Classification of food vegetable oils by fluorimetry and artificial neural networks

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ABSTRACT

There is a large variety and trademarks of vegetable oils in Brazil. Vegetable oils have characteristics quite similar to each other and often cannot be distinguished by only observing the color, odor or taste. Methods for classification of these oils are often costly and time consuming and they usually take advantage of techniques from analytical chemistry and mathematical methods such as PCA (Principal Component Analysis), PCR (Principal Components Regression) or PLS (Properties of Partial Least Squares) and ANN (Artificial Neural Networks) to increase their efficiency. Due to the wide variety of products, more efficient methods are needed to qualify, characterize and classify these substances, because the final price should reflect the excellence of the product that reaches the consumer. This paper proposes a methodology to classify vegetable oils like: Canola, Sunflower, Corn and Soybean from different man- ufacturers. The method used is characterized by a simple mathematical treatment, a light emission diode and CCD array sensor to capture the spectra of the induced fluorescence in diluted oil samples. An ANN that has three layers, each one with 4 neurons is responsible to perform the spectra classifications. The methodology is capable of classifying vegetable oil and allows fast network training using very few mathematical manipulations in the spectra data with 72% a rate of success.

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1. Introduction

Rapid and nondestructive measurements of quality-related pa- rameters in foods are advancing due to progress in spectroscopy and development of new sensor technology including cameras and spectrophotometers (Skjervold et al., 2003). Advances in spec- troscopy now enable researchers to obtain information about chemical and physical components in food or biological materials at the molecular level. Various spectroscopic techniques (e.g., atomic absorption spectroscopy, Raman and Fourier-transform infrared spectroscopy, near infrared spectroscopy, nuclear magnetic reso- nance spectroscopy, mass spectroscopy, X-ray fluorescence spec- troscopy, ultra-violet spectroscopy) have been used to study structure-function relationships in foods (both liquid and solid) to improve overall food quality, safety and sensorial characteristics; to investigate fungal infections in plant materials (e.g., fruits, seeds); or to study the mobility of different chemical components in food materials. Processing, analyzing, and displaying these data can often be difficult, time-consuming, and problem-specific. Chemo- metrics is well established for calibrating the spectral data to pre- dict concentrations of constituents of interest (Ghosh & Jayas, 2009). The fluorescence signals are complex data with fluo- rophore fingerprints that overlap themselves. Their processing take a long time and require powerful hardware and software (Vasilscu, Marmureanu, & Carstea, 2011). Fluorescence spectroscopy has been investigated among other uses as a tool for quantification of com- ponents of meat (Wold, Lundby, & Egelandsdal, 1999), and also related to structural properties of meat and cheese (Dufour, Devaux, Fortier, & Herbert, 2001; Dufour & Frencia, 2001; Lopez & Dufour, 2001), polyphenolic content in beverages (Andreu- Navarro, Fernandez-Romero, & et al., 2012) and to differentiate yeast and bacterial cells (Bhatta, Goldys, & Learmonth, 2006).

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Chemometrics is related to all those processes which transform analytical signals and complex data into useful information (Mujica-Ascencio, Moreno-Garcia, Stolik-Isakina, & de la Rosa-Vazquez, 2010). Traditional analysis of fluorescence spectroscopy is either uni- or multi-variate. Univariate analysis focuses in finding a pair of excitation-emission wavelengths where there is only one or a few known components giving rise to the signal. Wold et al. (1999) introduced the advantages of combining fluorescent images with fluorescence spectra.

