into food products. These safer sources of antioxidants can be especially found in plants, where relevant research has notably increased in recent years [1]. The research into using natural antioxidants to stabilize edible oils has concluded [2,3] that most natural additives demonstrate greater thermal stability and antioxidant potency compared to synthetic ones in various edible oils. Essential oils, as natural antioxidant compounds, have a strong potency in edible oils. Essential oils are a large group of natural products that exhibit various biological properties, including antimicrobial, antioxidant, anti-cancer, antiviral, anti-parasitic, disinfectant, and insecticidal activities. These oils are extensively used in the medicinal, cosmetic, and food industries. [4]. *Thymus daenensis* Celak, a member of the Lamiaceae family, is commonly known as a medicinal plant, a flavoring agent (condiment and spice), and herbal tea in Iran [5]. It has been reported that *T. daenensis* leaves are rich in phenolic compounds with valuable antioxidant activity [6,7]. *Echinophora cinerea* Boiss. A member of the Apiaceae family, the common name "Khosharizeh," is an endemic species in Iran. Its fresh and dried aerial parts have been used as flavoring agents in cheese and yogurt [8]. The high radical

scavenging effects (IC_{50}: 49.7 ± 2.3 µg/mL) of *E. cinerea* essential oil in various antioxidant systems have been reported [9].

The conventional method for extracting essential oil from medicinal plants is hydro distillation (HD). This method leads to the degradation of some compounds due to thermal effects and the loss of volatile components [10]. The use of microwave [11] and ohmic heating [12] as promising alternatives has been introduced to mitigate the limitations of conventional extraction techniques and enhance the quality of the essential oils obtained. To mitigate the limitations of conventional extraction techniques and improve the quality of the essential oils obtained, new alternatives such as ohmic-assisted hydro distillation (OAHD) [12,13] and microwave-assisted hydro distillation (MAHD) [10,11] have been introduced. Given the limited number of studies on neuro-fuzzy modeling and the shelf-life of edible oils, especially regarding the use of medicinal plants, this research aims to introduce a new method for assessing an approach to verifying the oxidative stability of oils during storage using neuro-fuzzy systems and determining the optimal artificial neural network topology. For this reason, the essential oils of *Echinophora cinerea and Thymus daenensis* were extracted using two different methods: Microwave-assisted Clevenger extraction (MACE) and Clevenger extraction systems. Their chemical compositions were then compared. The antioxidant activities of essential oils in soybean oil were evaluated using Neuro-Fuzzy modeling and compared to those of butylated hydroxytoluene (BHT) as a synthetic antioxidant.

MATERIAL AND METHODS

Sample Preparations

Thymus daenensis and *Echinophora cinerea* were obtained from the Medicinal Plants Research Institute of Iran. The samples were identified and authenticated with the numbers 6093 and 4507. The aerial parts of plants were dried in the shade at 40 °C for 2 days and then ground. 60 grams of plant powder was mixed with water (600 ml) in a 1000 ml volumetric flask for essential oil extraction using each method (n=4).

Extraction of Essential Oil by Water Distillation Extraction Method

The Clevenger system consisted of an electric heater, a volumetric flask (1000 ml), a condenser, and a collector. In this method, essential oil was extracted using Clonger's water distillation design for 4 hours, and the essential oils were collected and stored at 4°C until the analysis. During the first 30 min of the process, the amount of essential oil extracted was recorded at 1-minute intervals, followed by measurements every 10 minutes. The amount of essential oil extracted during the last 60 min of the extraction was recorded every 30 min [14].

Extraction of Essential Oil by Microwave Assisted Water Distillation Extraction (MACE)

A microwave oven (Samsung, model: CF3110N-5, Korea) was modified for MACE. The modified MACE system was similar to the Clevenger system, but a microwave was used as the heating source instead of a heater. The volumetric flask was placed inside the microwave oven with a condenser apparatus through a hole above the system. The microwave output was 900 W with a 2450 MHz frequency, and its inner cavity dimensions were 400×300×250 mm. The extraction time was 2 hours. To prevent the super-boiling of the mixture, after 5 min initial irradiation, the system was set up as 5-sec power "ON," 15-sec power "OFF." The essential oils were collected and stored at 4 °C until the analysis. During the first 30 min of the process, the amount of essential oil extracted was recorded at 1-minute intervals, followed by recording every 10 min, and the amount extracted during the last 60 min of the extraction was recorded every 30 min [14].

Essential Oil Extraction Yield

The percentage yield was calculated by dividing the volume of essential oil extracted (mL) into weight of dry plant materials (g), multiplied by 100 [15]:

$$Yield = \frac{m_{Essential\ oil}}{m_{dried\ plant}} \times 100 \qquad (1)$$

Chemical Composition of Essential Oil by GC and GC-Mass Analysis

GC (Gas chromatography) analysis was performed [8] by a Perkin Elmer 8500 gas chromatograph with a BP-1 capillary column (39 m × 0.25 mm) and the film thickness of 0.25 µm, coupled with the FID detector. The

carrier gas was helium with a 2 ml/min flow rate. The oven, injector, and detector temperatures were 280 °C. The mass spectroscopy was carried out by Hewlett Packard 6890 gas chromatograph equipped with an HP-5MS capillary column. The oven temperature program was set the same as GC condition, following a Hewlett Packard 6890 MS detector (ionized potential 70eV and source temperature 200 °C). The results were interpreted and reported according to the comparison with Retention Indices (RI) relative to the homologous series of n-alkanes and using libraries of Wiley 275. L and Wiley 7n.1, and also comparing the fragmentation patterns of the mass spectra with data in literatures [16].

