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Rheological analysis for detection of extra virgin olive oil adulteration with vegetable oils: predicting oil type by artificial neural network

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RESEARCH ARTICLE

Abstract

Food adulteration is a major concern in the food industry. High prices and increasing demand have made the adulteration of extra virgin olive oil (EVOO) a major concern for consumers. The purpose of this study was to detect EVOO adulteration by using rheological parameters. EVOO adulteration was identified with the addition of different types of vegetable oils (hazelnut, sunflower, and canola) at a ratio 25, 50 and 75% by weight. Refractive index (RI; 20 °C) and fatty acid composition of oils were also measured. Dynamic and steady rheological tests were managed. RI value of the EVOO was 1.4698. Addition of different vegetable oils increased the RI of the blended samples. Steady and dynamic test results indicated that EVOO adulteration can be detected by rheological tests. Also, η, G' and G" were used to verify the adulteration of EVOO with different types of vegetable oils by using artificial neural network (ANN). The developed ANN was able to reveal the relationship between η, G' and G" and studied oil type. Results show that ANN achieved a satisfactory prediction for vegetable oil adulteration of the studied oil type. This study provides a valuable insight to a method that allows the detection of extra olive oil adulteration in a more time and cost-efficient way.

Keywords: extra virgin olive oil, vegetable oils, adulteration, rheology, artificial neural network

1. Introduction

Olive oil is one of the most commonly adulterated food products of the world due to its relatively low production amount and higher prices as compared to vegetable and seed oils. Due to its nutritional value, olive oil consumption has considerably increased in recent years. Dishonest and corrupt producers may add to olive oil other types of oil that are cheaper in order to increase their profits. This deceitful practice is harmful to the consumer in terms of the price they have to pay such as higher cost, lower nutritional value and potential health risks associated with adulterated oil.

Olive oil is the principal fat source of Mediterranean diet which helps to prevent cardiovascular diseases, lipid abnormalities, high blood pressure, diabetes and obesity and, thereby the cause of coronary heart diseases (Covas *et al.*, 2015; Guasch-Ferré *et al.*, 2015; Martín-Peláez *et al.*, 2017; Urpi-Sarda *et al.*, 2012). In addition, it also plays a preventive role against different forms of cancer (Hashim *et al.*, 2014; Owen *et al.*, 2004; Storniolo and Moreno, 2016). Extra virgin olive oil (EVOO) is obtained from the olive trees fruit by cold pressing techniques without any thermal or chemical treatments. Adulteration is the replacing of high price ingredients with low and cheap components. It is a major issue in food products (Tay *et al.*, 2002).

EVOO has a higher price compared to other vegetable oils. Due to its higher price, it is very common to see EVOO adulteration with vegetable oils. The most common EVOO adulterants are sunflower, soy, corn, rapeseed, hazelnut and peanut (Firestone, 2001). Sunflower oil is generally used to adulterate EVOO due to its similar composition and low price. Hazelnut oil is more expensive than sunflower oil, but still cheaper than EVOO. Hazelnut oil is often used in EVOO adulteration due to its similarity in chemical composition (Vichi *et al.*, 2001). The high degree of similarity of hazelnut oil to EVOO make it very

difficult to detect at lower concentration levels (Ozen and Mauer, 2002). Beyond economic fraud, consumption of adulterated virgin olive oil may also threaten consumer health. In the 1980's, Toxic Oil Syndrome occurred as a result of consumption of rapeseed oil denatured with 2% aniline. The oil fraudulently consumed by humans caused more than 400 deaths and 20.000 casualties in Spain (Ruiz-Méndez *et al.*, 2001). Therefore, it is required to prevent adulteration of olive oil continuously.

Several tests have been used to detect adulteration in olive oils. The technique most commonly used is chromatography which provides information about the composition of the natural constituents of the oil and possible adulterants (Andrikopoulos *et al.*, 2001; Aparicio and Harwood, 2013; Christopoulou *et al.*, 2004). The conventional method of analysing oil is time consuming and may be destructive (Tay *et al.*, 2002). The fatty acid composition can only afford some but not conclusive data about the possible adulteration. In recent years, rheological tests have been extensively used due to advantages in speed and expense. Rheology is a field used to determine flow characteristics of liquid, viscous and semi liquids of foods (Marcotte *et al.*, 2001). Rheological properties take part in expressing the heat transfer or the design, evaluation and modelling of food treatment (Marcotte *et al.*, 2001). Viscosity is the fundamental parameter obtained in the rheological study of liquid foods, used to identify the fluid texture (Alonso *et al.*, 1990). Deng *et al.* (2018) used microfluidic evaluation of oil quality based on viscosity and interfacial tension to detect olive oil and frying oil adulteration.

Artificial neural networks (ANN) are mathematical computing models which are based on the structure and functions of the nervous system and the brain. The brain consists of large numbers of neurons that are interdepended. Due to their structure and properties ANN has some advantages such as being flexible, adaptable and applicable to a variety of problems and situations (Gonzalez-Fernandez *et al.*, 2019). Advantages of ANN compared to other approximation methods make them a favourite for prediction and control in food science. In this field, their first use was for thermal processing applications of foods (Sablani *et al.*, 1995) and in recent years its use has been continuously increasing. Goñi *et al.* (2008) used ANN which has provided a simple and accurate prediction method for freezing and thawing times, and is also valid for wide ranges of food types, sizes, shapes and working conditions. Cerit *et al.* (2017) estimated antioxidant capacities of different food varieties by using ANN allowing them saving time, labour and experimental costs. Jiménez *et al.* (2008) studied the moisture and fatty acid contents of olive pomace from the first cold extraction. They predicted moisture and fat content with 98.99 and 99.68% accuracy by using ANN, respectively. Silva *et al.* (2015) evaluated the stability of olive oil regarding auto-oxidation and photo-oxidation during processing at different levels of solar exposure and in two different types of packaging (tins and polyethylene terephthalate (PET) bottles). Their predictions using ANN was found to be more than 90% accurate. Aroca-Santos *et al.* (2016) identified and quantified binary blends of olive oil with four different EVOO varieties with a simple method based on combining visible spectroscopy and non-linear ANN. Their model successfully classified the EVOO varietal (100% identification rate).