During the last years, the Artificial Neural Networks and Channels Relationships methods have been frequently used to process spectral signals (Almhdi, Valigi, Gulbinas, Westphal, & Reuter, 2007). Artificial Neural Networks (ANNs) for modeling and simulating have become popular in energy sources, chemical engineering, water treatment, and control domain, among others (Jaafarzadeh, Ahmadi, Amiri, Yassin, & Martinez, 2012). ANN and their application in food chemistry and food science are described by Goyal (2013) and Marini (2009). ANN can be described as a mathematical model of a specific structure, consisting of a number of the single processing elements (nodes, neurons), arranged in inter-connected layers. An active neuron multiplies each input vector by its weight, sums the products and passes the sum through a transfer function to produce the output (Bieroza, Baker, & Bridgeman, 2011). The ANN is made up of a group of inter-connected artificial neurons. It consists of an input, hidden and an output layers. Each layer is also composed of neurons. Each neuron transforms input and sends outputs to other neurons to which it is connected. Weights and bias are determined from the receiving neurons. The network is trained with a subset or dataset of observations and optimized based on its ability to predict a set of known outcomes (Klaypradit, Kerdpiboon, & Singh, 2011).

Marine, Dyer, and Jochemsen (2007) employed a combination of two different neural networks architectures for the resolution of simulated binary blends of olive oils from different cultivars. Cámara, Fernández-Ruiz, Redondo, Sánchez-Mata, and Torrecilla (2012) modeled Lycopene degradation kinetics in tomato-based products using a radial basis network (RBN) obtaining a mean prediction error lower than 2.62% and a correlation coefficient higher than 0.983, they concluded that the RBN mathematical approach proposed can be considered as a reliable tool to monitor the stability of lycopene in tomato products (juices, sauces and ketchups) during its shelf life and may be a useful tool for tomato industry. Scott, James, Ali, O'Hare, and Rowell (2003) used ANNs for pattern recognition of olive oil fluorescence spectra. Chen et al. (2012) used ANN to classify honeys by their near infrared spectra. Groselj, Vracko, Pierna, Baeten, and Novic (2008) studied olive oil adulteration with hazelnut oils using the Counter-propagation Artificial Neural Networks (CP-ANN) model. Torrecilla, Rojo, Oliet, Domínguez, and Rodríguez (2010) used self-organizing maps and learning vector quantization networks as tools to identify vegetable oils and detect adulterations of extra virgin olive oil.

In the food industry, food safety and quality are considered important issues worldwide that are directly related to health and social progress. Consumers are increasingly looking for trusted brands of food products, and expect manufacturers and retailers to provide high quality products (Gori, Cevoli, Fabbri, Caboni, & Losi, 2012).

Increased consumer awareness of food safety and quality issues has led to the development of new and increasingly sophisticated techniques for food product authentication. However, most of these techniques are time consuming, and require extensive sample preparation and hazardous chemicals as well as skilled and experienced operators (Gori et al., 2012). These disadvantages have prompted for the adoption of new and simpler methods such as fluorimetry.

Due to the large variety of brands and trademarks of vegetable oils in the Brazilian market, it is common not to know for sure if the substance that is being bought is really a product without adulteration. Vegetable oils have characteristics quite similar to each other and often cannot be distinguished by only observing of the color, odor or taste. In this work we propose a method to classify vegetable oils: Canola, Sunflower, Corn and Soybean using a very few mathematical manipulation and ANN — Artificial Neural Networks as a tool to differentiate the fluorescence spectrum of these oils.

2. Material and methods

2.1. Instrument developed

The instrument used in data acquisition is a spectrofluorometer type LED/CCD that has been developed in the Laboratory of Optical Properties (LAPO) at the Federal University of Bahia (UFBA)-Brazil. This equipment is able to obtain fluorescence spectra between 350 nm and 1050 nm. This equipment was patented in 2012 and can be found in the QUIMIS catalog under the ticker Q-798FIL.

The spectrofluorometer schematic diagram is shown in Fig. 1. As a source of excitation light, a LED (Light Emitting Diode) is used. The sample holder can receive standard cuvettes of 3.5 mL, micro cuvette or microscope slides. Fluorescence light collection is made at 90° from the light source excitation at a fixed distance. The LED emits light with short wavelength on the sample, which, in its turn, emits fluorescent light at another wavelength. The detector captures both the fluorescence light from the sample and the scattered light of the LED optical system.