Evaluation of the Antioxidant Activity of Essential Oils in Soybean Oil

Evaluation of Antioxidant Effects by Storage Oxidative Stability Test

Two concentrations of *T. daenensis* and *E. cinerea* essential oils (500 and 1000 mg/kg) were prepared in soybean oil. The essential oils were dissolved completely in soybean oil. 30 ml of soybean oil samples were transferred into 50 ml open glass bottles. The bottles were placed in a forced air circulation oven with a temperature of 55 °C for 20 days. Soybean oil without any additive (negative control) and containing 100 and 200 mg/kg BHT (positive controls) were prepared as controls. Periodically (every 4 days), the samples were removed from the oven, flushed with nitrogen, and stored at -20 °C until the analysis [13].

Evaluation of Antioxidant Effects by Oil Stability Analysis

Schaal oven test was applied to assess the oxidative stability of soybean oil. Peroxide, Thiobarbituric acid (TBA), and p-anisidine values using spectrophotometer WPA (S2000, UK) at the wavelength of 532 and 350 nm were used to assess the stability of soybean oil during oven storage for 20 days. The peroxide, p-anisidine and TBA values evaluation were performed according to AOCS Cd 8b-90 [17], AOCS Cd 18-90 [18] and AOCS Cd 19-90 [19] procedure.

Statistical Analysis

In order to assess the oxidative stability of soybean oil containing 0, 500, and 1000 ppm of essential oils, a Neuro-Fuzzy modeling has been applied. To evaluate the relation between input factors (concentration, time) with output factors (peroxide, TBA, and p-anisidine values), after expanding the data using fuzzy logic, an artificial neural network with the following characteristics has been designed:

Types of designed networks: Feed Forward Back Propagation (FFBP) and Cascade Forward Back Propagation (CFBP), Training algorithm: Levenberg- Marquardt, transfer function of hidden layer: Hyperbolic tangent sigmoid, transfer function of output layer: Linear, Number of hidden layer: 1, Number of neurons in hidden layer: 5, Number of training cycle: 1000 epoch, Evaluation factors: Correlation coefficient (R) and mean square error.

In addition, in this process, 60 percent of data was used for training, 15 percent for validation, and 25 percent of data was used for testing the designed network. The transfer function of hyperbolic tangent sigmoid for optimization of the hidden layer was as follows, formula (2):

$$Y_j = \frac{2}{(1+\exp(-2x_j))} - 1 \qquad (2)$$

Where, x_j: The total of weighted inputs for each neuron of layer j, which was calculated as the following formula:

$$x_j = \sum_{i=1}^{m} w_{ij} \times Y_i + b_j \qquad (3)$$

m: The number of neurons for output layer

W_{ij}: Weight between layer i and layer j

Y_i: The output of neurons for layer i

b_j: Bias for neurons of layer j

The Neuro-Fuzzy modeling and preparing of its graphs was carried out by Matlab software (version 7.10.0.499 R2010a), and other statistical analyses (ANOVA) were performed by Minitab 16 (State College, PA, USA) using full factorial design. All experiments were performed in triplicate, and the mean values were reported. The graphs for the Schaal oven test were drawn using Microsoft Excel version 2010.

RESULTS AND DISCUSSION

3.1 Comparison of the extraction effects on the extraction yield and time

The results indicated that the onset of essential oil accumulation, total extraction time, and yield varied between the HD and MACE methods. The onset of essential oil accumulation occurred at 24 minutes in the HD method and 8 minutes in the MACE method. There is a highly significant ($P<0.05$) difference in the total extraction time between the two methods: just over 58 min for MACE and almost 235 min for HD ($P<0.05$). The results of this study were consistent with previously published findings, which concluded that microwave irradiation is a rapid extraction method [20]. The results indicated that the extraction yield of *Thymus daenensis* was 2.34% and 2.26% using the MACE and HD methods, respectively. For *Echinophora cinerea*, the percentages were 1.37% and 1.18%, respectively. The significant ($P<0.05$) reduction in extraction time and the insignificant ($P\geq0.05$) increase in extraction yield in the case of MACE may be attributed to the high-pressure gradient formed inside the plant material. Microwave absorption results in significant internal heating, creating higher internal pressures that enhance oil extraction from the leaves. The glands and oil-producing receptacles can swell and burst due to internal heating. The decrease in processing time with increased power can simply be attributed to the higher extraction rates observed at higher powers (2008).

Golmakani and Rezaei (2008) also reported similar findings for the extraction yield of essential oils obtained by HD and MACE [11]. These results were supported by the research conducted by Gavahian et al. (2012) and Seidi Damyeh (2016a), which found no significant difference in the essential oil extraction yield obtained by HD and OAHD [12,14].