In the literature, there has been no studies conducted on the identification of adulterated olive oil based on its rheological changes and modelling by ANN. In this study, we aimed to detect EVOO adulteration by using steady and dynamic rheological tests. Results of shear rate, G' and G'' were used to verify the adulteration of EVOO with different types of vegetable oils by using ANNs.

2. Material and methods

Materials

Vegetable oil types (EVOO, sunflower oil, canola oil and hazelnut oil) were collected from a local market in Kayseri, Turkey. The oil samples were mixed at different concentrations listed in Table 1. The total amount of oil mixed for the analysis was 100 g. Three replicates were analysed for each adulteration levels.

Refractive analysis

The refractive index (RI) of the oils and their mixtures were decided at 20 °C with an automatic refractometer (Reichert AR 700, Depew, NY, USA). RI measurements were repeated for 3 times.

Table 1. Concentrations of vegetable oils blended with EVOO.[1]

Runs	EVOO (%)	S (%)	C (%)	H (%)
1	25	75	-	-
2	50	50	-	-
3	75	25	-	-
4	25	-	75	-
5	50	-	50	-
6	75	-	25	-
7	25	-	-	75
8	50	-	-	50
9	75	-	-	25

[1] C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil.

Fatty acid composition analysis

The fatty acid compositions of the oil samples were determined according to the Agilent application catalogue (David *et al.*, 2005). 100 mg of oil samples were weighed and transferred to test tubes. The samples were dissolved in 3 ml *n*-hexane. 100 µl of 2 N KOH is added. The tubes were covered and vortexed for 30 s. Then test tubes were centrifuged at 24 °C, 6,000 rpm for 5 min (Nüve NM 110, Ankara, Turkey). One ml of the clear supernatant transferred into autosampler vials. The fatty acid compositions of the samples were analysed by gas chromatograph (Agilent 6890 N; Agilent, Palo Alto, CA, USA) equipped with a flame ionisation detector and 100 m × 0.25 mm Supelco HP 88 capillary column (Agilent, Santa Clara, CA, USA). 250 °C and 1 µl were used as an injection temperature and volume. The oven temperature was selected 130 °C for 1 min and then programmed as increasing by 6.5 °C/min to 170 °C, increasing by 2.75 °C/min to 215 °C and maintained at this temperature for 12 min, and finally kept at 230 °C for 5 min. The carrier gas was hydrogen with a flow rate of 1.3 ml/min; the split rate was 1/50. The fatty acids were identified by comparison of retention times to known standards. The results were expressed as percent of the total fatty acid weight (%). The fatty acid composition analysis was repeated for 2 times.

Rheological analysis

Rheology tests of the oil samples were carried out using a stress/strain controlled rheometer (Haake Mars III, Karlsruhe, Germany) equipped with a cone-plate configuration (diameter 50 mm, gap 0.5 mm) and a temperature module (Haake Mars, TM-PE-P). Shear measurements were carried out between 0.1 and 100 s^{-1} at 25 °C. A total of 25 data points were recorded at 10-s intervals during the shearing. Each measurement was repeated for 3 times. Shear stress values versus shear strain values were plotted. The data obtained from the measurements were fitted to a Newtonian model. The model was calculated according to the following equation:

$$\sigma = \eta \times \gamma$$

where σ is the shear stress (Pa), γ is the shear rate (per second) and η is the viscosity of the sample.

In order to decide linear viscoelastic region of the oil samples, the amplitude sweep test was carried out at 1 Hz between 0.1 to 10 Pa range. The frequency sweep test was carried out between 0.1 and 10 Hz range at 25 °C. The complex modulus is defined by the equation below:

$$G^*(\omega) = G'(\omega) + iG''(\omega)$$

where G^* is the complex shear modulus, G' is the storage modulus and G'' is the loss modulus

The linear viscoelastic test was repeated for 3 times.

Statistical analysis

In order to see the influence of vegetable oil addition, RI, viscosity and their interactions, ANOVA was performed (Minitab, 17; State College, PA, USA). Bivariate correlations between oil physical properties, fatty acid composition and rheology parameters of the oil samples were performed by Pearson's test (Minitab 17). Also, multiple regression analysis was performed (Minitab 17).

Artificial neural network

ANN is a nonparametric information processing methodology stimulated from human brain (Kheirkhah *et al.*, 2013). It is a kind of supervised learning method that contains parallel nonlinear processing units in a highly interconnected network. Feedforward multi-layered neural network is the most used class of the neural networks. A feed forward neural network is structured from basic elements called as input layer, hidden layer(s), output layer, neurons and weights. Input layers provide information to the model, hidden layers obtain the relationship between inputs and outputs by processing the incoming signals of inputs, and output layers provide estimation values of results. Hidden layers are responsible for performing nonlinear relationship of ANN. All layers consist of neurons called processing units and weights are the connections of neurons between layers. Weights between the neurons have also structured the relationships between inputs and outputs. This procedure is called training. Learning capability of a neural network is tested in the testing step. ANN can be applied when there is no theoretical evidence about the functional form. Therefore, ANN is a data-based, not model-based method (Santin *et al.*, 2004). Furthermore, it can be applied to many specific applications from different fields for pattern recognition, function approximation, data classification and forecasting purposes (Azadeh *et al.*, 2010). Interested readers can be forwarded to Bishop (1995) for a comprehensive study of the subject.

Selecting the proper architecture is a critical problem in ANN and to overcome with this problem, 500 different ANN architectures with different numbers of layers, neurons and activation functions are trained by using the STATISTICA 10.0 software package (StatSoft Inc., Tulsa, OK, USA). In this study, multilayer perceptron (MLP) which is a special type of feed forward neural networks are used to predict the oil adulteration.

