Utilization of laser-induced breakdown spectroscopy, with principal component analysis and artificial neural network in revealing cheating of similarly looking fish fillets

Omnia Hamdy
Cairo University
Zienab Abdel-Salam
Cairo University
Mohamed Abdel-Harith (mharithm@niles.edu.eg)
Cairo University

Research Article

Keywords: LIBS, PCA, ANN, Fish cheating

Posted Date: March 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1418452/v1

Abstract

Fish is an essential source of protein and many nutrients necessary for the welfare of human health. However, the deliberate mislabeling in fish fillet types of high similarity is common in popular markets to make use of the relatively high price difference. This, of course, is a type of explicit food adulteration. Therefore, in the present work, spectrochemical analysis and chemometric methods have been adopted for disclosing this type of fish species cheating for the benefit of the customers and the market reliability. As a spectrochemical analytical technique, Laser-Induced Breakdown Spectroscopy (LIBS) has been utilized to differentiate between the fillets of the low-priced Tilapia and the expensive Nile Perch. Currently, LIBS is available for *in situ* measurements, namely in the markets and fish distribution centers, making it a distinguished method for such tasks compared to other conventional analytical methods. Furthermore, the acquired spectroscopic data have been analyzed statistically using principal component analysis (PCA) and artificial neural network (ANN). The obtained results demonstrated the potential of using LIBS as a simple, fast, accurate, and cost-effective analytical technique combined with a proper statistical analysis method for the decisive discrimination between fish fillets species.

1. Introduction

Balanced nutrition is essential for optimal health. Fish is a very healthy food containing vital proteins, fats, and vitamins necessary for good nutrition, health, and immunity [1]. Moreover, it is more available and relatively inexpensive than the other animal protein sources in various countries[2]. However, the fishery products' regional price, especially fish fillets, varies according to the type and availability of the utilized fish. As a result, the fish fillet is exposed to massive modifications in its shape and appearance, increasing the probability of mislabeling or substitution with the aim of cheating for illegal economic gains[3,4]. Tilapia (Oreochromis niloticus) and Nile Perch (Lates niloticus) are the most common fish fillet types in the Egyptian market [5,6]. However, due to the vast production of Tilapia in Egypt with limited exportation to the external markets [7], its price is almost half that of the Nile Perch fillet [8]. Consequently, substituting such high-priced fish types with the cheaper ones mostly when sold as fish fillet is sometimes common. Therefore, it is essential to disclose the substitution and mislabeling, mostly deliberate, which is an actual adulteration, in the species used in fish fillet production.

Genetic and immunological assays methods [9–13] have been used for fish substitution detection. However, these destructive methods are costly, time-consuming, and require special laboratories with many samples. On the other hand, multimode hyperspectral imaging[14] and spectroscopic techniques have been widely used in pharmacology[15], biology [16], and the food industry to inspect the quality and substitution probability of their products[17,18].

Laser-Induced Breakdown Spectroscopy (LIBS) is a well-known spectrochemical elemental analysis technique that has been utilized in many biological applications since more than twenty years ago. In this technique, Q-switched nanosecond laser pulses are focused onto the sample surface, inducing a plasma

plume that consists of a collection of ions and swirling electrons at enormously elevated temperatures (> 6000 K). In cooling down the plasma plume, it gets rid of the previously absorbed laser energy by emission photons at different wavelengths. The emitted light is collected by a suitable optical system and fed to the entrance slit of a spectrometer equipped with a light detector (mostly ICCD) for dispersion and detection of the light spectrum.

The obtained emission spectrum includes the characteristic spectral lines of the elements present in the plasma plume and, thus, in the sample material in the case of stoichiometric ablation, considering the self-absorption and the matrix effect. The LIBS technique is detailed experimentally and theoretically in many books and review papers[19–21]

Laser-induced breakdown spectroscopy (LIBS) is characterized by being a simple, fast, and non-destructive method; besides, it can be used in situ via the nowadays commercially available portable or mobile LIBS systems. LIBS has already been utilized to detect adulteration in milk[22], beef [23], and butter [24], in addition to investigating meat substitution[25] and red wine classification [26]. Furthermore, LIBS data has been statistically analyzed using chemometric techniques such as partial least squares, artificial neural networks, and principal component analysis [27–30]. Recently, neural networks, as a supervised technique, have been considered efficient classifiers for many non-linear problems. Therefore, it has been significantly utilized to evaluate the classification rate LIBS data[31].

In the present work, laser-induced breakdown spectroscopy has been employed to discriminate between samples of commercial Tilapia and Nile Perch fillets. Moreover, LIBS has also been used to test the difference in tenderness between the two types of fish fillets. The spectroscopic data has been validated via conventional analysis of the investigated fish fillet samples. In addition, the chemometric PCA and ANN methods were exploited to analyze the obtained spectroscopic results statistically.

