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Qualitative analysis of edible oil oxidation using an olfactory machine

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Abstract

Oil oxidation is an undesirable series of chemical reactions involving oxygen that degrades the quality of an oil. Oxidation eventually produces rancidity in oil, with accompanying off-flavors and smells. An electronic nose was used in this study to detect the adulterations in edible oils. The acidity, peroxide, anisidine and Totox values of the edible oil samples were measured according to the official American Oil Chemist Society (AOCS) standard. The results were analyzed using cluster analysis, principal component analysis, support vector machine, quadratic discriminant analysis, and Partial least squares regression technique. In the sensor array, the TGS2602, and MQ136 sensors had the highest values of the Loudness coefficient and the MQ9, TGS822, TGS813, and TGS2620 had the lowest values. Based on the results obtained, the accuracy of the three methods; Support vector machine (SVM), Quadratic discriminant analysis and Partial least squares were 97%, 98.33%, and 100%, respectively. The results for the linear vector kernel support machine, training accuracy and validation for C-SVM and Nu-SVM were 98, 97, 97 and 95%, respectively. The results also indicated that the proposed method can be used as an alternative to the official AOCS methods to innovatively detect the edible oil oxidation with high accuracy.

Keywords AOCS · Chemometrics · Electronic nose · Oil

Introduction

Lipid oxidation is one of the major causes of food spoilage especially in those containing oil [1]. Therefore, this parameter has been considered as one of the important qualitative criteria in the food industry. Oxidation may occur from processing to the storage of the edible oil. In addition to the production of peroxides, aldehydes, ketones, acids, and other small molecules, it can decrease the quality of the food products. Oxidation degree can be influenced by storage conditions. When the oil is exposed to light and high temperatures, its oxidation will be increased [2].

The consumers' preference for use of a specific type of edible oil maybe attributed to its aroma, taste and nutritional values. Regarding the serious health-threatening concerns,

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² Department of Mechanical Engineering of Biosystems, Razi University, Kermanshah, Iran validation of the edible oil is one of the major issues in food product analysis [2].

American oil chemists' society (AOCS) has developed various methods to evaluate the oxidation status of the oil for example to assess peroxide value (PV), acidity value (AV), Anisidine value (AnV) and Totox value. PV and AV have been widely employed in the edible oil industry and food processing. These chemical tests are not difficult; however, they are time-consuming, destructive as mentioned earlier. These methods also impose potential risks on human and environmental health due to their solvent wastes [3]. Spectroscopic methods such as MIST and FTIR have been also developed in recent years to assess the lipid oxidation. These methods, however, require substantial spectral pre-processing to develop complex models [4].

Nowadays, extensive efforts have been dedicated to developing an electronic nose (E-nose) which can mimic the human smelling process and offer fast and low-cost information to evaluate the food products freshness as well as monitoring the processes and controlling their quality [2]. The key advantages of the e-nose based solutions are ease of use, high sensitivity, high productivity, fast response, low cost, aptness of working with harmful chemicals, and practicability of superseding with panel of experts. E-nose is a new technique among the various technologies available for detecting adulteration in foodstuff and can provide ample information for authorities for decision making [5].

The electronic nose includes a series of electrochemical sensors that can detect simple or complicated smells [6]. Digital outputs of the E-nose sensors should be analyzed to derive their useful information. Cluster analysis (CA), principal component analysis (PCA), linear discriminant analysis (LDA) and artificial neural network (ANN) and support vector machines (SVM) are among the methods most usually used in connection with e-noses [7, 8]. E-nose could be used for real-time monitoring of the volatile components of food to evaluate different attributes of the product.[9, 10]. Despite some achievements in the development of low-cost and compact electronic noses, these devices are still expensive and have been developed for multiple tasks [5].

The application of the E-nose has drastically increased in the recent decade and has led to significant achievements in the food industry. Among these researches, evaluation of the edible oil authenticity to detect oxidation spoilage can be mentioned [11-15]. Moreover, E-nose was employed to detect lipid oxidation in soy oil [1], oxidation degree of ultra-virgin olive oil [16, 17] and auto-oxidation of canola oil [18], vinegar [19], tofu brine [20], large yellow croaker [21], Spinyhead croaker [22], Chicken [23], Wine [24], Magnolia biondii Pamp [25], and edible olive oil [26]. Xu et al. used a new method in 2016 to qualitatively analyze the edible oil oxidation by electronic nose. They employed an electronic nose in combination with CA, PCA and LDA methods to detect the oxidized and non-oxidized oils which resulted in inaccuracies of 95.8%, 98.9%, and 100%, respectively [2].