3. Results and discussion

The RI values of the EVOO, hazelnut, sunflower and canola oil samples and their mixtures are given in Table 2. According to the results, the RI value of EVOO was found as 1.4698. The RI values of vegetable oils were higher than EVOO, such as 1.4750, 1.4734, and 1.4711 in sunflower, canola and hazelnut oils, respectively. Addition of vegetable oils to EVOO caused a significant reduction in the RI (P<0.05). The increment in the EVOO concentration caused a decrement in RI values of all the vegetable oil mixtures. The RI of a vegetable oil is an easy test for the identity or the purity of an oil. Iodine value, the saponification value and colorimetric reactions as well as RI , density and viscosity measurements can be used for the identification of oil adulteration (Boekenoogen, 1968; Christy *et al.*, 2004). RI value of the vegetable oil is related to their structure. The double bonds and especially conjugated double bonds in oil structure, cause an increase in the RI value (Simpson and Hamilton, 1982). According to our results, the RI value changed significantly within the oil type (P<0.05). The highest RI value was obtained in sunflower oil which had the highest polyunsaturated fatty acids (PUFA). RI measurements were significantly different between EVOO and adulterants (sunflower, canola and hazelnut) (P<0.05). RI of hazelnut and EVOO was significantly different with the exception of the 25% adulterated sample. The same difference was observed in canola adulterated samples.

Table 2. Refractive index of EVOO, vegetable oils and oils adulterated with EVOO.[1,2]

Oil samples	Refractive index
Adulterants	
Sunflower oil	1.4750^{a}±0.0000
Canola oil	1.4734^{b}±0.0000
Hazelnut oil	1.4711^{c}±0.0000
Adulterated oil samples	
EVOO+S	
Control sample (EVOO)	1.4698^{d}±0.0001
75% S	1.4728^{a}±0.0001
50% S	1.4710^{c}±0.0001
25% S	1.4712^{b}±0.0001
EVOO+C	
Control sample (EVOO)	1.4698^{c}±0.0001
75% C	1.4711^{a}±0.0021
50% C	1.4701^{b}±0.0101
25% C	1.4698^{c}±0.0001
EVOO+H	
Control sample (EVOO)	1.4698^{c}±0.0001
75% H	1.4708^{a}±0.0060
50% H	1.4699^{b}±0.0060
25% H	1.4698^{bc}±0.0060

[1] C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil.
[2] Means with different letters in the same column are significantly different at the 5% level.

Oil samples' fatty acid compositions are presented in Table 3. The percentages of total saturated, MUFA and PUFA levels of all samples ranged from 13.37 to 24.44%; 28.59 to 73.09% and 9.06 to 46.97%, respectively. The hazelnut, EVOO and canola oil samples consisted of higher levels of unsaturated fatty acids in contrast to sunflower oil. The unsaturation of the samples arise mainly from monounsaturated fatty acids (MUFA) whereas unsaturation of sunflower oil arises from PUFA. Although, the predominant fatty acid of hazelnut, canola and EVOO was oleic acid (C18:1), the predominant fatty acid in sunflower oil was linoleic acid (C18:2). Results are similar to findings of previous studies (Kim *et al.*, 2010; Shahidi, 2005). The fatty acid compositions of the blended oils were changed by adding the different vegetable oils at different concentration. According to the results, MUFA levels of EVOO decreased from 61.26 to 43.42% by addition of sunflower oil at 25 to 75% concentrations, respectively. The addition of hazelnut, sunflower and canola oil significantly affected MUFA levels of EVOO. Also, the addition of hazelnut, sunflower, and canola oil significantly increased the PUFA level of EVOO (P<0.05).

Steady shear measurements of the vegetable oils and their blended samples were carried out at 25 °C to determine flow behaviour. The viscosity of the samples are expressed as the slope of shear stress versus shear rate. Flow behaviour of the EVOO, sunflower, canola and hazelnut oil are presented in Figure 1. The studied vegetable oils showed Newtonian behaviour. In this flow type, the viscosity of the samples did not change with shear rate. Newtonian flow behaviours of fresh vegetable oils caused by long chain molecules in their structure (Maskan, 2003; Santos *et al.*, 2004). The characteristics of Newtonian flow explained by the equation given as:

$$\sigma = \eta \times \gamma$$

where σ is the shear stress (Pa), η is steady shear viscosity (Pa.s) and γ shear rate (s^{-1}).

Table 4 shows the Newtonian model parameters for adulterants and adulterated EVOO samples with changed levels of hazelnut, sunflower and canola oil. The viscosity of the oils differed depending on the species of oil. The hazelnut oil has the highest viscosity value, followed by EVOO, canola and sunflower oils (Table 4). (Kim *et al.*, 2010). The similar sequence for the vegetable oils' viscosity was obtained by Kim *et al.* (2010). They also found a positive correlation between the oil viscosity and C18:1 or C18:2. The oil viscosity increased with the portion of the 18:1 fatty acids increased whereas the oil viscosity decreased with

Table 3. Fatty acid composition of EVOO, vegetable oils and oils blended with EVOO (%).