The LED used as the excitation light source was manufactured by Nichia Corp. Am. (part number NSPu510CS) type ub9. The choice of this particular LED is due to its known ensured traceability (a nearly Gaussian emission profile centered at 382 nm, with a width of 13.054 nm) that guarantees the quality of the light source and ensure standard exchange for maintenance.

2.2. Samples

At local shops in the city of Salvador, one can find 10 trademarks of vegetable oils. Some of them produce more than one kind of vegetable oil. Choosing four vegetable oils (Canola, Sunflower, Corn and Soybean) they can be grouped at 6 different trademarks for each vegetable oil. Twenty dilutions were produced for each oil,
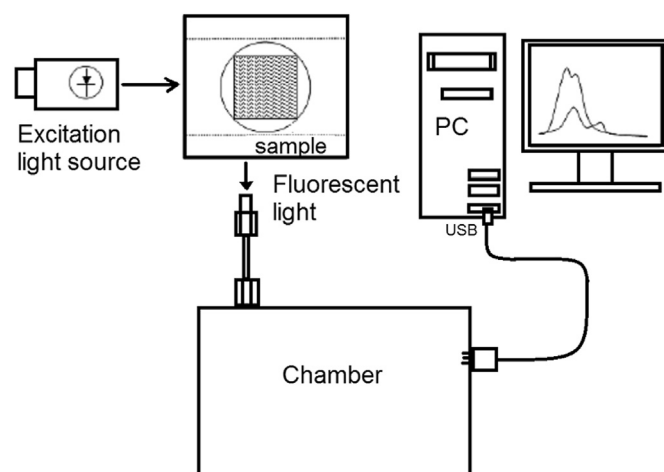

Fig. 1. Diagram of the spectrofluorimeter LED/CCD Q-798FIL.

generating a total of 480 samples. They were used to train the neural network. To build the validation set we produced, with the same methodology, 160 extra samples from another lot, using 2 trademarks for each oil. Each sample was achieved by dilution of the vegetable oil in heptane (C7H16), with a volume of 30 mL and concentration of the vegetable oil ranging from 5% to 100% in steps of 5%.

The heptane was chosen because of its large power of oil dilution and it is relatively easy to find with high purity and absence of residues that could contaminate the samples (Souza et al., 2004). Besides that, heptanes do not show fluorescence induced by the excitation light used. The heptane used is marketed and distributed by VETEC INC. under code 189, type PA (mixture of isomers).

To validate the network and the classification method developed, oils of the same brands used during training were used but from different manufacturing batches.

2.3. Data acquisition and treatment

The fluorescence spectra were acquired using as source of excitation a 382 nm light-emitting diode. In order to perform the fluorescence light collection the CCD was kept active for 1000 milliseconds. This period is called integration time which it is the time for charging the MOS structure of each CCD pixel. The CCD works like an "almost capacitor" being charged with the electrons coming from its active outer layer where photoelectric effect occurs. This process results in the accumulation of information brought by the fluorescence photons, in the form of electrical charge. At the end of the integration time the device (or CCD pixel array) is emptied of its contents. The voltage accumulated in each pixel is applied to the input of an analog to digital converter (ADC) that converts the voltage value in a digital word, generating a two-dimensional array with the wavelength data (pixel number) and the intensity of light (quantity of charge in each pixel).