According to previous reports and to the best of our knowledge, this study is the first research conducted on the use of E-nose combined with chemometrics methods for selection of an optimized gas sensors array to the detection of oil oxidation. The model performance was assessed by the official AOCS method. CA, PCA, SVM, QDA, and PLS techniques were applied for the qualitative differentiation oxidation in oxidized and non-oxidized oils.

Materials and methods

Sample preparation

In this study, first, liquid mixed edible oils (sunflower, canola, and soy) with new production data and expiry dates were prepared from the local market in Kermanshah, Iran. The samples were kept in a dry and dark place at room temperature (to minimize the physical and chemical changes) until the tests were conducted. Then, two oil samples were prepared using fresh and oxidized oils. Then, 20 mL of each sample was transferred to a 50-ML glass container at ambient temperature $(23 \pm 2 \ ^{\circ}C)$. The samples were equilibrated to the headspace for 50 min in a capped vessel. In total, 2 types of oil (oxidized and non-oxidized) with 30 replicates for each sample were used.

Electronic nose

In this study, the electronic nose constructed in Razi University [27] was employed to detect oxidation edible oil. The employed system included two sections: hardware and software. The hardware section encompassed the data collection system, sensors, sensors chamber, sampling chamber, voltage supply, joints and accessories, electric valves, air pump, and filter. The applied E-nose is schematically illustrated in Fig. 1. This system is equipped with a diaphragm pump (TYAP-127) with a flow of 1.3 L/min and a voltage of 12 V to transfer the sample smell (headspace) to the sensors chamber. For automatic control of the system and attaining the smell pattern, the E-nose was equipped with 3 onefourth-inch two-way electronic valves (UNI-DO 2/2) with stimulation voltage of 12 V whose voltage was decreased to 5 V by a circuit [28]. The responses of the eight sensors were surveyed by a data collection system connected to a PC. The sensor array used in this study was composed of eight metal oxide semiconductor (MOS) sensors whose attributes are listed in Table 1.

The sampling process involved three stages: baseline correction, sample smell injection, and measuring and cleaning the sensor chamber with fresh air. Regarding the unique time of E-nose in each of these stages for each application, these stages will be re-timed by changing the application. In this study, the proper time was obtained after several tests and investigations of the response of the sensors. In the baseline correction stage, oxygen was passed over the sensors for 200 s until the array response reached equilibrium. Upon injection of the sample smell to the sensors chamber, the output voltage of each sensor will be changed depending on the sensor type and sensitivity. This stage often lasts for 150 s. In the last stage, oxygen was again passed over the sensors for 200 s to return the sensor's response to the baseline and prepare the system for the subsequent test.

The sensor's responses were recorded and saved by a data collection system connected to a computer (NI USB 6009) which used a graphic link programmed by LABVIEW 2013 software.

Feature extraction

The first step in data analysis is the pre-processing of the obtained signals to extract the data from the sensor's response, improve the quality of the created database and prepare the data for the pattern analysis and detection stage [29]. The

Fig. 1 Schematic of olfactory system used. a Carbone active filter, b Sample, c Valve, d Pump, e Sensor array, f Date acquisition card, g PC and h Air outlet



Table 1The used sensors inelectronic nose

Sensor type	Main applications	Typical detection ranges (ppm)		
MQ3	Alcohol	10–300		
TGS822	Steam organic solvents	50-5000		
MQ-136	Sulfur dioxide (SO2)	1–200		
MQ-9	CO and combustible gas	Co 10–1000, Cg 100–10,000		
TGS813	CH4, C3H8, C4H10	500-10,000		
MQ135	Steam ammonia, benzene, sulfide	10–10,000		
TGS2602	Sulfide Hydrogen sulfide, ammonia, toluene	1–30		
TGS2620	Alcohol, Steam organic solvents	50-5000		

proper choice of the pre-processing method is of crucial importance and could have a crucial impact on pattern detection performance [30]. Although the sensor technology can be to some extent effective on the method of preprocessing, three major stages could be considered for data preprocessing: baseline correction, compaction, and normalization [29]. The baseline correction stage is to compensate the nose and drift and increase the sensor response quality which is often employed for intrinsically large or small signals [31]. Various methods (i.e. discriminant, relative, and fractional) have been developed for baseline correction which can be employed depending on the type of applied sensors, sensor application, and the researchers' preference [31]. Here, the fractional method was employed to correct the baseline. This method can also be used for data normalization and it has been widely employed for MOS sensors [29]:

$$Y_{S}(t) = X_{S}(t) - X_{S}(0)$$
(1)