Oil samples	C 16:0 palmitic acid	C 16:1 palmitoleic acid	C 18:0 stearic acid	C 18:1 oleic acid	C 18:2 linoleic acid	C 20:0 arachidic acid	Total saturated fatty acids	Total MUFA	Total PUFA
Extra Virgin olive oil	14.27^{a}±0.2	nd	7.68^{d}±0.0	68.99^{b}±0.6	9.06^{d}±0.2	nd	21.95^{b}±0.2	68.99^{b}±0.6	9.06^{d}±0.2
Adulterants									
Sunflower oil	7.31^{b}±0.1	nd	17.13^{a}±0.1	28.59^{d}±0.4	46.97^{a}±0.2	nd	24.44^{a}±0.1	28.59^{d}±0.4	46.97^{a}±0.2
Canola oil	5.97^{d}±0.3	nd	8.23^{c}±0.0	62.54^{d}±0.5	19.10^{b}±0.2	4.16±0.06	18.36^{c}±0.3	62.54^{d}±0.5	19.10^{b}±0.2
Hazelnut oil	6.37^{c}±0.1	nd	9.94^{b}±0.2	69.47^{a}±0.7	14.23^{c}±0.5	nd	16.31^{d}±0.2	69.47^{a}±0.7	14.23^{c}±0.5
Adulterated oil samples									
EVOO+S									
Control sample (EVOO)	14.27^{a}±0.2	nd	7.68^{a}±0.0	68.99^{a}±0.6	9.06^{d}±0.2	nd	21.95^{a}±0.2	68.99^{a}±0.6	9.06^{d}±0.2
75% S	8.35^{d}±0.1	nd	7.53^{b}±0.2	43.42^{d}±0.5	40.71^{a}±0.2	nd	15.88^{c}±0.2	43.42^{d}±0.5	40.71^{a}±0.2
50% S	9.91^{c}±0.1	nd	5.38^{c}±0.3	52.50^{c}±0.4	32.21^{b}±0.2	nd	15.29^{d}±0.3	52.50^{c}±0.4	32.21^{b}±0.2
25% S	11.51^{b}±0.1	nd	4.99^{d}±0.1	61.26^{b}±0.6	22.24^{c}±0.1	nd	16.50^{b}±0.1	61.26^{b}±0.6	22.24^{c}±0.1
EVOO+C									
Control sample (EVOO)	14.27^{a}±0.2	nd	7.68^{a}±0.0	68.99^{b}±0.6	9.06^{d}±0.2	nd	21.95^{a}±0.2	68.99^{b}±0.6	9.06^{d}±0.2
75% C	7.17^{d}±0.2	nd	4.22^{d}±0.2	66.25^{d}±0.7	18.35^{a}±0.4	4.01^{a}±0.01	15.40^{d}±0.2	66.25^{d}±0.7	18.35^{a}±0.4
50% C	9.15^{c}±0.3	nd	4.81^{b}±0.4	68.14^{c}±0.6	15.01^{b}±0.2	2.89^{b}±0.05	16.85^{c}±0.4	68.14^{c}±0.6	15.01^{b}±0.2
25% C	11.06^{b}±0.1	nd	4.72^{c}±0.1	69.92^{a}±0.4	12.39^{c}±0.3	1.91^{c}±0.06	17.69^{b}±0.1	69.92^{a}±0.4	12.39^{c}±0.3
EVOO+H									
Control sample (EVOO)	14.27^{a}±0.2	nd	7.68^{a}±0.0	68.99^{d}±0.6	9.06^{d}±0.2	nd	21.95^{a}±0.2	68.99^{d}±0.6	9.06^{d}±0.2
75% H	7.73^{d}±0.4	nd	5.64^{b}±0.5	72.05^{c}±0.8	14.58^{a}±0.4	nd	13.37^{d}±0.5	72.05^{c}±0.8	14.58^{a}±0.4
50% H	8.98^{c}±0.2	nd	5.13^{d}±0.3	73.09^{b}±0.7	12.80^{b}±0.4	nd	14.11^{c}±0.3	73.09^{b}±0.7	12.80^{b}±0.4
25% H	10.88^{b}±0.3	nd	5.48^{c}±0.2	72.69^{a}±0.7	10.94^{c}±0.3	nd	16.36^{b}±0.3	72.69^{a}±0.7	10.94^{c}±0.3

[1] C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; MUFA = monounsaturated fatty acids; nd = not detected; PUFA = polyunsaturated fatty acids; S = sunflower oil.
[2] Means with different letters in the same column are significantly different at the 5% level.

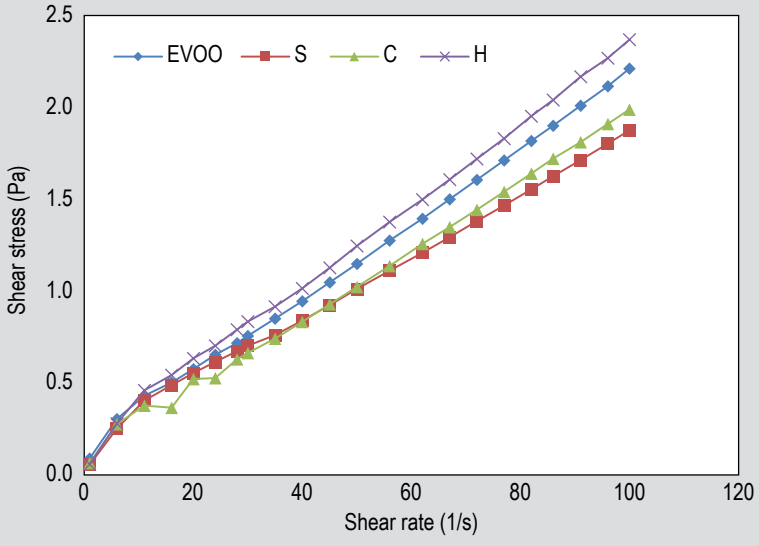

Figure 1. Flow behaviour of the vegetable oils at 25 °C (C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil)

Table 4. Newtonian model parameters defining flow behaviour of samples.[1,2]

Oil samples	R^2	Viscosity (mPa.s)
Adulterants		
Sunflower oil	0.998[a]±0.035	20.23[d]±0.009
Canola oil	0.998[a]±0.013	20.38[c]±0.002
Hazelnut oil	0.998[a]±0.077	24.33[a]±0.000
Adulterated oil samples		
EVOO+S		
Control sample (EVOO)	0.998[a]±0.035	22.97[b]±0.001
75% S	0.999[a]±0.017	20.97[c]±0.000
50% S	0.999[a]±0.049	23.82[a]±0.001
25% S	0.998[a]±0.042	22.97[b]±0.003
EVOO+C		
Control sample (EVOO)	0.998[a]±0.035	22.97[c]±0.001
75% C	0.998[a]±0.032	23.13[d]±0.000
50% C	0.998[a]±0.009	23.58[a]±0.002
25% C	0.999[a]±0.012	22.84[b]±0.003
EVOO+H		
Control sample (EVOO)	0.998[a]±0.035	22.97[d±]0.001
75% H	0.999[a]±0.009	26.88[a]±0.001
50% H	0.999[a]±0.005	26.40[b]±0.001
25% H	0.998[a]±0.001	23.37[c]±0.001

[1] C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil.
[2] Means with different letters in the same column are significantly different at the 5% level.

the portion of the 18:2 fatty acids increased. According to our results, the viscosities of hazelnut, EVOO, canola and sunflower oil were found as 24.33 mPa.s, 22.97 mPa.s, 20.38 mPa.s and 20.23 mPa.s, respectively. The 18:1 fatty acid portions of the hazelnut, EVOO, canola and sunflower oil were found as 69.47, 68.99, 62.54 and 28.59, respectively. The existence of double bonds prevent fatty acid molecules from piling up together because each double bond with a *cis* configuration causes a twist in the straight chain. Therefore, fatty acids with more double bonds are more fluid (Kim *et al.*, 2010).

