Artificial Neural Network Model AIP-SYMOMATH 2015

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Artificial Neural Network Model for Photosynthetic Pigments Identification using Multi Wavelength Chromatographic Data

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Abstract. The development of rapid and automatic pigment characterization method become an important issue due to the fact that there are only less than 1% of plant pigments in the earth have been explored. In this research, a mathematical model based on artificial intelligence approach was developed to simplify and accelerate pigment characterization process from HPLC (high-performance liquid chromatography) procedure. HPLC is a widely used technique to separate and identify pigments in a mixture. Input of the model is chromatographic data from HPLC device and output of the model is a list of pigments which is the spectrum pattern is discovered in it. This model provides two dimensional (retention time and wavelength) fingerprints for pigment characterization which is proven to be more accurate than one dimensional fingerprint (fixed wavelength). Moreover, by mimicking interconnection of the neuron in the nervous systems of the human brain, the model have learning ability that could be replacing expert judgement on evaluating spectrum pattern. In the preprocessing step, principal component analysis (PCA) was used to reduce the huge dimension of the chromatographic data. The aim of this step is to simplify the model and accelerate the identification process. Six photosynthetic pigments i.e. *zeaxantin*, *pheophytin a*, *α-carotene*, *β-carotene*, *lycopene* and *lutein* could be well identified by the model with accuracy up to 85.33% and processing time less than 1 second.

INTRODUCTION

Generally, natural colorant refers to biological pigment which is extracted from living organisms. Therefore, biological pigment is considered to be safer for human and more environmentally friendly compare to synthetic pigment. Nowadays, interest in natural colorant is increasing along with the growing interest within the Industrialized Nations in natural (Green) products [1]. Hence, researches about biological pigment exploration has grown extensively [2,3]. One of the common problems in such research related to the pigment characterization.

The existence of a pigment in certain mixture is revealed by chromatography. It separates mixture into individual components by letting them creep slowly past another substance which is typically a liquid or solid. There are three common methods in chromatography which is paper, column and gas chromatography. In liquid-column chromatography, the mixture is placed at one end of the column and an extra added substance called an eluent is poured in to help it travel through. Separated pigments are detected at the exit of the column by a flow-through device (detector). This detector will collect the UV light absorption data in various wavelengths in order to identify and measure the total amount of the pigment, this is due to the fact that each pigment reflected light as the result of wavelength-selective absorption. Output of chromatography is chromatogram, which is visualized in the form of line graph. It depicts the total amount of the pigment by time in the certain wavelength. In the case of an optimal

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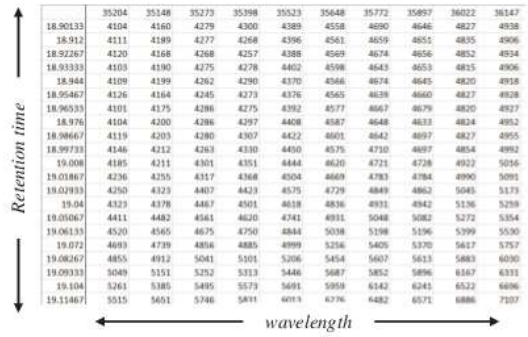
separation, different peaks or patterns on the line graph correspond to different pigment of the separated mixture. High performance liquid chromatography - diode array detector (HPLC-DAD) is a popular method to conduct sophisticated chromatography [4-6]. It is used to separate, identify, and quantify each pigment in a mixture simultaneously. Output of the HPLC is two dimensional matrix that depict the total amount of detected pigment during the operation time in various wavelengths).