Independent of the fluorescence technique and type of measurement i.e, spectrally resolved or integrated over a small wavelength interval, the final signal is affected by both the fluorescent analyte(s) and the instrument (Resch-Genger et al., 2005). In cases where the contribution of the lamp scatter profile is minimal, for instance, transparent solutions measured using right-angle geometry, the intensity enhancement of fluorimetric light results in a significant increase in sensitivity for fluorescence based analysis and detection. However, the advantages of fluorimetric measurement are compromised if the contribution from lamp scatter is appreciable as in the case of translucent solutions. The lamp scatter profile and the fluorescence spectral profile are, however, additive. Thus the lamp scatter profile can be independently obtained for the translucent media and this profile can be subtracted from the overall fluorescence spectrum to obtain a pure fluorescence spectrum. Therefore, software (Microcal Origin 7.0) was used for treatment of the experimental data in order to obtain the fluorescence spectrum free of LED influence. All samples were exposed to light during 15 s to collect the spectral data for each one. Some mathematical operations were performed:

1. Initially, the LED spectrum A was subtracted from the total spectrum obtaining the sample fluorescence called FLU.
2. Afterwards, this FLU was divided by a constant C.

In this work, C = 10,422.95 meaning the highest fluorescence intensity obtained for the total of 640 samples (100% corn oil). This division serves to obtain normalized spectra (NOR) facilitating comparisons.

The spectra were limited to the range running from 400 to 700 nm, since the 640 samples studied show no relevant information at 700 nm.

At Filardi, Silva, Santos, Pepe (2012) they used the full spectrum of the samples running from 350 to 1050 nm and the same mathematical treatment of the data used in this work and his input ANN used a set of 88 points of a biodisel sample fluorescence spectrum. However, the convergence of the network was very slow and could take several hours. To avoid this delay, it was designed a new arrangement of the data to be used as input parameters, where a selection of the characteristics of greatest importance in the fluorescence spectrum was made. After several attempts the characteristics were chosen: intensity of the fluorescence light at 400 nm, the wavelength of the maximum intensity of fluorescence of the sample, intensity of the fluorescence light at 700 nm, sum of the intensities observed at 400 and 700 nm.

2.4. Artificial neural network training

In assembling the ANN, the number of neurons in the hidden layer varied from 3 to 13 in order to achieve the greatest possible of success in classifying samples. It was observed that from 8 neurons in the hidden layer the cases of success were dwindling. The number of neurons in the hidden layer with the highest number of successful cases was four. Various ranges of weights were tested and the highest performance was between 1 and 4000 nm. Using shorter intervals for the weights, the network errors were very disparate.

Fig. 2 illustrates the model of the ANN used in this work, for this training method it was applied the feedforward supervised method. The ANN has three layers, each one with 4 neurons. The output layer describes the substance to be classified as follows: the output of each neuron assumes a binary value 0 (false) or 1 (true) and combinations with more than one neuron with true value are not allowed. Therefore, the possible situations are [1 0 0 0] for Canola, [0 1 0 0] for Sunflower, [0 0 1 0] for Corn and [0 0 0 1] for Soybean.

The neural network was programmed in MATLAB®. Many different arrangements and network settings were tested as already said and the strongest performance was the setting with 4 neurons in each layer.

3. Results and discussion

In order to acquire the spectrum A, described above, an empty bucket was placed in the sample holder. In other words, a device with no sample inside. The spectrum of the LED excitation is shown
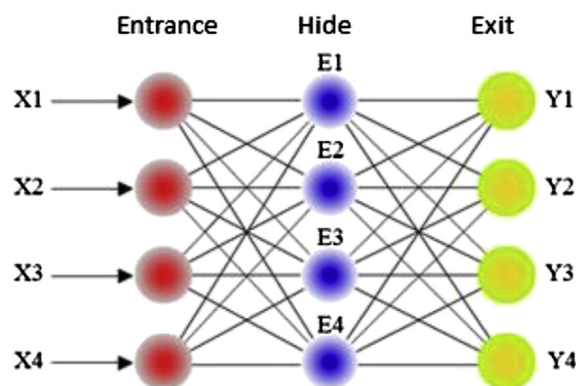

Fig. 2. Artificial Neural Network model.

in Fig. 3.The light reaching the detection fiber is the light scattered by the walls of the bucket. This figure shows that the spectrofluorimeter used is capable of measuring fluorescence light between 350 nm and 1050 nm. For some samples, the observed spectra clearly showed that the presence of the LED light (382 nm) was dependent of the amount of scattered excitation light without interacting with the fluorescent medium. For the different dilutions of the oils, the scattered light from the LED was more prevalent for small concentrations. This phenomenon can be seen in Fig. 4 where the spectra of samples from canola oil (trademark Bom Preço) at dilutions from 5 to 100% are plotted.