2. Materials & Methods

2.1 Sample collection and preparation

One hundred fish fillet samples (50 from each fish species) have been purchased from different commercial marketplaces in the vicinity of Cairo University. Fillets have been collected from various fishes of the same species to have independent samples. Each sample weighs approximately 100–120 g of muscle tissue. There are no treatments processes applied to the specimens before performing the experimental measurements. The sample's transportation to the laboratory took about 60 min.

2.2 Laser-Induced Breakdown Spectroscopy (LIBS)

A standard LIBS setup has been used in the current work in which a Q-switched Nd: YAG laser (Brio, Quantel, France) produces laser pulses of 50 mJ/pulse at a wavelength λ = 1064 nm of pulse width 5 ns and repetition rate of 20 Hz. The laser pulses have been focused in the air under atmospheric pressure onto the sample's surface using a 10 cm focal length plano-convex quartz lens. An x-y micrometric

translational stage was used to control the sample holder's position in front of the focused laser beam. A 2-m length fused silica optical fiber having 600 µm core diameter was used to collect the light emitted from the laser-induced plasma. The collected light is fed to the entrance slit of an echelle spectrometer (Mechelle 7500, Multichannel, Sweden) coupled to an ICCD camera as a detector (DiCAM-Pro, PCO, Computer optics-Germany). Accumulated 50 spectra were collected from 10 laser shots on each of 5 new spots separated by 1 mm on the same fish fillet specimen to compensate for any surface inhomogeneity in the sample. Emission spectra treatment and identification of the spectral lines have been performed by the commercial software LIBS++. A detailed description of the used LIBS instrumentations is given elsewhere[32].

2.3 Laboratory determination of total protein and total Fats:

The fish fillets samples total protein and fat have been estimated using a conventional meat analyzer (food Scan TM Pro meat analyzer, Foss Analytical A/S, Model 78810, Denmark) at the Faculty of Agriculture's science park center, Cairo University. The automated system gave the percentage of the total protein, the fats, and the average in each Tilapia and Nile Perch fillets sample.

2.4 Statistical analysis

2.4.1 Principle component analysis (PCA)

PCA has been chosen as a multivariate statistical analysis method to reduce the dimensionality of the data set and retain as much information as possible. PCA, therefore, simplifies the visualization of the distribution of samples, the observation of outliers' spectra and reduces the variables. Thus, score plots elucidate similar, dissimilar, typical, or outlier data sets. Moreover, loading plots were used to indicate the origin of the principal components' spectral information enclosed. To construct the principal components, the first principal component (PC1) explains the maximum available variance, while the second principal component (PC2) represents the following highest variance, and so on. In this work, the spectroscopic data were analyzed statistically via PCA via the commercial software (OriginPro 2018 (64-bit) SR1 b9.5). The fifty LIBS spectra of the two types of fish fillet samples were used in the PCA analysis.

2.4.2 Artificial Neural Network (ANN)

Pattern Recognition Neural Networks (PRNN) are feed-forward networks trained to classify inputs according to target classes. In the current implementation, the obtained LIBS spectra have been used as the input of the PRNN, with two outputs (1 or 0) for tilapia or Nile perch fillet samples, respectively. Additionally, the scaled conjugate gradient (SCG) algorithm has been used for training the neural network[33]. In the Matlab platform, the network training function named "trainscg" has been used to update the weight and bias values based on the SCG method[34].

Due to the spectral lines' large number in the LIBS spectrum, the PCA technique minimizes the spectra' dimensions. Furthermore, optimizing the input variable simplifies the NN model and increases its accuracy. Therefore, two NN models have been constructed; the first model has been fed with the whole

spectra pixels (28207 input variable). The other model used a specific spectral range (from 300 to 500 nm) as recommended from the PCA results. 50 LIBS spectra for each fish species (Tilapia and Nile Perch) have been utilized to construct the network. The input data sets have been randomly divided into percentages of 70%, 15%, and 15% for training, validation, and test; such a ratio is appropriate for the relatively small dimensional datasets [35]. The construction of the two models is illustrated in Fig. 1.

The utilized NN models have been initially implemented using 10 neurons in the hidden layer. Then, a different number of neurons have been tested, 10, 15, 20, using the validation set to get the best performance[36].

3. Results And Discussion

3.1 Evaluation of LIBS spectra

Figure 2(a) shows the LIBS spectra of Nile Perch and Tilapia fish fillet. Each spectrum is an average of 50 spectra for every species. It is evident from the figure that there is a significant difference between the two fish types' spectra, especially in the CN spectral band around 386 nm. Moreover, the carbon line at 247.8 nm and the molecular carbon band C_2 at 516 nm show up clearly in the case of the Tilapia spectrum, contrary to that of the Nile Perch. The Fe and Mg spectral lines' intensity difference could also discriminate among the two fish fillet species.