Commonly, an expert is needed to determine particular pattern on chromatogram in order to characterize the existing pigments. Consequently, the characterization process is time consuming and subjective. Moreover, there is another problem related to the data processing time due to the huge size of HPLC chromatogram matrix. Using the common analysis method, pigment identification is an exhausting task with high probability of inaccuracy [7-,8]. Therefore, several researchers apply various classification method to substitute expert judgement. Classic mathematical or statistical classification model shows promising result [9-12]. Most of the models have high accuracy. But, since mathematical/statistical model is difficult to developed, some researchers try artificial intelligence approach. This approach could handle the classification of a complex nonlinear data. Although the classification accuracy is lower than the mathematical/statistical model, it is much more simple to developed. Moreover, the artificial intelligence model could perform self training in order to increase its ability. Support vector machine was proven better than k-nearest neighbour to classify biological samples from chromatogram data of two dimensional liquid chromatogram [13]. But, those method was proven to be inferior to artificial neural network to recognize nonlinear data [14-16]. Therefore, in this research a model based on artificial intelligence approach was developed to automate the HPLC chromatogram matrix analysis. The model was developed based on artificial neural network classification concept. Using this concept, the model could performs self-learning mechanism to improve its performance during application. Hence, the model could provide fast and accurate pigment identification.

Six photosynthetic pigments were modeled using artificial neural network architecture. Those pigments are *zeaxantin*, *pheophytin a*, *α-carotene*, *β-carotene*, *lycopene* and *lutein*. In order to train the model, as much as 570 sample data (95 data for each pigment) were used. Those training data was obtained from well identified chromatogram data as a result of column chromatography method using various eluent. After the training process the model will have the ability to identify the existence of the six pigments in new chromatogram data. In advance, along with the information technology development this model will be embedded in online laboratory analysis system. User of the system will be able to run self-service chromatogram analysis for pigment identification by simply upload the chromatogram data.

MULTIWAVELENGTH CHROMATOGRAPHIC DATA

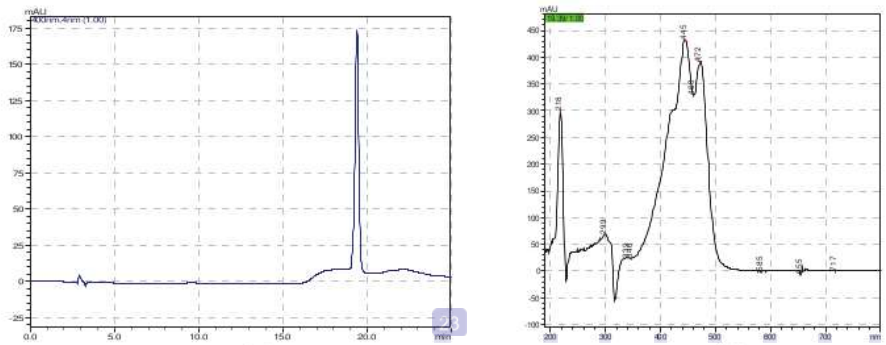
Figure 1 illustrate an example of multi wavelength chromatographic data. Each cell within the matrix represent the amount of the UV light (with corresponding wavelength) that is absorbed by the pigment which is collected at corresponding time.



	35204	35148	35273	35398	35523	35648	35772	35897	36022	36147
18.90133	4104	4160	4279	4300	4389	4558	4690	4646	4827	4938
18.912	4111	4189	4277	4268	4396	4561	4659	4651	4835	4906
18.92267	4120	4168	4268	4257	4388	4569	4674	4656	4852	4934
18.93333	4103	4190	4275	4278	4402	4598	4643	4653	4815	4906
18.944	4109	4199	4262	4290	4370	4566	4674	4645	4820	4918
18.95467	4126	4164	4245	4273	4376	4565	4639	4660	4827	4928
18.96533	4101	4175	4286	4275	4392	4577	4667	4679	4820	4927
18.976	4104	4200	4286	4297	4408	4587	4648	4633	4824	4952
18.98667	4119	4203	4280	4307	4422	4601	4642	4697	4827	4955
18.99733	4146	4212	4263	4330	4450	4575	4710	4697	4854	4992
19.008	4185	4211	4301	4351	4444	4620	4721	4728	4922	5016
19.01867	4236	4255	4317	4368	4504	4669	4783	4784	4990	5091
19.02933	4250	4323	4407	4423	4575	4729	4849	4862	5045	5173
19.04	4323	4378	4467	4501	4618	4836	4931	4942	5136	5259
19.05067	4411	4482	4561	4620	4741	4931	5048	5082	5272	5354
19.06133	4520	4565	4675	4750	4844	5038	5198	5196	5399	5530
19.072	4693	4739	4856	4885	4999	5256	5405	5370	5617	5757
19.08267	4855	4912	5041	5101	5206	5454	5607	5613	5883	6030
19.09333	5049	5151	5252	5313	5446	5687	5852	5896	6167	6331
19.104	5261	5385	5495	5573	5691	5959	6142	6241	6522	6696
19.11467	5515	5651	5746	5831	6013	6276	6482	6571	6886	7107