Chemical Properties of Essential Oils

Upon reviewing the results, no significant difference ($P>0.05$) was observed in the chemical compositions of essential oils extracted by the conventional Clevenger and MACE methods. Table 1 shows that thymol (39.8%) and p-cymene (19.2%) are the primary components of *T. daenensis* essential oil, while thymol and p-cymene constitute 13.3% and 15.8% of *E. cinerea* essential oil**.** The major compounds in E. cinerea essential oil were α-phellandrene (16.6%), α-pinene (16.5%), p-cymene, and thymol. The extraction procedure had no significant effect ($P> 0.05$) on the chemical composition of essential oils. However, the microwave extraction method identified a higher number of compounds compared to the Clevenger extraction method. On the other hand, the concentration of compounds such as thymol, which plays a significant role in antioxidant properties, was higher in the microwave method than the Clevenger method. Due to the internal heating of MACE through electrical flow and the occurrence of electroporation under the influence of an electric field, easier extraction of some components is observed. In recent years, MACE has been used for the extraction of phenolic compounds such as flavones [21], vanillic acid, gallic acid, p-coumaric acid, catechin, ferulic acid, tocopherols, and tocotrienol [22].

The results of this study were consistent with those of other studies, suggesting that microwave techniques are more effective than traditional methods for extracting essential oils without affecting their chemical compositions. The study of spearmint, basil, and thyme essential oils revealed that microwave extraction had no significant ($P>0.05$) impact on the chemical compositions of the essential oils. The chemical profiles of the essential oils extracted by microwave were found to be the same as those obtained through the hydro distillation method [23]. The extraction of thyme (*Zataria multiflora*) essential oil using microwave technology as a green method had no detrimental effects on the chemical components of the essential oils [11]. The safety of microwave techniques on the chemical composition of essential oils was confirmed in two species of savory essential oils [24]. Furthermore, some researchers have confirmed the superiority of the microwave technique over the hydro-distillation method for extracting cardamom essential oil [25].

Table 1 The chemical composition of essential oil by Conventional Clevenger (CC) and Microwave assisted Clevenger extraction (MACE) methods

T. daenensis				*E. cinerea*			
Compound	Retention index	Percentage		Compound	Retention index	Percentage	
		MACE	CC			MACE	CC
Camphene	946	0.71	0.54	α-Pinene	932	16.56	15.08
β-Pinene	971	0.15	0.16	Camphene	946	0.25	0.33
β-Myrcene	980	1.14	0.9	β-Pinene	971	1.1	1.5
α-Phellandrene	997	0.16	0.19	Sabinene	977	0.59	0.21
δ-3-carene	1006	0.05	-	β-Myrcene	980	0.82	1.1
α-Terpinene	1011	1.69	1.55	α-Phellandrene	997	16.69	17.02
P-Cymene	1015	19.22	20.01	α-Terpinene	1011	0.35	0.38
β-Phellandrene	1023	0.34	0.38	P-Cymene	1015	15.89	15.55
1,8-Cineol	1031	0.02	0.06	β-Phellandrene	1023	3.86	2.77
E-β-Ocimene	1044	0.04	-	1,8-Cineol	1031	0.25	-
γ-Terpinene	1052	8.3	8.01	γ-Terpinene	1052	2.63	3.11
Cis-sabinene hydrate	1071	0.28	0.18	Fenchone	1075	0.84	-
Terpinolene	1078	0.22	0.31	Linalool	1085	4.89	5.19
Linalool	1085	6.12	6.44	trans-Rose Oxide	1126	0.16	0.44
trans-Chrysanthemal	1124	0.16	0.19	trans-p-2-Menthen-1-ol	1127	0.15	-
Borneol	1152	4.11	4.33	Pinocarvone	1137	0.56	-
4-Terpineol	1169	0.54	0.34	trans-Pinocarveol	1138	0.96	1.2
α-Terpineol	1180	0.13	0.08	trans-Verbenol	1145	0.38	0.43
cis-Geraniol	1209	0.07	-	Borneol	1152	1.18	0.97
cis-Citral	1222	0.11	0.19	unknown	1155	0.49	0.66
Thymol	1268	39.89	37.03	Cryptone	1156	0.16	0.34
carvacrol	1294	9.74	10.23	Terpin-4-ol	1169	0.37	0.28
				α-Terpineol	1180	1.33	1.21
				Citronellol	1208	3.83	4.97
				trans-Myrtanol	1259	0.22	0.36
				Thymol	1268	13.36	12.54
				unknown	1273	1.89	2.12
				carvacrol	1294	5.98	6.89

Neuro-Fuzzy Modeling

An artificial neural network (ANN) is a machine-learning model whose structure and function are inspired by the behavior of the human brain. The primary components of a neural network are the neurons. These neurons are arranged in input, output and one or more hidden processing layers. Neuro-fuzzy system model combines a neural network and a fuzzy inference system, using the neural network to determine the parameters of the fuzzy inference system. Fuzzy logic also improves the generalization capability of a neural network by producing more reliable output when extrapolation is required beyond the limits of the training data. Intelligent networks have been successfully utilized to predict changes in the properties and quality of food products during processing and storage. These networks have already been used to simulate processes such as fermentation, the drying behavior of various foods, osmotic dehydration, and cross-flow microfiltration. Additionally, to predict the oxidation of Menhaden fish oil during storage, the rheological properties of Iranian bread dough and the freshness index of modified atmosphere-packed rice snacks during the storage period were examined [26].