In which $Y_s(t)$ is the normalized response, $x_s(o)$ denotes the baseline and $x_s(t)$ represents the sensor response.

Data analysis methods

To analyze the pre-processed data, CA, PCA, PLS, QDA, and SVM techniques were applied. 70% of the data were used for training while 30% of them were employed for testing. The cluster method is a classification method to allocate similar entities and objects to the groups or clusters. Considering a series of objects and some of their similarity values, their ranking in the classification clusters or groups could be defined, CA is a technique aimed to divide the data to specific groups based on their similarity or distance [32]. The results of a hierarchical clustering method are often represented as a dendrogram [33]. The distance between different observations in the dendrogram can be measured to determine the similarity of the observations in terms of each property. This method calculates the distance between the individual data and the centroid by an intra-group covariance matrix. Each point will be then allocated to the nearest cluster [32]. In this research, Ward's method (with the help of square Euclidian distance) was used to determine the membership cluster based on the nearest centroid ordering method.

PCA is an unsupervised pattern detection method with a perpendicular linear transforms, which transform the data to the new coordination system in such a way that the largest variance will be placed on the first axis, the second largest one will be placed on the second axis and so on. In this way, the data of a series could be simply visualized. Analysis of the major component s could reduce the data dimension. In this way, the components of the dataset with the highest impact on the variance will be preserved. This method has been widely employed to represent the E-nose response to simple and complicated smells and can provide some qualitative information for pattern detection [34, 35].

PLS is a linear and supervised multivariate calibration method that attempts to find factors (i.e., latent variables), which capture as much variance as possible in the predictor block X-matrix, under the constraint of being correlated with the predicted block Y-matrix [36].

Linear differentiation analysis (LDA) creates a linear combination of the attributes resulting in classification. This function increases the inter-group variance to the intra-group variance ratio. Transformations and transferring in this function are as follows: when a new observation is introduced, it reaches its maximum value to predict the inter-group differences [37]. Three methods including linear, quadratic, and mahalanobis are used for data classification by the LDA approach. LDA method was used for data classification along with quadratic analysis, which is abbreviated as QDA. This method has been widely applied in statistics, pattern identification, and machine learning to find a combination of unique attributes.

This is one of the most accurate data mining methods with two-class monitoring and classification with high generalizability which can be employed for classification of linear and nonlinear data [38]. In support vector machine (SVM), C-SVM and Nu-SVM are used to classify the data. The difference between these two methods is in their expression of the problem as an optimization problem and selection of Nu, C, and γ parameters to minimize the error function. C parameter in the C-SVM method is chosen to reduce the complexity of computations and reduce the noise in the data. The higher the C value, the lower the training error will be. However, the generalizability of the machine will be also declined. The increase of Nu in Nu-SVM during an increase of class separation will enhance the error [38].

One of the most powerful techniques in the field of chemometrics is factor analysis. This is a multi-variable method, which reduces the data dimension and minimizes the number of perpendicular vectors to offer important information. Partial lowest square is one of the main factor analysis approaches. The main factor or part is a linear combination of the major variables in the matrices. Instead of an $i \times j$ matrix (i is the number of characteristics and j is the number of sensors), its component can be defined as a linear combination of J factor; which finally gives rise to new variables for the matrices [39]. In the PLS method, instead of finding a plane of maximum variances between the responses and non-independent variables, a relationship is established between the major components. This method forms a linear regression model between the predicted variables and those observed in the new space [40]. Finally, a new regression model will be constructed between the input and output results by a PLS modeling process. The model performance can be evaluated by R^2 and RMSE [41, 42]:

$$R^{2} = 1 - \frac{\sum_{i=1}^{N} (S_{k} - T_{k})^{2}}{\sum_{i=1}^{N} (S_{k} - T_{m})^{2}}$$
(2)

$$RMSE = \frac{1}{N} \sum_{i=1}^{N} |S_k - T_k|$$
(3)

where, S_k , T_k , and Tm are measured, predicted, and average predicted. All the analyses (PLS, SVM, LDA, and PCA) were implemented using Unscrambler × 10.4 software (CAMO AS, Trondheim, Norway).