FIGURE 1.Example of multiwavelength chromatographic data

Researcher analyses the data by visualized it as a line graphs called chromatogram and spectrum (Fig. 2). Each column in multi wavelength chromatographic data could be used to produce chromatogram and each row could be used to produce spectrum. The peak in the chromatogram indicates existence of any pigment in the mixture. This existence is captured by the detector along analysis time. Each pigment will flow through the column in different speed, therefore it will be captured by the detector at the different time as well. Hence, several peaks in the chromatogram indicate that the mixture contains several pigments. Meanwhile, the pattern in spectrum is used to confirm the identity of the pigment. This is due to the fact that each pigment will perform unique spectrum pattern.



(a) (b)

FIGURE 2.Visualization of of multi wavelength chromatographic data from sample containing zeaxhantin: (a)Chromatogram, (b)Spectrum

However, some patterns have small differences such that it is difficult to get absolute confirmation. Therefore, both retention time and spectrum is used to determine a probability of identifying a pigment in a mixture. Former HPLC analysis commonly uses a spectrum from a fixed wavelength. It discards much of the information contained at other wavelength. Therefore, different approaches have been proposed to utilize chromatographic data [9-10]. Instead of using a fixed wavelength, the new approach involves several wavelengths simultaneously. This new approach was adopted in this research.

MATERIALS AND METHOD

Six photosynthetic pigments from carotenoid family were used as the target ofidentification. Those pigments are *zeaxantin*, *pheophytin a*, *α-carotene*, *β-carotene*, *lycopene* and *lutein*. In order to create training data,pigments are extracted from fresh vegetables such as corn, spinach, tomato, watermelon and carrot. Standard pigment were also used as control. As much as 100 samples for each pigment were prepared to produce multi wavelength chromatographic data using HPLC SIL-20ACXR prominence auto sampler. Each sample was run for 80 min and the absorption of UV light was recorded at 189 nm – 800 nm wavelength. Table 1 shows the range of retention time for each pigment. Therefore, the dimension of each matrix will be about 7502x493. Those matrices was then divided into 2 dataset, 570 for training set and 30 for test set. Training set will be used to develop the artificial neural network classification model and test set will be used to analyse the performance of the model (accuracy of the classification result). There are three main procedures to develop the identification system i.e., data preprocessing, model building and validation.

TABLE 1. Retention Time of The Pigments

Pigment	RT (min)
zeaxanthin	18 – 20
pheophytin a	28 – 31
α-carotene	27 –30
β-carotene	31 –34
lycopene	6–8
lutein	15 – 17

Data Preprocessing

As stated in previous section, calculation of the multiwavelength chromatographic data leads to data processing problem because of the matrix size. Therefore, a method is needed to handle this problem. Priciple Component Analysis (PCA) was proven could reduce the matrix dimention while keeping the important information contained on it [11]. Hence, this method was applied in this step.PCA provide a way of identifying patterns in data. It is expressing the data in such a way as to highlight their similarities and differences. Since patterns in data can be hard to find in high dimension data and graphical representation of it is not available, PCA is a useful tool for analyzing such data [17]. The information in the original variables (refer to the columns of the matrix) is compressed into a smaller number of uncorrelated variables called principal components (PCs) using Eq.1:

$$X = CS^T + E \tag{1}$$

where X is the final compressed matrix (this matrix will be used in the classification process), C is the scores matrix, S^T is loading matrix transpose and E is an error matrix. The first column of matrix X will be $PC_{(1)}$, accounts for the larget amount of the total variation of the data. $PC_{(1)}$ is linear combination of the observed variables (Eq. 2).