Two primary mechanisms lead to the formation of CN and C_2 spectral bands; the first is the recombination of the molecular carbon present in the fatty acids in the fish fillet tissues with the nitrogen from the amino acids in the same tissues or ambient air. On the other hand, fragmentation is essential for producing C_2 in the molecules comprising double bonds carbon (the fatty acids) [37,38]. Thus, the direct ablation of native CN and C_2 molecules from the fillet tissue samples represent the second mechanism [39,40]. In this way, LIBS could be exploited to evaluate fats in the fish fillet samples by detecting CN and C_2 spectral bands in such samples' emission spectra, as illustrated in Fig. 3.

The error bars represent the standard deviation of the experimental data of each group.

As shown in Fig. 2(b), the high intensity of the CN band in the LIBS spectrum of the Tilapia (The upper graph) reflects its high-fat content compared to the Nile Perch samples. Similarly, the C_2 spectral band's intensities and the strong atomic line of carbon at 247.8 nm shown in Fig. 2 (The middle and lower graphs) confirm the same results.

LIBS results cannot discriminate between different types of fats, but they can help evaluate the total fats in different fish samples. For example, Yvon G. et al.[41] found a clear relationship between C_2 emission from the plasma and the concentration of saturated fatty acids in the oil via LIBS. Therefore, the fish fillet samples' total protein and fats content have been estimated conventionally using a classical meat analyzer to confirm the LIBS results as presented in Fig. 3.

The results presented in Fig. 4 show that fat content in Nile Perch samples is much less than that in Tilapia. However, protein percentage is almost the same in both fish species. Such analysis results agree with the LIBS results where the intensity of the CN and C_2 spectral bands and the carbon atomic line at 247.8 nm in the LIBS spectra are higher in the Tilapia samples than in Nile Perch. Fatty acids, as mentioned before, are the primary origin of the spectral bands of the molecular cyanide and carbon bands, besides the atomic carbon spectral line.

Meat tissue tenderness and softness differ for different species of fish fillet. As in the hardness of solids, fish fillets' tenderness can be estimated spectroscopically from the ratio of ionic to atomic spectral lines intensities in the relevant LIBS spectra. Accordingly, the magnesium and iron spectral lines in the LIBS spectra of the fish fillet samples have been used to estimate the tenderness of the two fish types' tissue. The bar graphs in Fig. 3 show the values of the ratio MgII/MgI and FeII/FeI obtained for the different fish samples spices, Nile Perch and Tilapia. This figure depicts that the intensity ratio of Tilapia spectral lines is less, which means this fish type's tenderness is higher than the other type. Hence, these results can justify the above findings, which indicated the higher fat content of the Tilapia compared to the Nile Perch, which makes its texture tenderer.

It is worth mentioning that freezing and thawing affect the sensory profile negatively and, therefore, the quality of fish fillets through the formation of ice crystals reducing tenderness and reduced water holding capacity, which is the case for the imported Nile Perch contrary to the locally provided Tilapia[42,43].

3.2 PCA of LIBS Spectra

It is well-known that PCA, as a chemometric method, can be used to convert high-dimensional multivariate LIBS data to be evaluated on a low-dimensional scale. PCA has been applied frequently in food analysis during the last two decades[44]. PCA was exploited in the discrimination between NILE Perch and Tilapia fish fillet by analyzing their LIBS spectra in the present work. The multivariate statistical analysis results via the PCA are depicted in Fig. 6. PCA analysis was performed for the whole wavelength range of the LIBS spectra from 200 to 900 nm. As mentioned before, fifty replicates were analyzed for each sample species. Therefore, the spectra of all the fifty replicates were used in the PCA analysis. Figure 4(a) shows that the samples were well-separated into two groups in the obtained score plot. The first two principal components, PC1, and PC2, were chosen considering their highest cumulative variance. The first two PCs' score plots for the whole spectral range (200–900 nm) provided excellent discrimination between Nile Perch and Tilapia, explaining 92.7% of the total variance. The first and second PCs captured 90.1% and 2.6% of the variance, respectively. The obtained score plot shows the PCA method's potential in classifying the LIBS spectra of the two fish types under investigation.

Figure 3(b) depicts the corresponding loadings plots of 230 to 650 nm spectral range. In the upper graph, it is clear that the spectral features of C, CN, and C_2 are in the positive sector of PC2, contributing to Tilapia, as shown in Fig. 4(a). On the other hand, Fe, Mg, Ca, and Na lines are present in the positive sector of PC1, confirming the obtained Nile Tilapia results in Fig. 4(b). Ca and Na lines show up in the negative sector of PC2, too, confirming the part of the corresponding score plot of the Nile Perch.