Due to the same chemical composition of essential oils, the essential oils extracted by MACE were chosen for the Schaal oven test. Table 2 shows the effect of the number of neurons in a hidden layer on the model estimation efficiency for the oxidation rates of samples during a 20-day Schaal oven test. Regarding the mean squared error (MSE) and correlation coefficient (R) values across different topologies, the feed forward back propagation network with a 2-4-3 topology (2 neurons for the input layer, 4 neurons for the hidden layer and 3 neurons for output layer) (MSE:0.0725 and R:0.9971), and cascade forward back propagation with topology of 2-3-3 (MSE:0.0340 and R:0.9972) were selected as optimized models for *T. daenensis* antioxidant activity.

Table 2 The effects of the neurons number in a hidden layer on the models' efficiency estimation regarding samples' oxidation rates during 20 days Schaal oven test.

Number of neurons in hidden layer	*T. daenensis*				*E. cinerea*			
	FFBP		CFBP		FFBP		CFBP	
	R	MSE	R	MSE	R	MSE	R	MSE
1	0.9705	0.7155	0.9577	0.7212	0.9703	0.4578	0.9907	0.2067
2	0.9708	0.5421	0.9925	0.1737	0.9955	0.1654	0.9865	0.2280
3	0.9963	0.0646	0.9972	0.0340	0.9974	0.0434	0.9962	0.0691
4	0.9971	0.0725	0.9972	0.0697	0.9961	0.0668	0.9975	0.0512
5	0.9963	0.0818	0.9970	0.0615	0.9975	0.0518	0.9975	0.0225

FFBP; Feed Forward Back Propagation, CFBP; Cascade Forward Back Propagation, R; Correlation coefficient, MSE; Mean Squared Error