$$PC_{(1)} = w_{(1)1}X_1 + w_{(1)2}X_2 + \cdots + w_{(1)p}X_p \tag{2}$$

Second column of matrix X is $PC_{(2)}$, is that weighted linear combination of the observed variables which is uncorrelated with the first linear combination and which accounts for the maximum amount of the remaining total variationnot already accounted for by $PC_{(1)}$. In general the m-th PC is that weighted linear combination ofthe X's which has the largest variance of all linear combinations thatare uncorrelated with all of the previously extracted PCs (Eq. 3). It is possible to extract PCs as much as the total number of the observed variables: but, as the aim of the most principal component is data reduction, only several of the first PCs will be used in the next analysis. Therefore, At the end of the process, X will have smaller dimension.

$$PC_{(m)} = w_{(m)1}X_1 + w_{(m)2}X_2 + \cdots + w_{(m)p}X_p \tag{3}$$

Training data as well as test data will be pre-processed using this method prior to the classification step.

Training Data

Training data is needed during the training (supervised learning) of the artificial neural network. During the training, the same set of data is processed many times as the connection weights are ever refined. Both the inputs and the outputs are provided to the network. The network then processes the inputs and compares its resulting outputs against the desired outputs. Errors are then propagated back through the network and the weights are adjusted. Training set for the whole pigments is a 570x451 matrix (data was captured from 570 samples, reading at 451 different wavelengths) obtained from the stacked chromatogram vector. Those vectors are taken from multiwavelength chromatogram data from each pigment sample. Prior to principal component process, column reduction was done to the matrix. This reduction was refer to the relevant wavelength, which is a particular range of wavelength that theoretically have maximum absorption for particular pigment. In this research the relevant

wavelength for the six pigments is 350 nm - 800 nm. Thus, all data outside the relevant wavelength was discarded, resulting a 570x367matrix. The column of this matrix was then reduced by principal component, a 570x5 matrix was then ready to be an input for artificial neural network model.

Test Data

Performance of the model was justified by "out sample" accuracy using test data. Actually, test data was prepared togather with training data. But, during artificial neural network training only training data was used. Test data will be used after the best model was determined in order to test its "out sample" accuracy. Hence, the best model was "never seen" the test data before. Multiwavelength chromatographic data from 30 samples was taken as test data. The same preprocessing process for training data was also done for the test data, resulting a 30x5 matrix. Figure 3 ilustrates the preparation process of the training and test data.