3.3 Artificial Neural Network (ANN) results

The accuracy rate of the constructed ANN models is presented in Table 1. As depicted in the table, when using the LIBS spectrum's whole spectral lines as input variables for the ANN, the abstained accuracy rate was 100%, 93.3%, 80%, and 96% for training, validation, test, and all data sets respectively. While all data sets accuracy rate increased to 98% upon using the optimized input variables with the same number of neurons.

Table 1
ANN implantations result at different input variables and the number of neurons

Spectral range	Number of neurons	Accuracy rate			
		Training	Validation	Test	All
Whole spectrum	10	100%	93.3%	80%	96%
From 300 to 500 nm	10	100%	93.3%	93.3%	98%
From 300 to 500 nm	15	100%	100%	93.3%	99%
From 300 to 500 nm	20	100%	100%	100%	100%

Optimizing the number of neurons in the ANN model's hidden layer increases the accuracy rate significantly to 100% using 20 neurons. A sample of the error histogram obtained from the implemented NN models is presented in Fig. 5. The error shows the difference between the desired and the real output produced by the network. The "Instances" in the figure refers to the number of training, validation, and test sets samples. It is clear from the figure that the fitting data errors are distributed near the zero-error region, verifying the good performance of the implemented network.

4. Conclusions

LIBS has been utilized to disclose adulteration of similar fish fillets from the low-priced Tilapia and the high-priced Nile Perch. The obtained spectra of the two fish types showed a pronounced difference reflecting the high-fat content in the Tilapia compared to the Nile Perch by the appearance of high CN, C_2 bands, and atomic line at 247.8 nm in the Tilapia spectrum. The high-fat content in Tilapia has been confirmed by the conventional analysis of the samples using a classical meat analyzer. Moreover, the ionic to the atomic ratio of the Fe and Mg spectral lines in both fish fillets samples depicted the high tenderness of the Tilapia. The obtained spectra have been evaluated using PCA and ANN methods. Excellent discrimination has been achieved in the PCA score plot between Tilapia and Nile Perch. The implemented ANN classification accuracy rates reach 100% upon using LIBES spectral range (300–500 nm) with 20 hidden neurons. For the first time, to the best of our knowledge, this work demonstrates the potential of using the commercially available compact and portable LIBS systems with the proper

chemometric method for disclosing adulteration of looking similar fish fillets species. These measurements could be performed in situ, namely in markets and fish distribution centers.

Declarations

Conflicts of interest

The authors have no competing interests to declare relevant to this article's content.

References

1. Rucitra, A. L. The application of material handling in fish fillet production process of red snapper in PT X. *IOP Conf. Ser. Earth Environ. Sci.* **230**, 012061-1–7 (2019).
2. Mohanty, B. P. *et al.* Fish in human health and nutrition. in *Advances in fish research* vol. 7 189–218 (2019).
3. Etienne, M. *et al.* Identification of fish species after cooking by SDS-PAGE and urea IEF: A collaborative study. *J. Agric. Food Chem.* **48**, 2653–2658 (2000).
4. Filonzi, L., Chiesa, S., Vaghi, M. & Marzano, F. N. Molecular barcoding reveals mislabelling of commercial fish products in Italy. *Food Res. Int.* **43**, 1383–1388 (2010).
5. Desta, D. T. *et al.* Proximate Analysis of Nile Tilapia, (Oreochromisniloticus), Fish Fillet Harvested from Farmers Pond and Lake Hawassa, Southern Ethiopia. *Int. J. Res. Dev. Technol.* **11**, 94–99 (2019).
6. Njiru, M. *et al.* Increasing dominance of Nile tilapia, Oreochromis niloticus (L) in Lake Victoria, Kenya: Consequences for the Nile perch Lates niloticus (L) fishery. *Aquat. Ecosyst. Heal. Manag.* **11**, 42–49 (2008).
7. Shaalan, M., El-Mahdy, M., Saleh, M. & El-Matbouli, M. Aquaculture in Egypt : Insights on the Current Trends and Future Perspectives for Sustainable Development. *Rev. Fish. Sci. Aquac.* **26**, 99–110 (2018).
8. Macfadyen, G. *et al.* Value-chain analysis - An assessment methodology to estimate Egyptian aquaculture sector performance. *Aquaculture* **362–363**, 18–27 (2012).
9. Lockley, A. K. & Bardsley, R. G. DNA-based methods for food authentication. *Trends Food Sci. Technol.* **11**, 67–77 (2000).
10. Hsieh, H., Chai, T. & Hwang, D. Using the PCR – RFLP method to identify the species of different processed products of billfish meats. *Food Control* **18**, 369–374 (2007).
11. Basiita, R. K., Zenger, K. R., Mwanja, M. T. & Jerry, D. R. Gene flow and genetic structure in Nile perch, Lates niloticus, from African freshwater rivers and lakes. *PLoS One* **13**, 1–21 (2018).
12. Asensio, L., Garcı, T. & Gonza, I. Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). *Food Control* **19**, 1–8 (2008).