The spectra for the excitation light (LED) centered at 382 nm for all vegetable oils used (canola, sunflower, corn and soybean) at the concentration of the 100% are plotted in Fig. 5. The spectra were similar with peaks very close to each other, but the light intensity was quite different. Even very close peaks could be numerically distinguished. Therefore, the fluorescence spectra were very individual and could be used to identify the type of the oil.

Similar spectra were found by Nikolova, Eftimov, Perifanova, and Brabant (2012). These authors used these spectra to group the oils according to their chemical composition. Mbesse Kongbonga et al. (2011) used a Lorentzian profile deconvolution to group vegetables oils according to Vitamin E content also using their fluorescence spectra. In both cases, a large amount of mathematical process was used before the identification. In this work a ANN with few entrance parameters was able to simplify the vegetable oil classification.

Comparing the training time for this methodology to the methodology that was used before in all the spectra (Filardi et al., 2012) it was found that the network training was 1.2 times faster. As formerly describe the only mathematical treatment made with the spectra was to subtract the LED spectrum from the total spectrum and divide the result by 10.42295. The result of this mathematical treatment is shown in Fig. 6, where the spectra for the different vegetable oils used in ANN training are presented.

Among the 160 samples used in the network test, 115 samples were classified with accuracy and in 45 cases the network was not successful. One possible explanation for this level of the ANN error is the fact that some of the tested oils have radical carbon chains similar to each other; this may create a certain similarity in the fluorescence of these substances. Vegetable oils are composed primarily of triacylglycerols which, in refined oils, can reach over 99% (Costa, Mafra, & Oliveira, 2012). Therefore, one way to classify
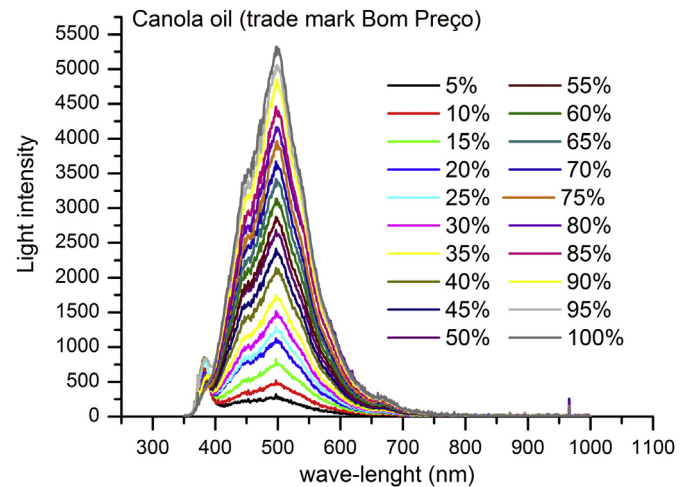

Fig. 4. Light spectra for Canola Oil (trademark Bom Preço) with concentrations ranging from 5% to 100%.

Vegetable oils is to determine the primary fatty acids in its composition and there are 6 possible classifications: lauric, palmitic, oleic, linoleic medium, high linoleic and erucic (Maurera, Hatta-Sakodab, Pascual-Chagmanb, & Rodriguez-Saonaa, 2012). However, the industrial processing may rearrange the fatty acids and bring some difficulty to classify highly processed vegetable oils using ANN (Vieira, Pierre, e Castro, 2005). On the other hand, the vegetable oils were not submitted to any kind of chemical analysis test to determine if they were adulterated, but it is worth emphasizing the importance of the quality of the samples, mainly the methodology for its collection and preparation. Other factors such as the optical adjusting characteristics of the fluorimeter and the parameterization of the data acquisition software had to remain the same way throughout the process of fluorescence spectra acquisition. Based in the results found, it became clear that the methodology proposed in this work attended these quality criteria and, therefore, it could ensure a good classification in future works.