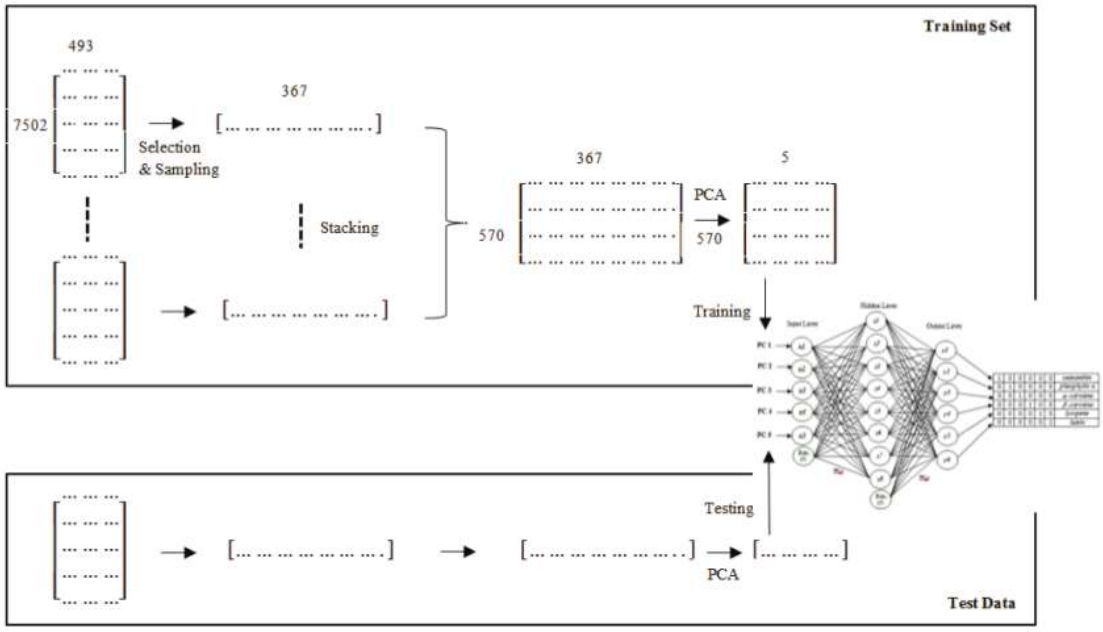


FIGURE 3. Data preparation procedure

Model Building

An artificial neural network is a network of neurons organized in layers. There are 3 main layers of artificial neural network model: (1) Input layer, in this layer *m* predictor variables are represented as *m* neurons; (2) Hidden layers, this layer is intermediate layer which is connected input neuron and output neuron using weghted connected hidden neurons , (3) Output layer, in this layer forcasts variables are representated as some neurons. The forecasts are obtained by a linear combination of the inputs. The weights in hidden neuron are selected in the neural network framework using a learning algorithm that minimizes a cost function such as mean square error (MSE). The simplest networks contain no hidden layers and are equivalent to simple linear regression. Once an intermediate layer is added with its hidden neurons, the neural network becomes non-linear [18].

In this research, there were 5 inputs variables. These variables were taken from the first 5 PCs created during reduction of the training data using principal component method. Those 5 PCs could captures as much as 90% of total variation of the training data whereas less PCs capture less variation and lead to classification inacurracy. Using the PCs, the chromatogram data being discard is minimized. In former artificial neural network model, the input data was chosen from the particular peak of the chromatogram meanwhile the other data was neglected [16]. This approach could easily lead to inaccuracy since peak position could be slightly alter due to variation in sample preparation.

Output variables were representated in 6 neurons since there are 6 pigments being the target of the classification. Hidden layers were determined by experiment, there were 50 experiments using single hidden layer and 500 experiments using double hidden layers. The best model was chosen by analys ing the minimum MSE and total running time. Figure 4 depicts the best model for the six pigments identification purpose and Table 2 describes the comparison of the model performance using single and double hidden layers. Table 2 shows that experiment using double hidden layer did not give good result, especially in running time (represented in iteration/epoch). Despite double layer could reach the minimum MSE, its running time is almost 3 time greater than single layer. It indicates that using double layer did not fulfill efficiency criteria. On the other hand, although experiment using single hidden layer did not give the least MSE, its running time is the best among all (see single layer with 8 nodes). Therefore the single hidden layer with 8 nodes was chosen as the best model.