13. Asensio, L. *et al.* Detection of grouper mislabelling in the fish market by an immunostick colorimetric ELISA assay. *Food Agric. Immunol.* **19**, 141–147 (2008).
14. Qin, J. *et al.* Detection of fish fillet substitution and mislabeling using multimode hyperspectral imaging techniques. *Food Control* **114**, 107234-1–14 (2020).
15. Biancolillo, A. & Marini, F. Chemometric Methods for Spectroscopy-Based Pharmaceutical Analysis. *Front. Chem.* **6**, 1–14 (2018).
16. PARKER, F. S. *Applications of Infrared Spectroscopy in Biochemistry, Biology, and Medicine*. (Plenum Press, 1971). doi:10.1007/978-1-4684-1872-9.
17. Wang, K., Sun, D., Pu, H. & Wei, Q. Trends in Food Science & Technology Principles and applications of spectroscopic techniques for evaluating food protein conformational changes : A review. *Trends Food Sci. Technol.* **67**, 207–219 (2017).
18. Arabi, D. S. *et al.* Utilization of Spectrochemical Analysis and Diffuse Optical Techniques to Reveal Adulteration of Alike Fish Species and Their Microbial Contamination. *Food Anal. Methods* (2022) doi:10.1007/s12161-021-02212-z.
19. Miziolek, A. W., Palleschi, V. & Schechter, I. *Laser Induced Breakdown Spectroscopy*. (Cambridge: Cambridge University Press, 2006).
20. Cremers, D. A. & Radziemski, L. J. *Handbook of Laser-Induced Breakdown Spectroscopy*. (John Wiley & Sons, 2013).
21. Hahn, D. W. & Omenetto, N. Laser-induced breakdown spectroscopy (LIBS), part II: Review of instrumental and methodological approaches to material analysis and applications to different fields. *Appl. Spectrosc.* **66**, 347–419 (2012).
22. Moncayo, S., Manzoor, S., Rosales, J. D., Anzano, J. & Caceres, J. O. Qualitative and quantitative analysis of milk for the detection of adulteration by Laser Induced Breakdown Spectroscopy (LIBS). *Food Chem.* **232**, 322–328 (2017).
23. Velioglu, H. M., Sezer, B., Bilge, G., Baytur, S. E. & Boyaci, I. H. Identification of offal adulteration in beef by laser induced breakdown spectroscopy (LIBS). *Meat Sci.* **138**, 28–33 (2018).
24. Temiz, H. T., Sezer, B., Berkkan, A., Ugur, T. & Boyaci, I. H. Assessment of Laser Induced Breakdown Spectroscopy as a Tool for Analysis of Butter Adulteration. *J. Food Compos. Anal.* **67**, 48–54 (2018).
25. Bilge, G., Hasan Murat Velioglu b, Sezer, B., Eseller, K. E. & Boyaci, I. H. Identification of meat species by using laser-induced breakdown spectroscopy. *Meat Sci.* **119**, 118–122 (2016).
26. Moncayo, S., Rosales, J. D., Izquierdo-hornillos, R., Anzano, J. & Caceres, J. O. Classification of red wine based on its protected designation of origin (PDO) using Laser-induced Breakdown Spectroscopy (LIBS). *Talanta* **158**, 185–191 (2016).
27. Koujelev, A., Sabsabi, M., Motto-Ros, V., Laville, S. & Lui, S. L. Laser-induced breakdown spectroscopy with artificial neural network processing for material identification. *Planet. Space Sci.* **58**, 682–690 (2010).