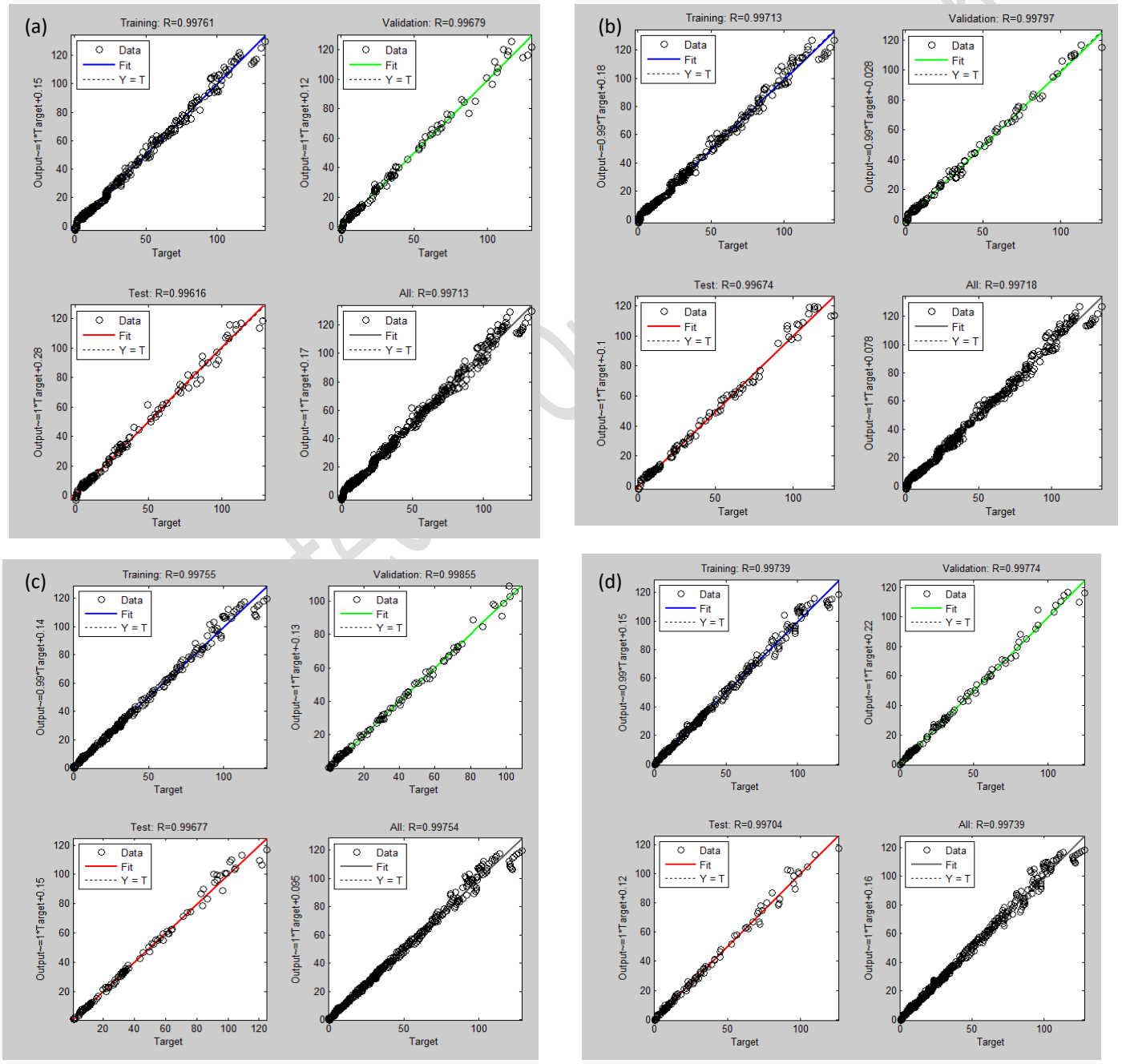

Fig. 1 The correlations between estimated data by the model and real data from the experiments in 4 states of training, validation, test, and overall, for the selected topologies of *T. daenensis* FFBP (a), *T. daenensis* CFBP (b), *E. cinerea* FFBP (c), and *E. cinerea* CFBP (d). FFBP; Feed Forward Back Propagation, CFBP; Cascade Forward Back Propagation.

Also, for *E. cinerea* antioxidant activity, feed forward back propagation network with the topology of 2-3-3 (MSE:0.0434 and R:0.9974) and cascade forward back propagation with the topology of 2-5-3 (MSE:0.0225 and R:0.9975) were used as an optimized model. The correlations between the estimated data by the model, and the real data from the experiments in 4 stages of training, validation, test, and overall, for the selected topologies were indicated in Fig. 1. Regarding the results, it could be concluded that Neuro-Fuzzy modeling could predict the oxidation rate of soybean oil and antioxidant activity of *T. daenensis* and *E. cinerea* essential oils with high correlation with real data.

Karaman *et al* (2011) survey the comparison of adaptive neuro-fuzzy inference system and artificial neural networks for estimation of oxidation parameters of sunflower oil added with some natural byproduct extracts. Peroxide values of sunflower oil samples containing different natural extracts were found to be lower compared to control sample [27]. Adaptive neuro-fuzzy inference systems (ANFIS) and artificial neural networks (ANN) were used for the construction of models that could predict the oxidation parameters and were compared to multiple linear regression (MLR) for the determination of the best model with high accuracy. Comparison of adaptive neuro-fuzzy inference system and artificial neural networks (MLP and RBF) for estimation of oxidation parameters of soybean oil added with curcumin investigated by Asnaashar et al. (2015) [26]. They started the adaptive neuro-fuzzy inference system (ANFIS) and multilayer perceptron (MLP) and radial basis function (RBF) functions of artificial neural network (ANN) with three inputs (temperature and concentration, time of sampling) and three outputs (PV, AV, and IV) were used for the construction of models that could predict the oxidation parameters. Intelligent networks have been successfully used to predict property and quality changes of food products during processing and storage. These networks have already been applied to simulate processing such as fermentation, drying behavior of different foods, osmotic dehydration, and cross-flow microfiltration. Also, to predict the Menhaden fish oil oxidation during storage, rheological properties of Iranian bread dough, and freshness index of modified atmosphere-packed rice snack during the storage period.

Antioxidant Activity of Essential Oils in Soybean Oil

The peroxide value of blank sample without any additives reached a maximum value of 39.4 meq/kg after 20 days of storage (Fig. 2). The peroxide value of soybean oil containing 500 and 1000 mg/kg of *T. daenensis* essential oil, 500 and 1000 mg/kg of *E. cinerea* essential oil, 100 mg/kg and 200 mg/kg BHT were 30.5, 38.5, 34.9, 30.1, 33.8 and 29.9 meq/kg; respectively. The oxidation rate for samples containing BHT was different from the other samples. In BHT samples, the peroxide values increased slowly during the first 12 days of storage, and consequently, there was a remarkable increase until the end of storage. On the contrary, other samples had fast-increasing trends during the first 12 days, and then, the trends were slower. The inhibition rates of oxidation in soybean oils containing *E. cinerea* increased at higher concentrations, which were comparable to BHT. On the 20th day, the peroxide value of soybean oils containing 1000 mg/kg *E. cinerea* was lower than 500 mg/kg BHT. The antioxidant activity of 500 mg/kg *T. daenensis* essential oil (30.5 meq/kg) was significantly ($P<0.05$) higher than 500 mg/kg *E. cinerea* essential oil (34.9 meq/kg), which was comparable to 100 mg/kg BHT ($P<0.05$), but interestingly, the antioxidant activity of 1000 mg/kg *T. daenensis* essential oil was less than its antioxidant activity at 500 mg/kg.