Chemical analysis of the oils

Lipid oxidation is a dynamic equilibrium process in which the hydro-peroxides are the key mediators in controlling the auto-oxidation progress. Hydro-peroxide can continue to produce oxidation secondary products and degrade. Some of these breakdown products are volatile. Upon production of hydro-peroxides in a specific stage, volatile and non-volatile substances are produced which are occur in the form of degradation and more accumulation of hydro-peroxides. The concentration of these volatile and non-volatile substances will increase which will be decelerated by the formation of the volatile substances [43]. The Chemical analysis includes measurement of different parameters, which will be discussed below. Peroxide index is a criterion to measure the hydro-peroxides. Free fatty acids are the consequence of enzymic hydrolysis of triglycerides in which heat and humidity play the role of catalysts. These compounds contribute to auto-oxidation and give rise to products which are the main cause of unpleasant taste and smell in the oil products [44]. Hydro-peroxides are the primary product of the oxidation in oils and fats which can be degraded to volatile and non-volatile secondary products. Peroxide index can be a proper indicator of the initial stages of oxidation [43]. AV or the free fatty acid value (w_{FFA}) indicates the level of free fatty acid in the oil in the form of oleic acid (%); while PV indicates hydro-peroxide level (meq O_2/kg) which can be formed through oxidation during the storage process. AV and PV measurements were conducted according to the official AOCS methods [44]. Anisidine index indicates the secondary products of oxidation produced as the result of peroxide destruction [45]. AnV can be used to assess the aldehyde content (especially unsaturated α and β aldehydes) [46]. Totox index can be also calculated by the following equation [47]:

$$Totox = 2 \times (PV) + AnV \tag{4}$$

According to AOCS standards, the oils with $PV \le 10 \text{ meq/}$ kg and $AV \le 0.6 \text{ mg/g}$ are defined as the non-oxidized oils, while the oils with PV 10 meq/kg and AV > 0.6 mg/g are considered as the oxidized [2]. According to the strict standard regulation in Iran, the oils with acidity index above 0.6 and peroxide levels more than 5 are considered as the spoiled oil [48]. Chemical analyses were conducted in three replicates for each sample. All the experiments were carried out in Mahidasht Kermanshah Vegetable Oil Agricultural Industrial. The statistical analyses were conducted using a completely randomized factorial test.

Results and discussions

The voltage responses of the sensors were measured in 30 replicates for all the samples (Non-oxidized and oxidized). Finally, the responses of the sensor arrays were recorded for 60 samples. The maximum response of each sample was extracted as the descriptor of the obtained signals. Then

a 60×8 feature matrix (obtained from the samples) was used as the input for the data analysis. The responses of the applied sensors to different levels of oxidation of the edible oil are represent in Fig. 2. The difference in the output responses of the sensors in the measurement stage can be observed in the mentioned figure.

CA method results

The hierarchical CA method was used to classify 60 edible oil samples based on the responses of the 8-sensor array using squared Euclidean as the similarity distance and Ward's clustering method as the amalgamation rule. The dendrogram of the CA method is shown in Fig. 3. As this figure suggests, the edible oil samples were divided into 2 clusters with a distance of 4.9. The first cluster included non-oxidized oils while the oxidized oils were placed in the second cluster. Therefore, the CA method could offer an initial classification although the group divisions were different at different distances. Xu et al. [2] Classified the oxidized and non-oxidized oils with an inter-group distance of 5.01.

PCA method results

To detect oxidation in the edible oil samples, the PCA method was also applied. The score diagram of the two major components is represented in Fig. 4. This diagram is generally used to classify the separate data clusters to identify their patterns [29]. The first two major components described 99% variance of the dataset (PC1 = 98% and PC2 = 1%) for differentiating the different levels of oxidation. According to the Score diagram, there is a good distinction between oxidized and non-oxidized oil samples.