28. Haddad, J. El *et al.* Artificial neural network for on-site quantitative analysis of soils using laser induced breakdown spectroscopy. *Spectrochim. Acta Part B At. Spectrosc.* **78**–**79**, 51–57 (2013).
29. Andrea, E. D. *et al.* An artificial neural network approach to laser-induced breakdown spectroscopy quantitative analysis. *Spectrochim. Acta Part B At. Spectrosc.* **99**, 52–58 (2014).
30. Chen, J. *et al.* Convolutional neural network as a novel classification approach for laser- induced breakdown spectroscopy applications in lithological recognition. *Spectrochim. Acta Part B* **166**, 105801-1–7 (2020).
31. Yang, Y. *et al.* Classification and identification of brands of iron ores using laser-induced breakdown spectroscopy combined with principal component analysis and artificial neural networks. *Anal. Methods* **12**, 1316–1323 (2020).
32. Abdel-Salam, Z., Abdel-Salam, S. A. M. & Harith, M. A. Application of Laser Spectrochemical Analytical Techniques to Follow Up Spoilage of White Meat in Chicken. *Food Anal. Methods* **10**, 2365–2372 (2017).
33. Møller, M. F. A scaled conjugate gradient algorithm for fast supervised learning. *Neural Networks* **6**, 525–533 (1993).
34. Babani, L., Jadhav, S. & Chaudhari, B. Scaled conjugate gradient based adaptive ANN control for SVM-DTC induction motor drive. in *12th IFIP International Conference on Artificial Intelligence Applications and Innovations (AIAI)* 384–395 (2016). doi:10.1007/978-3-319-44944-9_33.
35. Rácz, A., Bajusz, D. & Héberger, K. Effect of Dataset Size and Train/Test Split Ratios in QSAR/QSPR Multiclass Classificatio. *Molecules* **26**, 1–16 (2021).
36. Sheela, K. G. & Deepa, S. N. Review on Methods to Fix Number of Hidden Neurons in Neural Networks. *Math. Probl. Eng.* (2013).
37. Baudelet, M. *et al.* Time-resolved ultraviolet laser-induced breakdown spectroscopy for organic material analysis. *Spectrochim. Acta - Part B At. Spectrosc.* **62**, 1329–1334 (2007).
38. Lucena, P., Doña, A., Tobaria, L. M. & Laserna, J. J. New challenges and insights in the detection and spectral identification of organic explosives by laser induced breakdown spectroscopy. *Spectrochim. Acta - Part B At. Spectrosc.* **66**, 12–20 (2011).
39. Abdel-Salam, Z., Abdelghany, S. & Harith, M. A. Characterization of Milk from Mastitis-Infected Cows Using Laser-Induced Breakdown Spectrometry as a Molecular Analytical Technique. *Food Anal. Methods* **10**, 2422–2428 (2017).
40. Abdel-Salam, Z., Alexeree, S. M. I. & Harith, M. A. Utilizing biosynthesized nano-enhanced laser-induced breakdown spectroscopy for proteins estimation in canned tuna. *Spectrochim. Acta - Part B At. Spectrosc.* **149**, 112–117 (2018).
41. Mbesse Kongbonga, Y. G., Ghalila, H., Onana, M. B. & Ben Lakhdar, Z. Classification of vegetable oils based on their concentration of saturated fatty acids using laser induced breakdown spectroscopy (LIBS). *Food Chem.* **147**, 327–331 (2014).
42. Soltanizadeh, N., Kadivar, M., Keramat, J. & Fazilati, M. Comparison of fresh beef and camel meat proteolysis during cold storage. *Meat Sci.* **80**, 892–895 (2008).

43. Ali, S. *et al.* Effect of multiple freeze-thaw cycles on the quality of chicken breast meat. *FOOD Chem.* **173**, 808–814 (2015).
44. Grané, A. & Jach, A. Applications of principal component analysis (PCA) in food science and technology. in *Mathematical and Statistical Methods in Food Science and Technology* (John Wiley & Sons, Ltd, 2014). doi:10.2310/7070.2005.34652.