Table 1 shows the responses of the ANN in the validation tests. There were two cases in which the method had 100% success (Corn — trademark Mazola and Soybean - trademark Liza), One can see
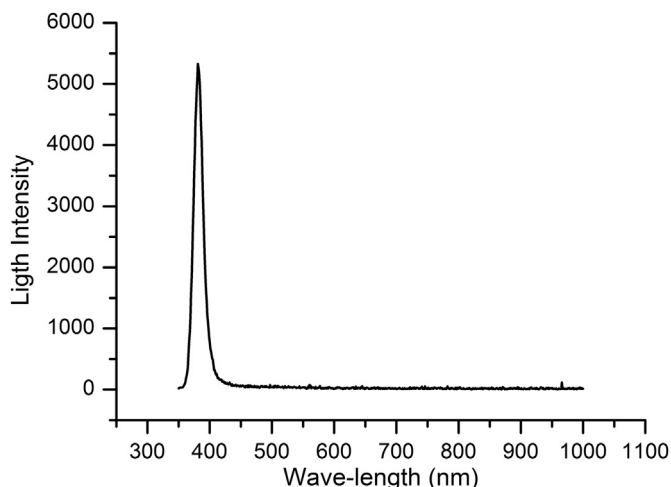

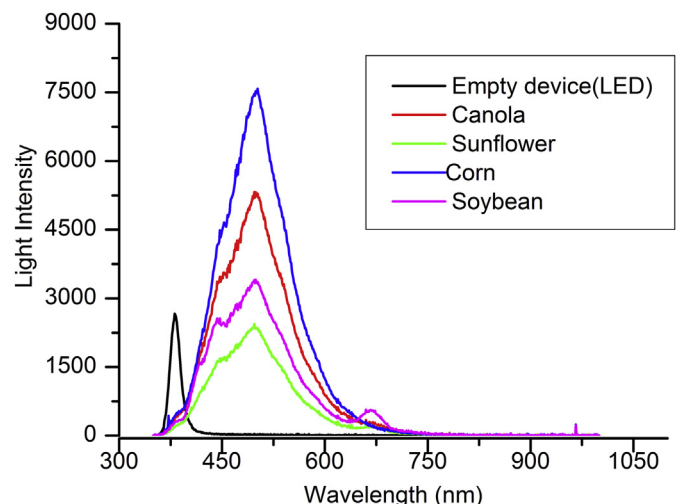**Fig. 3.** Light spectrum of the empty fluorimeter (LED spectrum).