Fig. 2 The responses of the electronic nose system to Different levels of oxidation on the edible oils: a Non-oxidized, b oxidized



Fig. 3 CA dendrogram responds to Non-oxidized and oxidized oil samples



Fig. 4 Score plot PCA analysis For different levels of adulteration

The role of each sensor in differentiating the samples can be studied by the Loading diagram [34]. For this purpose, the sensors were visualized in the loading diagram with their specific coefficients (Fig. 5). Higher loading of a sensor on a major component (more proximity to the outer circle) reflects its higher role in the detection and differentiation of the samples. According to the loading results, the sensors with the lowest impact of detection and differentiation can be eliminated. This can reduce the complexity of the data analysis and also decline the construction cost of the sensor array [34]. The loading diagram of the two major components is depicted in Fig. 5. Accordingly, TGS2602 and MQ136 had the highest loading coefficients and hence played the most significant roles in the sample differentiation. Regarding high loading coefficients and proximity of these values for MQ136 and TGS2602 sensors in the loading figure, the responses of the sensors to the smell pattern of the headspace are presented using mean and standard deviation (Fig. 6). As can be observed, TGS2602 and MQ136 had the highest contribution in smell identification, respectively and are the best choices for detecting oxidation in the oil.

Ayari et al. [27] Also reported similar results regarding the oxidation detection in animal oil and edible oil [27, 28]. A lot of research has been done on various products by PCA, which can be referred to Ridgetail white prawn (*Exopalaemon carinicauda*) [49, 50], Large Yellow Croaker (*Pseudosciaena crocea*) [21, 51], and *Collichthys lucidus* [22].

PLS method results

As TGS2602 and MQ136, sensors allocated the highest significance in the loading diagram in PLS analysis. Regarding the high correlation between the measured and predicted values, the PLS method is highly capable of the best sensors. Using the PLS method, TGS2602 and MQ136 showed the highest correlation coefficient which is line with the loading diagram. The performance of this model was determined by calculating the determination coefficient (R²) and root mean square error (RMSE) of Eqs. 2 and 3. In the PLS method, factor 1 and factor 2 covered 98% and 1% of the data variance (99% of total variance), respectively (Fig. 7).

Men et al. [52] used the PLS method combined with E-nose and managed to predict various ratios of old and



Fig. 7 Result of PLS regression in prediction of sensor response. a TGS2602, b MQ136

fresh frying oils with an accuracy of 84%. Oussama et al. [53] Employed the PLS method to classify different percentages of adulterations in olive oil with an accuracy of 99%. Mabood et al. [39] Applied the PLS method to predict different percentages of adulteration in commercial sweeteners in the fruit juice. Moreover, Ghasemi-Varnamkhasti et al. [54] employed the PLS approach to classifying different cultivars of caraway with a precision of 100%. Tohidi et al. [55] Also reported similar results. Results of the previous studies, as well as the findings of this study, confirm the high precision of this method and its applicability in adulteration detection and classification of food products.

QDA method results

In order to detect oil oxidation, the LDA method was used for data classification along with quadratic analysis, which is abbreviated as QDA. The models' input included the data obtained from eight sensors. All the data had a weight of one. QDA diagram of the E-nose signals for oxidation detection in oil is depicted in Fig. 8. ODA method exhibited an accuracy of 100%. As can be seen, the QDA method had the highest precision in the differentiation of fresh and oxidized oil, and they are properly classified into two groups. Based on the sensor's datasheet (Table 1), MQ136 can be used for the detection of SO2, and TGS2602 can be used for the detection of hydrogen sulfide, and ammonia which is related to the amount of sulfur which is added to the oil in the bleaching stage and ammonia is used as a convenient source of hydrogen for the hydrogenation of fats and oils. Finally, regarding the significant role of TGS2602 and MQ136 sensors and high prediction capability of the QDA method, quality control managers and researchers can employ a combination of the QDA method and mentioned sensors to detect oil oxidation.

Olsson et al. [56] Reported a classification accuracy of 81.1% for naturally infected barley samples. Donis-González et al. [57] Used different classifiers including support vector machine, LDA, QDA, and Mahalanobis distance that QDA had the highest overall performance accuracy. Mohammadi et al. [58] The best result for the classification of fruits was obtained by the QDA classification, with an accuracy rate of 90.24%.