A 75% addition of sunflower to EVOO, reduced the viscosity of the EVOO sample. As can be seen in Figure 2, shear stress values of the sunflower adulterated samples decreased as the increment of sunflower oil, confessing that sunflower oil addition caused decrement in viscosity value of EVOO. Deng *et al.* (2018) studied the olive oil adulteration with rapeseed oil and found that olive oil viscosity decreases with adulteration. However, the addition of canola oil did not change the shear stress values of the EVOO. As the level of hazelnut oil increased, shear stress values of the hazelnut adulterated oil samples increased (Figure 2). Addition of hazelnut oil to EVOO increased the viscosity of the control sample (Table 4). These results suggest that steady shear rheological analysis can be used in detection of adulterated vegetable oils. The viscoelactic region of the samples were determined by an amplitude sweep test, and all the samples resulted in the linear viscoelastic region, in accordance with others (Yalcin *et al.*, 2012). Figure 3 and 4 illustrate the elastic or storage modulus (G') and viscous or loss modulus (G'') of adulterants and adulterated oil samples as a function of the frequency. As can be seen, the G' and G'' values of all samples increased with frequency. However, G' values of the samples had irregular ups and downs which might have resulted due to the liquid characteristics of the oil samples. G' values of the oils showed a non-linear increase while G'' values presented a linear increase. The results indicate that adulterated oil samples' G'' values can be used for oil rheology characterisation. Furthermore, G'' values of the samples were higher than G' values, pointing out that oil samples had viscous nature. Based on the results, it can be concluded that oil samples displayed liquid-like behaviour due to the fact that G'' values were higher than G' values. G' values and G'' of the adulterants and adulterated oil samples were significantly different ($P<0.05$) (Table 5). EVOO adulterated 25% of hazelnut oil had the greatest G'' values compared to others (Table 5).

Table 5 shows the storage and loss modulus values of the samples. It can be seen that G'' values decreased linearly ($P<0.01$) as the adulteration level increased. G' values of the samples showed irregular ups and downs. Results suggest that G'' would potentially be a good indicator to detect adulteration of vegetable oil at the levels ranging between 25 and 75%.

Detection of oil adulteration is very important for the food industry. Therefore, numerous methods and techniques have been developed in recent years to identify olive oil adulteration. These methods are based on gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) (Christopoulou *et al.*, 2004), near-infrared, Fourier transform infrared (FTIR), Fourier transform-Raman methods (Lerma-García *et al.*, 2010; Yang and Irudayaraj, 2001), mid-infrared (IR) spectroscopy with chemometrics (Mid-IR) (Gurdeniz and Ozen, 2009), and Nuclear magnetic resonance (NMR) (Fragaki *et al.*, 2005). However, conventional methods are time consuming and destructive. Rheology has been extensively used having advantages in terms of speed and expense for the test. More recently, rheology is being used to determine the adulteration status of foods. Yilmaz *et al.* (2014) used the steady, dynamic and creep rheological analysis to detect honey adulteration by fructose and saccharose syrup. The natural honey sample was adulterated with the addtion of fructose and saccharose at a ratio of 0, 10, 20, 30, 40 and 50% by weight.