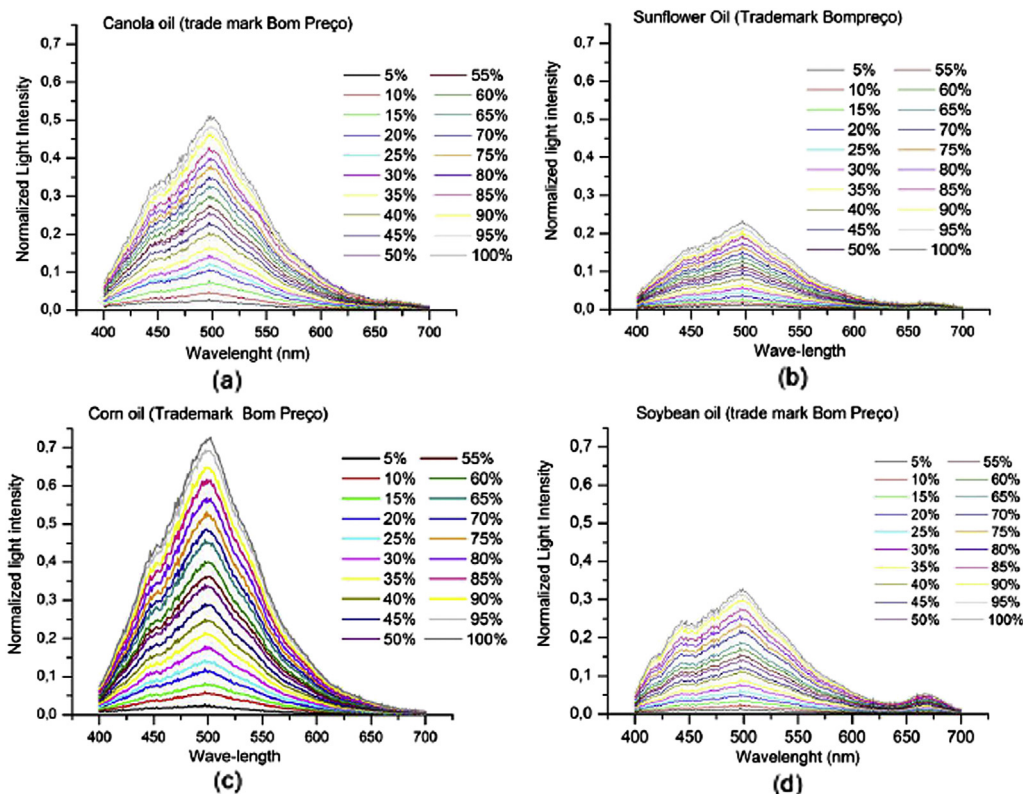**Fig. 5.** Light spectra of four vegetable oils and the empty device.

Fig. 6. Spectra of canola, sunflower, com and soybean oils (trade mark Bom Preço) after the mathematical.

that the best results are in corn and soybean oils. The worst result was in the sample of Sunflower oil (trademark Bom Preço), where only 5 samples were correctly classified. During the previous tests, it was observed that the fluorescence spectra of some Sunflower samples were quite similar to samples of canola oil and the network could not distinguish those.

To meet the market needs and to provide uniform product from raw variables, technical modification of oils and fats allow greater flexibility in the choice of raw materials and help to balance the trends between local availability and demand. Among modification techniques we highlight the hydrogenation, which is the most versatile and used processing method, which can change the fatty acid profile of a vegetable oil. Therefore, the processing can lead to oil coming from the same plant may have different physical and chemical characteristics. Processing can also approximate the physical and chemical characteristics of different vegetable oils (O'brien, 2010). This can be one of the possible causes whey for the ANN implemented confused samples of sunflower oil with canola oil, although only a deeper chemical analysis can prove this hypothesis.

Table 1
Responses of the ANN in 160 validation tests.

Trade mark	Oil	Total success in 20 samples
Bom Preço	Canola	17
Qualitá	Canola	10
Bom Preço	Sunflower	5
Liza	Sunflower	18
Salada	Corn	19
Mazola	Corn	20
Liza	Soybean	20
Soya	Soybean	19

4. Conclusion

A methodology based in artificial neural network was developed. This methodology allows fast network training and uses very few mathematical manipulations in the spectra data.

This is a promising technology from the standpoint of the quality control of vegetable oils and can be thought as a starting point to design future studies focused on the resolution of real blends of oils from different cultivars mainly for Corn and Soybean Oils. Sunflower and Canola oils need more studies to improve precision in their classification.

This new methodology by using the most relevant parameters of the fluorescence spectrum as input data for ANN confirms the need for systematic study of the details that characterize the regions of interest over the fluorescence spectrum. Thus, studying the fluorescence of the primary substances that make up the samples to be analyzed, becomes a crucial step in the process of analyzing the information from fluorescence spectra of vegetable oils and other more complex substances.

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