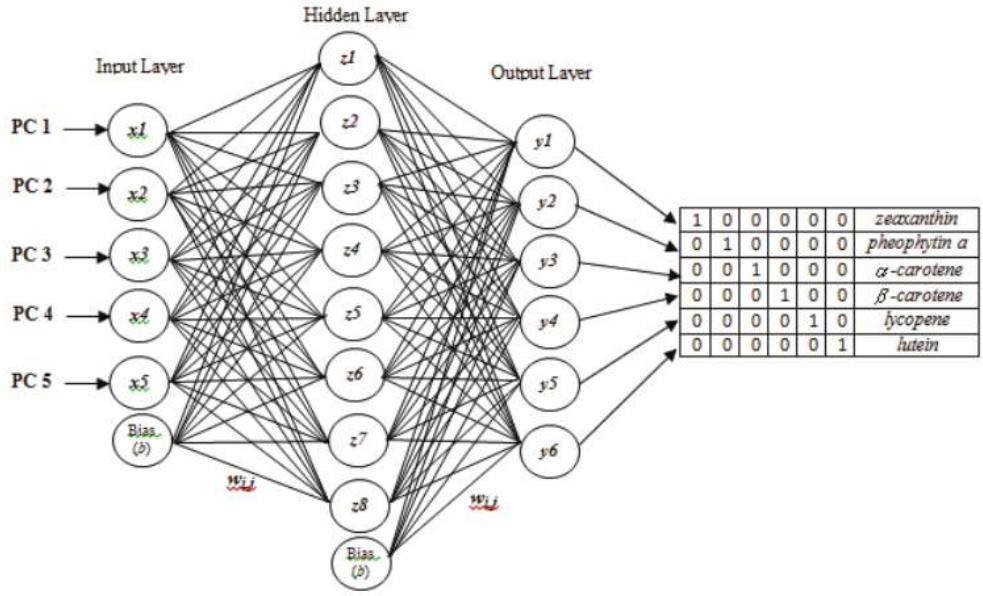


FIGURE 4. Artificial neural network model for pigment identification

Such type of artificial neural network is commonly called *multilayer feed-forward network*. Each layer of nodes receives inputs from the previous layers. The outputs of nodes in one layer are inputs to the next layer. The inputs to each node are combined using a weighted linear combination (Eq. 4). The result is then modified by a nonlinear function (the activation function) before being output. There are several kind of actifation function that could be chosen to give the best result, in this research sigmoid function was used (Eq. 5). Sigmoid function tends to reduce the effect of extreme input values, thus making the network somewhat robust to outliers.

$$z_j = b_j + \sum_{i=1}^{8} w_{i,j}.x_i \quad (4)$$

$$s(z) = \frac{1}{1+e^{-z}} \quad (5)$$

TABLE 2. Part of The Experiment Result in Designing Artificial Neural Network Model

Number of node for each layer	Mean Square Error ($.10^{-4}$)		Iteration (epoch)		In Sample Accuracy (%)
	Single Layer	Double layer	Single Layer	Double layer	
1	90.57	85.78	2204	2500	100%
2	85.99	84.60	2035	2900	100%
3	82.11	80.36	1956	3254	100%
4	78.91	11.32	1932	3200	100%
5	77.55	9.66	1856	3125	100%
6	69.32	8.03	1555	4870	100%
7	70.12	97.55	1365	4900	100%
8	**23.91**	100.61	**804**	5001	**100 %**
9	22.78	104.77	966	5503	100%
10	21.20	100.12	980	5468	100%

Initially, the weights ($w_{i,j}$) take random values, which are then updated using the observed data. Therefore, there is an element of randomness in the predictions produced by an artificial neural network. Hence, the network is usually trained several times using different random starting points, and the results are averaged. Backpropagation learning algorithm was used to train the network. Backpropagation uses error (actual value of the output and forecast differences) to update the weights sequentially, begin with the last hidden layer up to the first hidden layer. This process is repeated until error reach the desired value. Updating the network weight is done using information from the partial derivative $\partial C/\partial w$ of the cost function C with respect to any weight w (or bias b) in the network. It inform how quickly the cost changes when the weights and biases are changed.

Computer Application

A software named "SaptaCHROME" was developed in order to run rapid identification of a pigment from a chromatogram data matrix. Figure 5 depict the user interface of the software. User input the chromatographic data matrix by loading the data in .csv format (since HPLC output is also .csv format). Right after the .csv data was read by the software (by clicking the **identify Chromatogram's Data** button), a chromatogram is displayed. The chromatogram will shows the existence of any pigment by peak along the line graph. Each peak is marked by red bold arrow. As an example, chromatogram in Fig. 5 shows that there are 4 different pigments. The detail retention time for each peak is shown in the information box next to the chromatogram. To display detail of the pigment, user could click each red bold arrow. For example, in Fig. 5 user click the second peak from the left of the chromatogram, the best spectrum then displayed in the bottom of the chromatogram. The identity of the pigment, which is *zeaxanthin* is displayed in the result box.

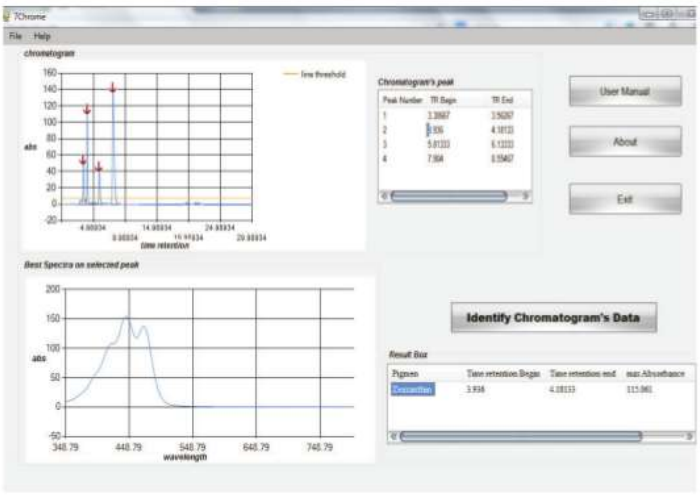


FIGURE 5. User interface of "SaptaCHROME"