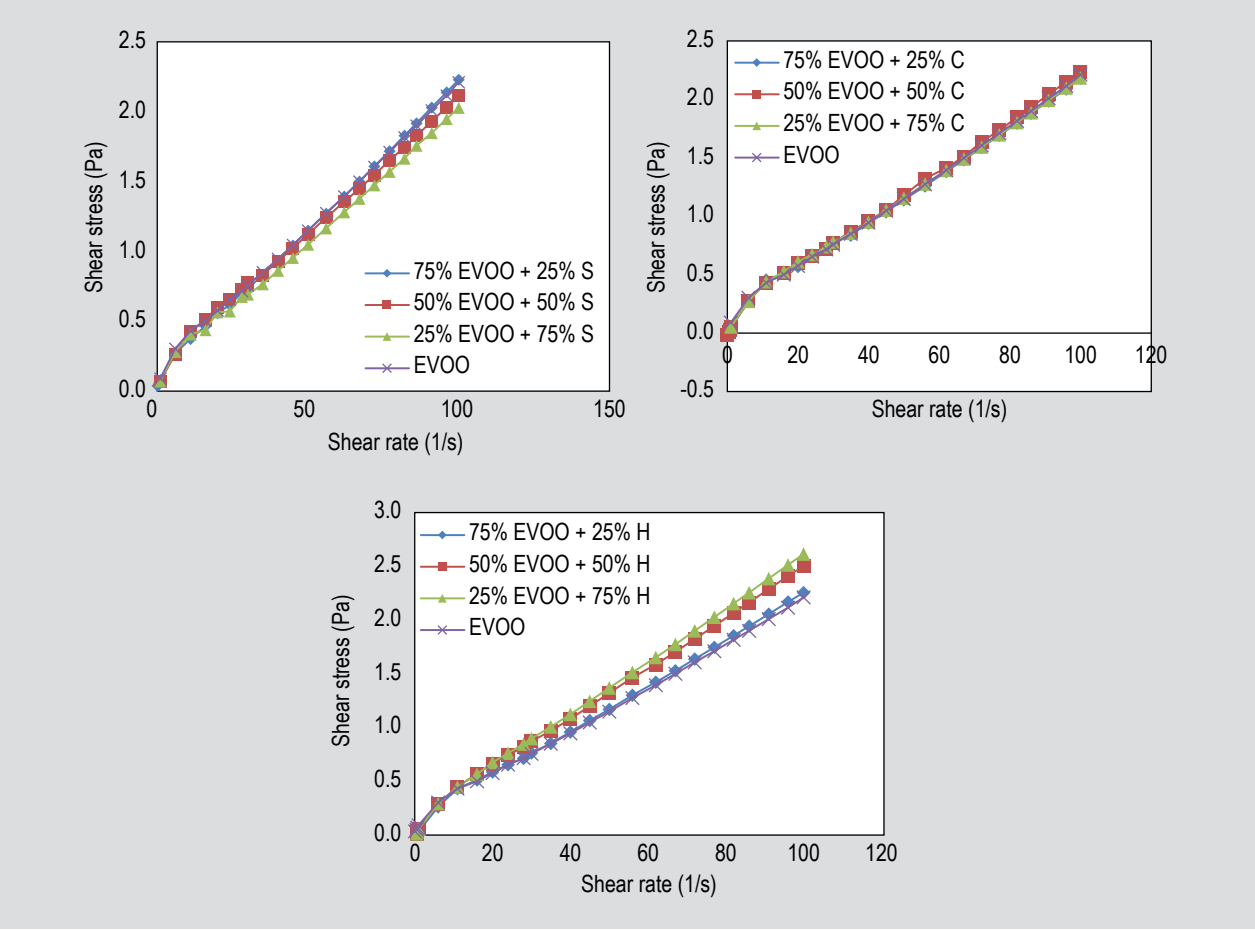

Figure 2. Shear stress (Pa) versus shear rate (1/s) data for adulterants and adulterated samples (C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil).

They found that syrup addition decreased viscosity (η), storage (*G′*) and loss modulus (*G″*) values of the control honey samples. They suggested that the use of steady, dynamic and creep analysis could be a novel approach to detect honey adulteration with fructose and saccharose syrup. El-Bialee and Sorour (2011) studied the detection of honey adulteration with starch, glucose, molasses and distilled water at 1, 3, 6, 12 and 24% concentrations by using physicochemical and rheological characteristic of honey samples. They concluded that pure honey samples exhibited Newtonian flow behaviour while adulterated samples showed non-Newtonian pseudoplastic behaviour. Valantina *et al.* (2013) added coconut oil and sunflower oil to EVOO at different concentrations. They studied the variation of rheological and ultrasonic parameters in the binary mixtures of the oils. They recommended that the feasibility of using rheology and ultrasonic techniques to evaluate the quality parameters of oils replacing of the high cost traditional analytical method with this simple method.

Pearson test was used to analyse correlations between oil physical properties, fatty acid composition and rheology parameters of adulterated oil samples. In Table 6, the analysis results are given. Significant positive and negative correlations were found between oil physical property, fatty acid composition and rheology (steady and dynamic) parameters. These parameters were η (viscosity), *G′* and *G″* proving that *G″* could be used for the EVOO adulteration with the studied concentration levels ranging between 25 and 75%.

Due to the significant correlations found between the RI, fatty acid composition and rheological properties (Table 6), multiple regression analysis was used to predict *G′*, *G″* and η values of the samples based on RI, MUFA and PUFA composition of the vegetable oils. Results of multiple regression analyses are shown in Table 7. The sunflower oil sample's *G″* value used for observation of the performance of the derived equations after determination of the relationship between the RI, MUFA and PUFA. RI, MUFA and PUFA values of the sunflower oil were found experimentally as 1.475, 28.59 and 46.97, respectively. The *G″* value of the sunflower oil was calculated as 184.28 by using the regression equation in Table 7, which was found as 183.00 by experimentally. It can be seen that predicted and experimental values were considerably close to each other.