Figures

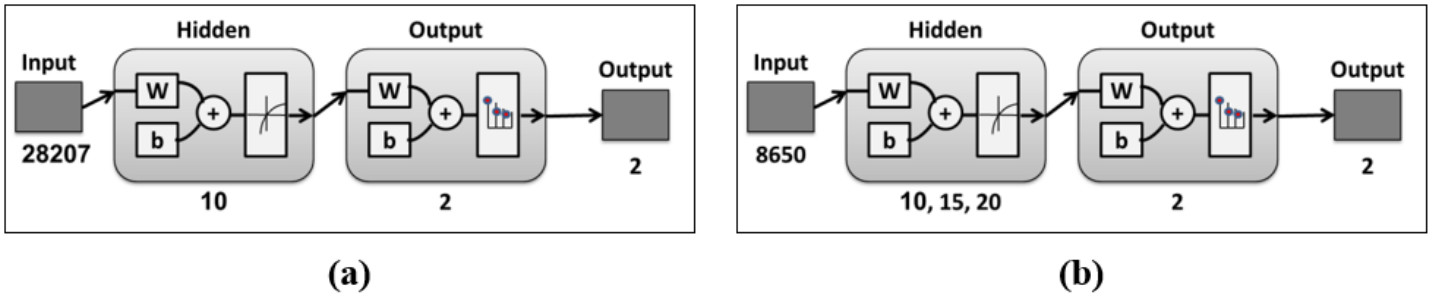
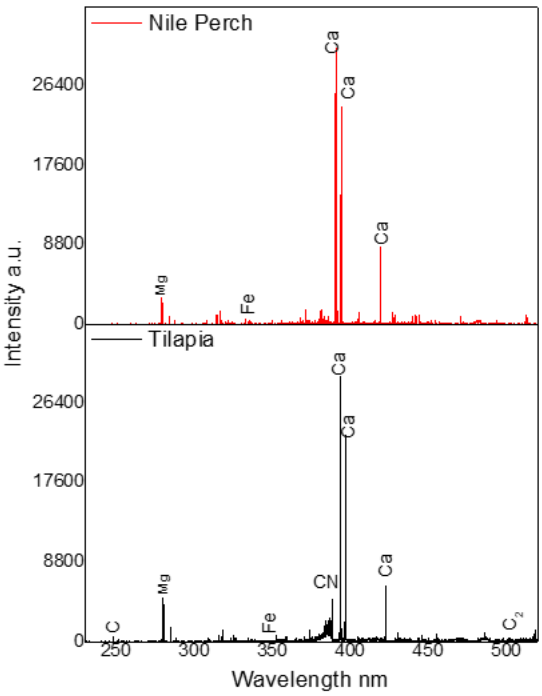
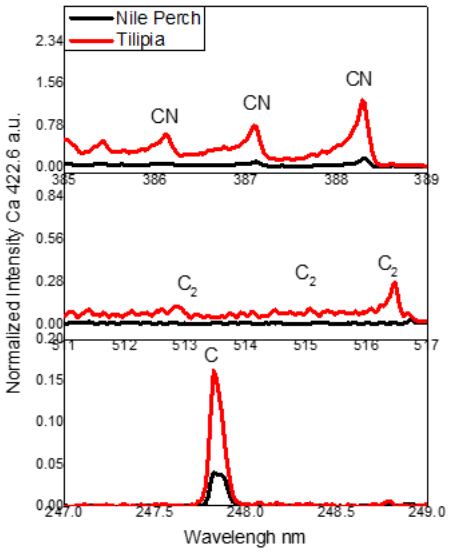


Figure 1

The Implemented Neural Network Models, (a) using the whole LIBS spectrum, (b) using LIBS spectrum from 300 to 500 nm only



(a)



(b)

Figure 2

(a) LIBS spectra of Nile Perch and Tilapia fish samples. (b) Comparison between the violet CN band (upper), the Swan C_2 band (middle), and C 247.8 nm atomic line (lower) in Tilapia and Nile Perch fish samples.

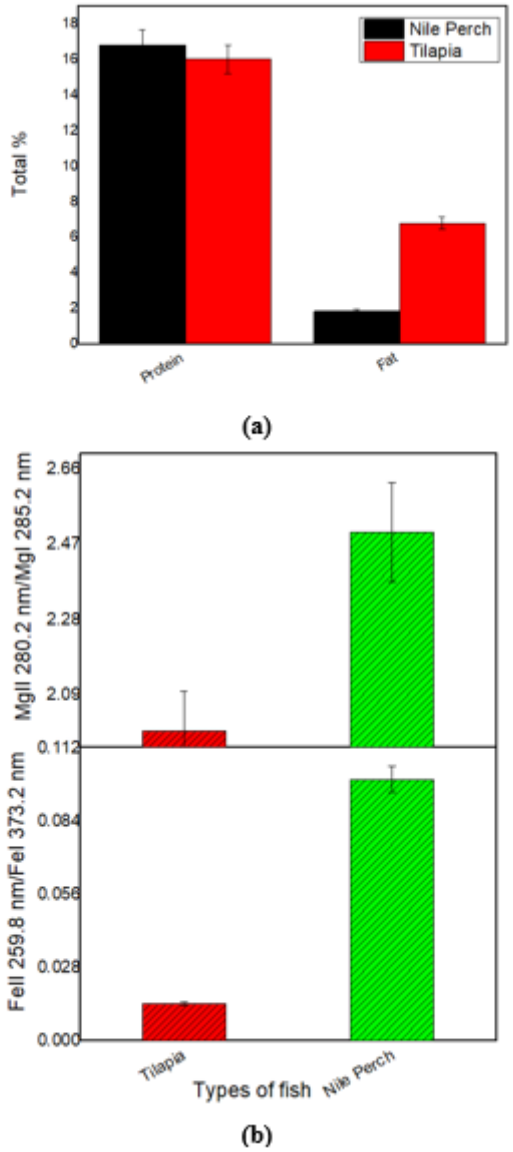


Figure 3

(a) A bar graph for the percentage of protein and fats in Nile Perch and Tilapia. The error bars represent the standard deviation of the experimental data of each group (b) LIBS intensity ratios of ionic to atomic spectral lines of Magnesium and Iron in Tilapia and Nile perch fish samples.