As it has been shown in Fig. 2, no TBA values (g/kg) of samples were comparable to BHT. TBA values (g/kg) of blank samples and samples containing essential oils exhibited an increasing trend until day 16, and then, interestingly, its trend was reversed. This phenomenon could be related to the oxidation of secondary autoxidation products and the formation of carboxylic acids [28]. The p-anisidine value is provided to estimate secondary oxidation products (principally 2, 4-alkadienals and 2-alkenals), which are generated during the decomposition of hydroperoxides. As Fig. 2 shows, the slow increasing trend in p-anisidine value was for the first 12 days of the experiment and then, a sharper increase was determined until the end of storage. Regarding p-anisidine value results, BHT, 1000 mg/kg *E. cinerea*, and 500 mg/kg *T. daenensis* showed the best antioxidant activity, respectively.

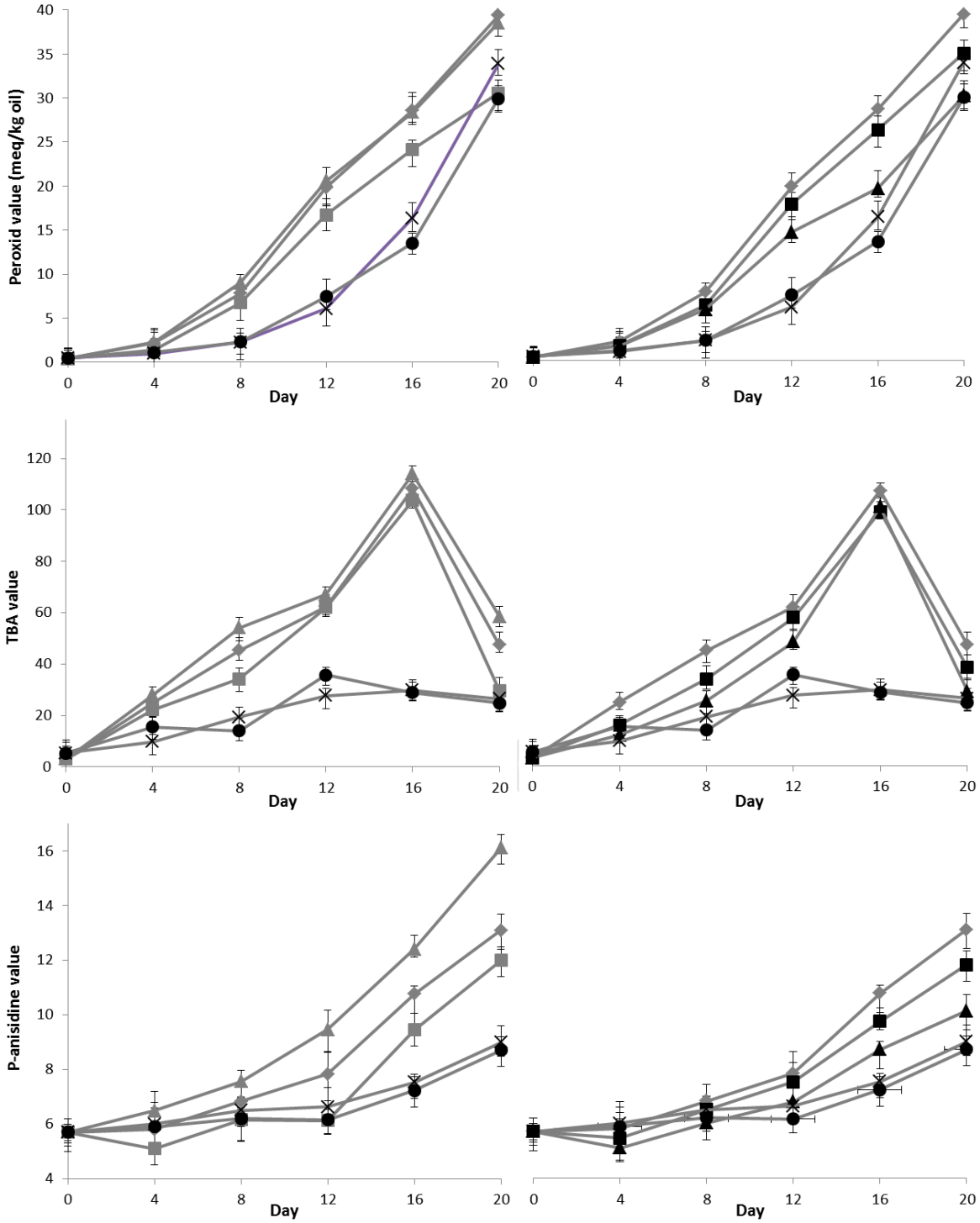

Fig. 2 The peroxide, TBA and p-anisidine values (the average of three replicates) of soybean oil samples containing *T. daenensis* at 500 (■), and 1000 (▲) mg/kg, *E. cinerea* at 500 (■) and 1000 (▲) mg/kg, BHT at 100 (×) and 200 (●) mg/kg, and soybean oil without any additive (◆) storage at 55 °C for 20 days.