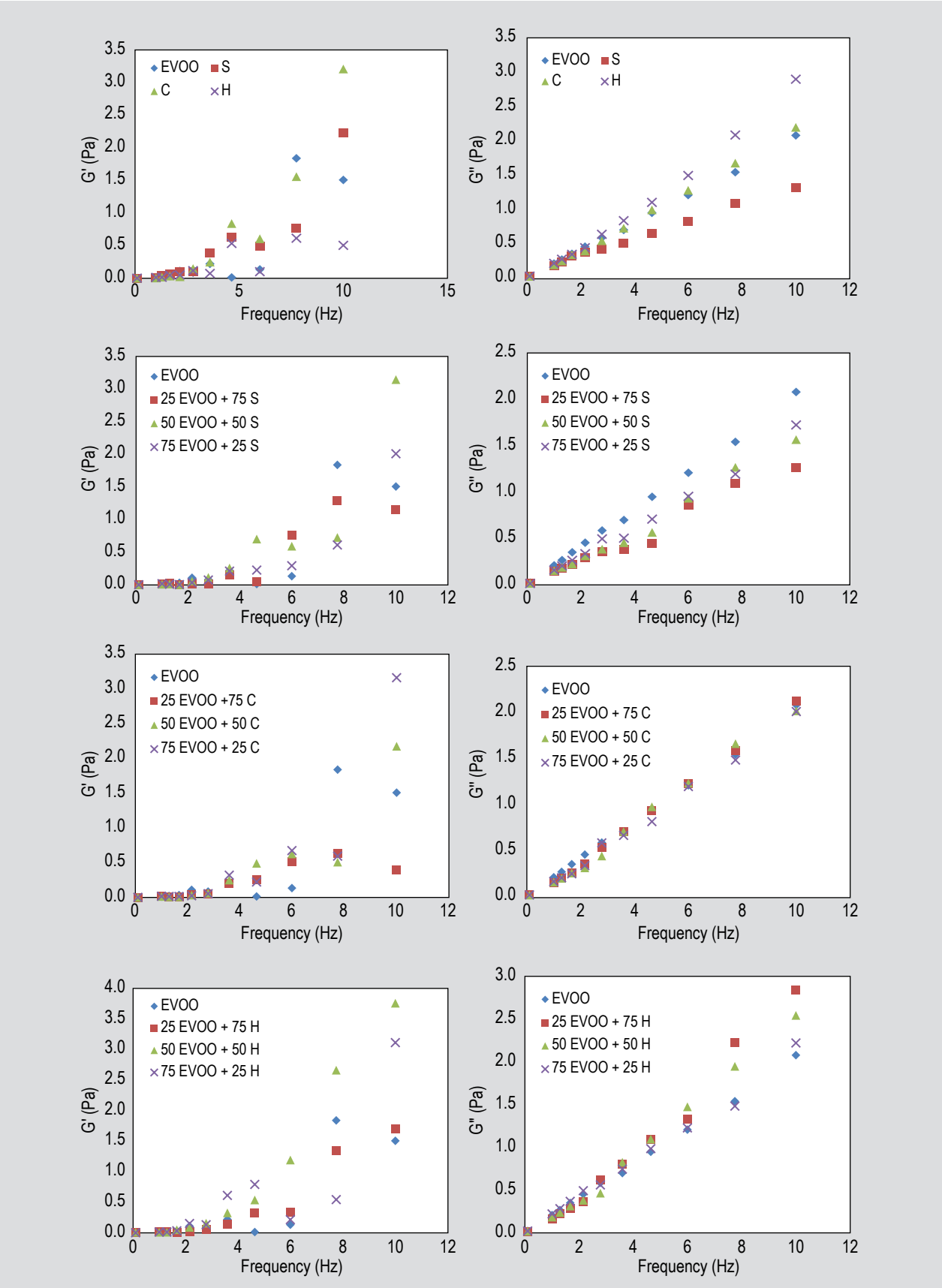

Figure 3. *G'* (storage modulus) and *G''* (loss modulus) values of adulterants and adulterated oil samples (C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil) as a function of frequency.

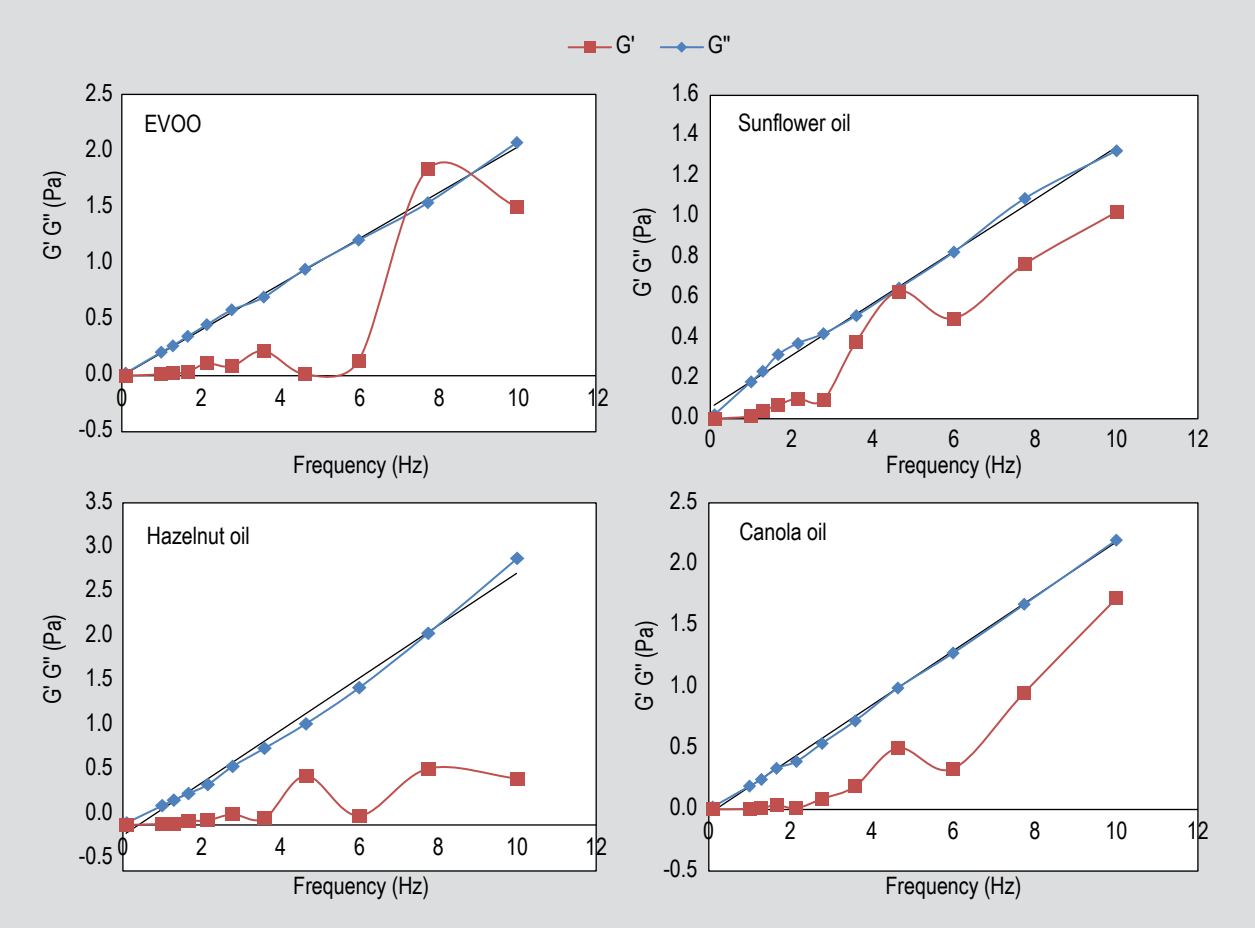

Figure 4. *G'* (storage modulus) and *G"* (loss modulus) values of the vegetable oils (C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil) as a function of frequency.

Table 5. Storage modulus (*G'*) and loss modulus (*G"*) values of oil samples.[1,2]

Oil samples			*G'* (mPa)	*G"* (mPa)
Adulterants		Sunflower oil	11.81^{a}±0.002	183.00^{d}±0.003
		Canola oil	4.45^{b}±0.000	190.70^{c}±0.003
		Hazelnut oil	10.57^{d}±0.000	210.50^{a}±0.002
Adulterated oil samples	EVOO+S	Control sample (EVOO)	11.77^{c}±0.009	208.80^{a}±0.003
		75% S	18.08^{a}±0.008	148.00^{d}±0.006
		50% S	10.60^{d}±0.003	153.03^{c}±0.004
		25% S	17.45^{b}±0.004	159.03^{b}±0.000
	EVOO+C	Control sample (EVOO)	11.77^{d}±0.009	208.80^{a}±0.003
		75% C	15.79^{a}±0.003	153.96^{d}±0.002
		50% C	14.58^{c}±0.002	156.38^{c}±0.004
		25% C	14.98^{b}±0.003	162.66^{b}±0.009
	EVOO+H	Control sample (EVOO)	11.77^{c}±0.009	208.80^{b}±0.003
		75% H	13.09^{b}±0.001	167.23^{d}±0.001
		50% H	8.68^{d}±0.001	185.43^{c}±0.004
		25% H	13.58^{a}±0.001	221.77^{a}±0.001

[1] C = canola oil; EVOO = extra virgin olive oil; H = hazelnut oil; S = sunflower oil.
[2] Means with different letters in the same column are significantly different at the 5% level.