SVM method results

For classification of the samples based on SVM, C-SVM, and Nu-SVM were employed. Nu, C, and y parameters were found by trial and error and validated by minimization. The four types of kernel functions including linear, polynomial, radial basis function, and sigmoid were employed. 70% of the data were used for training while 30% of them were employed for testing. The weight of all inputs was equal to one. The results of the SVM method are listed in Table 2. In Nu-SVM and C-SVM methods, linear function showed the highest accuracy in the detection of oxidation in oil. The results show that the C-SVM method is able to classify oxidized and non-oxidized oil with an accuracy of 98% for the training data and 97% for the Validation data. Also in Nu-SVM, method is able to classify oxidized and non-oxidized oil with an accuracy of 97% for the training data and 95% for the Validation data. In addition, the linear function represented better results in detection than polynomial, sigmoid and radial basis function. Tohidi et al. [59] Classified different percentages of formalin, hydrogen peroxide and sodium hypochlorite in milk with accuracies of 94.64, 92.85 and 87.75%,



Table 2Results and comparisonof Nu-SVM and C-SVM modelssubjected to the kernel functions

Kernel function	C-SVM				Nu-SVM			
	c	γ	Accuraccy training	Accuraccy validation	Nu	γ	Accuraccy training	Accuraccy validation
Linear	0.75	_	98	97	0.1	1	97	95
Polynomial	0.5	0.125	96	95	1	1	95	95
Radial basis function	0.255	1	97	91	10	1	96	94
sigmoid	0.01	0.1	96	96	10	0.1	96	94

 Table 3
 Analysis Of Variance for the Chemical parameters of edible oil

	Sources	Degrees of freedom	Mean square
p-Anisidine value	Treatment	1	16.105**
	Error	4	0.006
Proxide value	Treatment	1	87.554**
	Error	4	0.00025
Acetic acid value	Treatment	1	0.001**
	Error	4	0.00022
Totox value	Treatment	1	216.612**
	Error	4	0.012

**p≤0.01

respectively with the use of SVM method. Chen et al. [60] Evaluated the quality of green tea using the SVM method with an accuracy of 100% for the training data and 95% for the test data. Nouri et al.[61] To classify different percentages of cocoa in chocolate, they obtained a 100% detection accuracy. Also, in another study, they found 90% accuracy in detecting the quality of pomegranate fruit infected with fungal disease [62].

Chemical analysis of the oil

The major components of the oil samples were analyzed according to official AOCS methods after E-nose analysis. Variance analysis on the impact of samples on the acidity, peroxide, anisidine, and Totox is summarized in Table 3. It can be seen that the effect of the 4 components became significant at the probability level of 1%. The mean comparison Non-oxidized and oxidized oil for Anisidine 11.840 and 8.563, Peroxide 1.693 and 9.333, Acetic Acid 0.055 and 0.075 and Totox were 12.227 and 27.230, respectively. Totox index is a criterion of total oxidation including initial and secondary products of oxidation [43]. As the peroxide index is not a reliable index for the oil's oxidation and it can be broken during the heat procedures, Totox index was used to calculate the oil oxidation [63]. As expected, spoiled oils had a higher Totox index compared to the fresh samples.

Conclusions

In this study, a portable 8-sensor E-nose was employed to investigate the oxidation degree of the edible oils. These results indicated that the application of E-nose in combination with CA, PCA, SVM, QDA, and PLS technique could be a promising approach in the successful detection of oxidation in the edible oils. Eight sensors of the electronic nose exhibited different response signals to the oxidized oils, which were different from the non-oxidized one. Based on the findings of this study, it is suggested to use E-nose along within this study, a portable 8-sensor E-nose was employed to investigate the oxidation degree of the edible oils. These results indicated that the application of E-nose in combination with CA, PCA, SVM, QDA, and PLS technique could be a promising approach in the successful detection of oxidation in the edible oils. The E-nose, which is similar to the human olfaction system, requires no specific design to detect the volatile compounds. This technique is superior over the conventional official method for the detection of oxidation degree in the edible oils as it is a non-destructive and time-saving method that reduced the use of toxic organic solvents. The result s of this study showed that the application of the proposed E-nose could decrease the dependence on the evaluator individuals or time-consuming data analysis to differentiate the oxidized oils from the non-oxidized ones.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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