Regarding the decreasing antioxidant activity of *T. daenensis* at higher concentrations, the same results from TBA and p-anisidine values as peroxide values were obtained. It could be concluded that *T. daenensis* essential oil had better antioxidant activity at low concentrations, comparable to BHT and 1000 mg/kg *E. cinerea* essential oil, which could be used as suitable candidates for synthetic antioxidants. This agrees with the radical scavenging capacity results that *T. daenensis* essential oil showed stronger reducing power than *E. cinerea* essential oil and BHT. These results indicate that essential oils rich in phenolic monoterpenes (thymol, carvacrol, and P-Cymene) are more potent reductants and radical scavengers than those rich in monoterpene hydrocarbons [29]. Thymol and carvacrol are the major volatile compounds of the essential oil fractions of oregano and thyme, and they are known for their high in vitro antioxidant activities. The current and potential

applications of thymol and carvacrol and their major compounds in food systems as antimicrobial and antioxidant additive antimicrobial and antioxidant additives have been reviewed comprehensively in the literature [30].

Carum nigrum essential oil (0.02%) in mustard oil at 60 °C for 28 days by evaluating the peroxide index and TBA showed that the essential oil was able to reduce the oxidation rate of mustard oil, and this antioxidant potency was comparable to BHT and BHA (0.02%). The antioxidant potency of garlic extract (0.1%) in sunflower oil at 65 °C for 24 days stabilized the oil, which was comparable to BHA and BHT [31]. In other investigations on grape peels and seeds as antioxidant agents, the higher content of phenolic compounds in red grape peels had higher antioxidant effects than its seeds in sunflower oil [32]. Thyme, marjoram, and pennyroyal essential oils reduced the oxidation rate in butter during storage [33]. Using rosemary [34], and thyme [35] essential oils in sunflower oil reduced the oxidation rates. According to the results of other investigations and this article, essential oil could be a good candidate as antioxidant agents in edible oils. Seidi Damyeh et al. (2016b) studied the Effect of the essential Oil Extraction Method from *Satureja macrosiphonia* on Its Biological Activities [15]. The antioxidant activity of these methods showed no significant difference from that of the other.

CONCLUSION

Our results showed that Neuro-Fuzzy modeling could be used as an effective method for predicting the oxidation rate of soybean oil and the antioxidant activity of the essential oils from *T. daenensis* and E. cinerea, with a high correlation to real data. MACE significantly reduced the total and initial extraction time, but it had an insignificant effect ($P>0.05$) on essential oil composition. The essential oils of *T. daenensis* and E. cinerea contain antioxidant compounds such as thymol, p-cymene, and α-phellandrene, which exhibit significant antioxidant activity. The optimal concentrations of *T. daenensis* and E. cinerea extracts in soybean oil for achieving better antioxidant activity are 500 and 1000 mg/kg, respectively.

REFERENCES

1. Keramat M., Golmakani M.T. Effect of *Thymus vulgaris* and *Bunium persicum* essential oils on the oxidative stability of virgin olive oil. Grasas y aceites. 2016; 67 (4), 162.
2. Bera D., Lahiri D., Nag A. Studies on a natural antioxidant for stabilization of edible oil and comparison with synthetic antioxidants. J. Food Engin. 2006; 74: 542-545.
3. Taghvaei M., Jafari S.M. Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J. Food Sci. Technol. 2015; 52: 1272-1282.
4. Karimian V., Sepehry A., Barani H., Nejad Ebrahimi S., Mirjalili M.H. Productivity, essential oil variability and antioxidant activity of *Ferula assa-foetida* L. oleogum-resin during the plant exploitation period. J. Essential Oil Res. 2020; 32: 1-11.
5. Nickavar B., Mojab F., Dolat-Abadi, R. Analysis of the essential oils of two *Thymus* species from Iran. Food Chem.2005; 90: 609-611.
6. Lee S. J., Umano K., Shibamoto T., Lee K. G. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. 2005; 91: 131-137.
7. Emami Bistgani Z., Ataollah Siadat S., Bakhshandeh A., Ghasemi Pirbalouti A., Hashemi M., Maggi F., Morshedloo M. Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak, Industrial Crops and Products. 2018; 121: 434-440.
8. Gholivand M.B., Rahimi-Nasrabadi M., Mehraban E., Niasari M., Batooli H. Determination of the chemical composition and in vitro antioxidant activities of essential oil and methanol extracts of *Echinophora platyloba* DC. Natural Product Res. 2011; 25: 1585-1595.
9. Saei-Dehkordi S.S., Fallah A.A., Saei-Dehkordi S.S., Kousha S. Chemical Composition and Antioxidative Activity of *Echinophora platyloba* DC. Essential Oil, and Its Interaction with Natural Antimicrobials against Food-Borne Pathogens and Spoilage Organisms. J. Food Sci. 2012; 77: 631-637.
10. Ferhat M.A., Meklati B.Y., Smadja J., Chemat F. An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. J. Chromatography. 2006; 1112: 121-126.
11. Golmakani M.T., Rezaei K. Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extractionof essential oils from Thymus vulgaris L. Food Chem. 2008; 109: 925-930.