RESULT AND DISCUSSION

The performance of the artificial neural network model was analyzed using the test data. Model prediction was compared to the information of the actual existing pigment in the sample. The result is shown in Table 3. Despite the in sample accuracy reach the maximum value (100%), the out sample accuracy is not the same. The best accuracy is performed when classify *zeaxanthin* and *pheophytin a* and the worst is performed when classify the *lycopene* and *lutein*. *Lycopene* and *lutein* were often wrongly classify as *zeaxhantin*. This phenomena was occur due to the pattern close similarity of the spectrum of those three pigments, see Fig. 6(a). Meanwhile, the *α-carotene* and *β-carotene* accuracy is moderately good. The *α-carotene* often wrongly classify as *β-carotene* and vice versa because these two pigments have similar spectrum, see Fig.6(b).

TABLE 3. Classification Accuracy

Pigment	Out Sample Accuracy (%)
zeaxanthin	100
pheophytin a	100
α -carotene	84
β -carotene	87
lycopene	74
lutein	67
Average	**85.33**

On the average, the artificial neural network model reach 85.33% of accuracy. Therefore, in general the model could be considered good. This result also shows that the compression data method using PCA could well encapsulated the unique pattern information of the carotenoid spectrum.

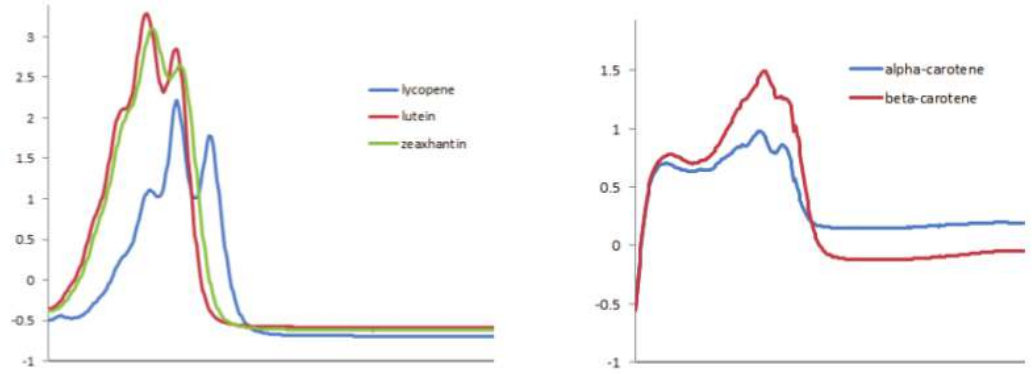


FIGURE 6. Spectrum Similarity: (a)Spectrum of *zeaxhantin*, *lutein* and *lycopene*, (b)Spectrum of *α-carotene* and *β-carotene*

CONCLUSION

The evaluation of artificial neural network model to simultaneously classify six carotenoid pigments reveals promising result. With a little adjustment on the model (especially in recognizing *lycopene* and *lutein* spectrum), the proposed method could be developed into more sophisticated computer application. Moreover, using this artificial intelligence method, more photosynthetic pigments could be recognized by simply update the training set database with various multi wavelength chromatogram data matrix from other photosynthetic pigments. In order to improve the accuracy, artificial neural network model could also be designed to dynamically stores the new data being analyze and keep it in database as new training data.

FUTURE WORK

Regarding that the model have the ability to self-improve its intelligence and it also easy to re-model as the pigment being identify is added, it will be developed as an main identification engine on the online laboratory system. The model will automatically analyze the chromatogram data which is uploaded by the user and give prediction of the existing pigments. Therefore the identification could be done real time.

Since samples used in this research is well prepared such that all experiment variable is controlled, further confirmation of the method should be done. Complex chromatographic profile from other plant extraction mixture will be a good material to study the weaknesses of the proposed method.

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