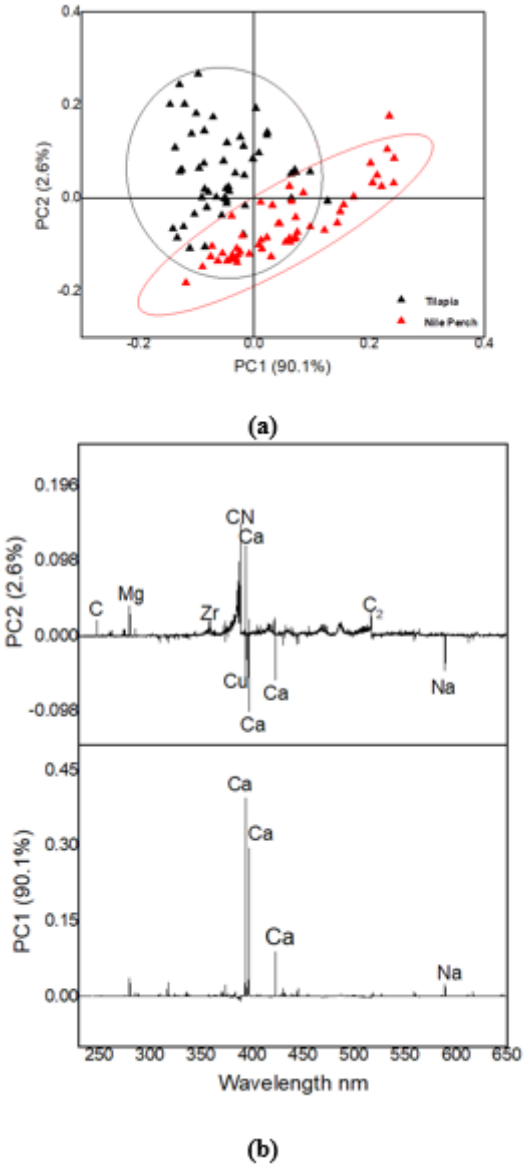


Figure 4

(a) PCA score plot based on LIBS spectra. (b) PCA loadings plots PC1 vs. PC2

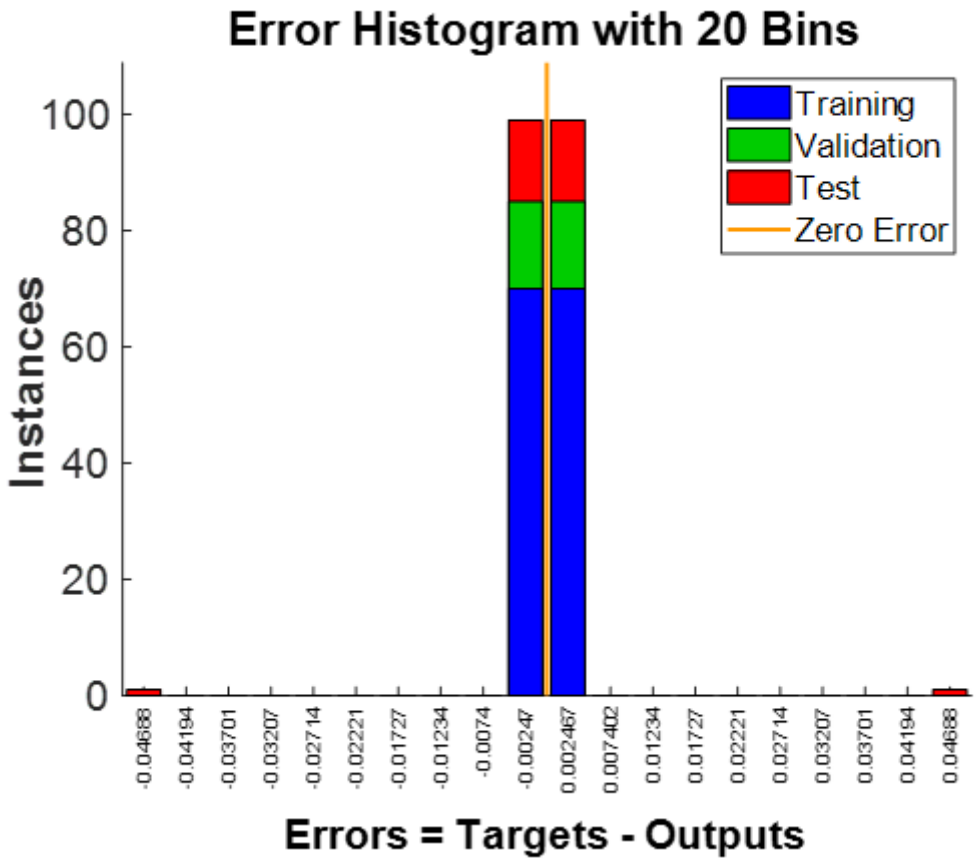


Figure 5

Error histogram for one of the ANN executions.