12. Gavahian M., Farahnaky A., Javidnia K., Majzoobi M. Comparison of ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from Thymus vulgaris L. Innovative Food Sci. Emerging Technol. 2012; 14: 85-91.
13. Hashemi M.B., Niakousari M., Saharkhiz M.J. Antioxidant activity of *Satureja bachtiarica* Bunge essential oil in rapeseed oil irradiated with UV rays. European J. Lipid Sci. Technol. 2011; 113: 1132-1137.
14. Seidi Damyeh M., Niakousari M., Golmakani M.T., Saharkhiz M.J. Microwave and ohmic heating impact on the in situ hydrodistillation and selective extraction of *Satureja macr*osiphonia essential oil. J. Food Processing and Preservation. 2016a; 40: 647-656.
15. Seidi Damyeh M., Niakousari M., Saharkhiz M.J., Golmakani M.T. Evaluating the Effect of Essential Oil Extraction Method from *Satureja macrosiphonia* on Its Biological Activities: Ohmic-and Microwave-Assisted Hydrodistillation. J. Food Processing and Preservation. 2016b; 40: 697-706.
16. Adams R., P. Identification of essential oil components by gas chromatography/mass spectrometry. (4th ed.). Allured Publishing Corp., Carol Stream, IL. 2012.
17. AOCS. Official Method Cd 8b-90. Peroxide Value Acetic Acid-Isooctane Method. Sampling and Analysis of Commercial Fats and Oils.2003.
18. AOCS. AOCS Official Method Cd 18-90: p-Anisidine Value. Official Methods and Recommended Practices of AOCS. 2017.
19. AOCS. AOCS Official Method Cd 19-90: 2-Thiobarbituric acid value direct method. Animal Models of Diabetic Complications Consortium: AMDCC Protocols. 2001.
20. Taghvaei M., Jafari S.M., Assadpoor E., Nowrouzieh S., Alishah O. Optimization of microwave-assisted extraction of cottonseed oil and evaluation of its oxidative stability and physicochemical properties. Food Chem. 2014; 160: 90-97.
21. Terigar B.G., Balasubramanian S., Boldor D., Xu.Z., Lima M., Sabliov C.M. Continuous microwave-assisted isoflavone extraction system: Design and performance evaluation. Bioresource Technology. 2010; 101: 2466-2471.
22. Zigoneanu I.G., Williams L., Xu.Z., Sabliov C.M. Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresource Technology. 2008: 99: 4910-4918.
23. Lucchesi M. E., Chemat F., Smadja J. Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. J. Chromatography A. 2004; 1043: 323-327.
24. Rezvanpanah S., Rezaei K., Razavi S.H., moini S. Use of Microwave-assisted Hydrodistillation to Extract the Essential Oils from *Satureja hortensis* and *Satureja montana.* Food Science and Technology Research. 2008; 14: 311-314.
25. Lucchesi M.E., Smadja J., Bradshaw S., Louw W., Chemat F. Solvent free microwave extraction of *Elletaria cardamomum* L.: A multivariate study of a new technique for the extraction of essential oil. Journal of Food Engineering. 2007; 79: 1079-1086.
26. Asnaashari E., Asnaashari M., Ehtiati A. Farahmandfar R. Comparison of adaptive neuro-fuzzy inference system and artificial neural networks (MLP and RBF) for estimation of oxidation parameters of soybean oil added with curcumin. J. Food Measurement and Characterization. 2015; 9: 215-224.
27. Karaman S., Ozturk I., Yalcin H., Kayacier A., Sagdic O. Comparison of adaptive neuro-fuzzy inference system and artificial neural networks for estimation of oxidation parameters of sunflower oil added with some natural byproduct extracts. J. Sci. Food Agric. 2011; 92: 49-58.
28. Akoh C.C., Min D.B. Food lipids chemistry, nutrition, and biotechnology. CRC London, New York. 2008; 121: 3810–3810.
29. Shahsavari N., Barzegar M., Sahari M.A., Naghdi Badi H. An investigation on the antioxidant activity of essential oil of *Zataria multiflora* Boiss. in soy bean oil. Journal of Medicinal Plants. 2008; 4: 56-68.
30. Gursul S., Karabulut I., Durmaz G. Antioxidant efficacy of thymol and carvacrol in microencapsulated walnut oil triacylglycerols. Food Chem. 2019; 278: 805-810.
31. Iqbal S., Bhanger M.I. Stabilization of sunflower oil by garlic extract during accelerated storage. Food Chem. 2007; 100: 246-254.
32. Shaker E.S. Antioxidative effect of extracts from red grape seed and peel on lipid oxidation in oils of sunflower. LWT - Food Science and Technology. 2006; 39: 883-892.
33. Amarowicz R., Zegarska Z., Rafałowski R., Pegg R.B., Karamac M., Kosinska A. Antioxidant activity and free radical-scavenging capacity of ethanolic extracts of thyme, oregano, and marjoram. European Journal of Lipid Science and Technology. 2009; 111: 1111-1117.
34. Hras A.R., Hadolin M., Knez Z., Bauman D. Comparison of antioxidative and synergistic effects of rosemary extract with α-tocopherol, ascorbyl palmitate and citric acid in sunflower oil. Food Chem. 2000; 71: 229-233.
35. Loziene K., Venskutonis P.R., Šipailiene A., Labokas J. Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. Food Chem. 2007; 103: 546-559.