Table 6. Pearson correlation coefficients (r) between oil physical properties, fatty acid composition and rheology (steady and dynamic) parameters of adulterated oil samples.[1,2]

Adulterated oil samples	Physical properties and fatty acid composition	Rheological parameters		
		Steady shear parameter[a]	Dynamic shear parameters[b]	
		η	*G′*	*G″*
EVOO adulterated with sunflower oil	Refractive index	-0.680*	0.079	-0.187
	Total unsaturation	0.365	0.500*	-0.828**
EVOO adulterated with canola oil	Refractive index	-0.562*	-0.822**	0.187
	Total unsaturation	0.061	0.365	-0.889**
EVOO adulterated with hazelnut oil	Refractive index	0.298	-0.021	-0.303
	Total unsaturation	0.665*	-0.121	-0.618**

[1] Correlations between oil physical properties (refractive index), fatty acid composition (total unsaturation) and rheology parameters: * = $P<0.05$; ** = $P<0.01$.
[2] EVOO = extra virgin olive oil; *G'* = storage modulus; *G"* = loss modulus; η = apparent viscosity.

Table 7. Regression equations for adulterated oil samples.[1]

Oil sample	Regression equation	R^2
EVOO adulterated with sunflower oil	*G'* = -8,929 + 6,008 RI + 1.486 MUFA + 0.7886 PUFA	88.91
	G" = 9,747 – 6,146 RI – 6.478 MUFA – 6.646 PUFA	97.70
	η = 1,663 – 1,115 RI – 0.024 MUFA + 0.039 PUFA	85.18
EVOO adulterated with canola oil	*G'* = 4,352 – 3,005 RI + 0.974 MUFA + 1.0612 PUFA	97.35
	G" = -17,171 + 12,169 RI – 6.15 MUFA – 9.549 PUFA	95.61
	η = 2,091 – 1,399 RI – 0.181 MUFA + 0.153 PUFA	37.83
EVOO adulterated with hazelnut oil	*G'*= -24,018 + 16,247 RI + 2.749 MUFA – 4.401 PUFA	77.43
	G" = -88,196 + 59,976 RI + 6.48 MUFA – 20.3 PUFA	46.98
	η = 9,340 – 6,324 RI – 0.616 MUFA + 2.13 PUFA	53.06

[1] EVOO = extra virgin olive oil; *G'* = storage modulus; *G"* = loss modulus; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; RI = refractive index; η = apparent viscosity.

As a result, multiple regression equation has predicted the *G″* value of the sunflower oil with an approximately 0.7% error.

Oil type of samples were selected as output of network and 3 properties of oil samples were used as inputs of networks (*G′*, *G″* and steady shear). We categorised oil types as '1' for EVOO, '2' for sunflower, '3' for canola and '4' for hazelnut oils. Experiments were partitioned randomly into two groups for training and testing purposes consisting of 70% and 30% concentrations, respectively. Cross entropy, which is a specific classification error function, is used to compare the performance of different ANN architectures. We adjusted the activation function of the output layer as softmax for classification purposes. The best classification was obtained in the MLP 3-4-13 topology.

In this study, a gradient-based technique, the Levenberg-Marquardt algorithm, a popular non-linear least squares method, was used in nonlinear regression and optimisation and applied to ANN modelling. The Levenberg–Marquardt algorithm was used because this technique is more powerful and faster than the conventional gradient descent technique (Hagan and Menhaj, 1994).

This network contains 3 layers: input, output and a single hidden layer. Also, there are 3 neurons (inputs) in the input layer, 4 neurons in the hidden layer and 13 neurons (the categories of oil type are handled as different outputs) in the output layer. The characteristics of the selected network is given in Table 8. On the other hand, classification accuracy is also a good measure to compare the performance of algorithms. In Table 9, the confusion matrix for the best network is given. Sensitivity analysis (SA) is one of the most

Table 8. Characteristics of multilayer perceptron (MLP) 3-4-13.

Topology	Training performance (%)	Test performance (%)	Training algorithm	Error function	Hidden activation	Output activation
MLP 3-4-13	100	81	Broyden–Fletcher–Goldfarb–Shanno	Cross entropy	Tanh	Softmax

Table 9. Confusion matrix of best networks.

Multilayer perceptron 3-4-13		Oil type
	Correct (%)	94.8
	Incorrect (%)	5.2

Table 10. Sensitivity analysis results of multilayer perceptron (MLP) 3-4-13.

Input variables	*G″*	*G′*	Steady shear
MLP 3-4-13	19.41	14.26	4.99

frequently used methods for analysing the significance of inputs of ANN models. The SA results of the MLP 3-4-13 network are given in Table 10. The findings indicate that *G″* is the most significant parameter for estimating the oil type of samples.

4. Conclusions

In this study, EVOO was adulterated with different levels of sunflower, canola and hazelnut oil at ratios of 25, 50 and 75% by weight. Steady and dynamic rheology tests were carried out to identify such adulterations at specific ratios. The rheology tests showed that extra virgin olive adulteration at these levels can be identified by using viscoelastic behaviour of oil. Significant correlations were found between the physical properties, fatty acid composition and rheology parameters of adulterated EVOO samples. Regression equations predicted *G′*, *G″* and η very close to experimental values of the samples. This was also confirmed with ANN modelling. Consequently, the results of this study revealed that ANN was a good predictor for EVOO adulteration with sunflower, canola and hazelnut oils at levels ranging from 25 to 75%. ANN results show that *G″* is the most significant rheology parameter for estimating the oil types. In conclusion, several methods can be used to detect oil adulteration. However, due to concerns about time and cost-efficiency, rheology can be effectively employed for detection of oil adulteration as shown in this study.

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