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Pesticide Residue Measurements in Raw Milk Based on Optical Spectrum and Neural Networks

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Abstract

Pesticide residue has become as one of the major food adulterants as a result , rapid, simple, spectrophotometric method for pesticide residue measurement (bifenthrin, glyphosate) in milk sample based on UV-Vis spectrophotometer and Artificial neural network had been developed .The developed system detecting the milk contaminants like pesticide residue and classified it based on absorption spectrum. The hybrid system consists of UV-Vis spectrophotometer and neural package. The detection and classification processes depend on the readout of the UV-Vis spectroscopy to achieve the detection and recognition of all the samples by using Levenberg-Marquardt back propagation (LM-BP) neural network algorithm. The reported classification rate accuracy reached above 99.98%.

Keywords: pesticide, milk, artificial neural network, optical spectrum

Introduction

Consuming milk on a daily basis makes it one of the most important types of food, according to food and agriculture organizations. The importance of milk comes from its high nutrition value and effects on the economy of many countries. The consumption of dairy products exceeds 6 billion around the world. The milk composition included carbohydrates, protein, lipids, vitamins and minerals [as explained by 1]. As a result, milk accomplishes effective biochemical and nutritional functions for children and elderly people. Many contaminant materials such as pesticide residue can reach the bovine milk from different sources.

The effect of pesticide on human health it's appear after exposure to pesticide directly or indirect by consuming contaminate food such as milk that contain dangerous chemical materials [as depicted by 2]. After exposure to pesticide the human suffer from several symptoms like headache dizziness, vomiting, damage the nervous system and as results reach the death.

Pesticide use has a number of negative consequences for the ecosystem, including insect resistance, food chain disturbance and ecological imbalance. In addition, many effect on environments. Due to the negative environments and health implications of using agricultural pesticide, there is a need to reduce their use [as mentioned by 3].

Pesticide

According to pesticide pollution the pesticide identified as "chemical compounds or mixture of substances with diverse chemical nature and biological activity" they are intended specifically and produced for the purpose of preventing, killing obstructing, sterilizing or reducing any undesired life that had been defined as pest [according to 4]. Also defined as chemicals compound that are either synthetic or natural that had been used to prevent or reduce pests. Reduce the population of pest insects, rodents, fungi, weeds and all forms of aquatic plants animals, viruses, bacteria and microorganisms. Insecticides are classified into several groups such as herbicides which are toxins that are used to suppress or reduce weeds and other unwanted plants. Insecticides can be used to suppress a wide range of insects.

Disinfectants are used to prevent the spread of bacteria and to control mice and rats with

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rodenticides. Classification of methods into groups was as Pyrethroid pesticide, Organophosphate pesticide, Organochlorine pesticide [according to 5, 6, and]. There are many ways to detect contaminants. Several methods have been developed for the detection of pesticide residues, each with its own set of advantages and disadvantages. Such methods as separation methods such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) are the common detection methods. Pesticides can be detected and quantified using a variety of practical methods and procedures according to the EPA such as ion chromatography, atomic absorption, wet chemistry, and ultraviolet spectrometry [as comes in 4, and 8]. Investigating the presence of organic pollutants from environmental samples can be very costly. For example, the cost of analysis glyphosate exceeds 150$ for water sample and 300$ for soil sample. This high cost can always have a significant impact on how monitoring. Studies are designed, particularly in the universities and agriculture sectors [as explained by 5]. Much analytical technique for pesticide detection and quantification had been developed in recent years and also alternative methods had been suggested to reduce the cost of detection. All of the separation methods are costly, necessitate long separation periods, and often necessitate the construction of a high complex gradient for separation. These pesticide need to be determine quickly and cheaply by depending on different spectrophotometric methods [6].

The aim of this study was to propose simple, cheap, reliable, high accurate method to detect pesticide residue. The propose method for detect and estimate the quantities of residual pesticide in the food and dairy product by depending on optical properties of the sample.

Optical properties of milk

The composition of milk is as water 87%, fat 3.7, lactose 4.8, protein 3.4, and other components represent 0.7% like acid citric and minerals. The interaction of light –milk depends on the relation between the size of the suspended particles and the used wavelengths of the incident light. Since the lipids particles have size from 0.1 up to 10 μm in diameter and the casein protein is between 50 – 680 nm in diameter [as mentioned by both 3, and 7]. The light milk interaction governs by Lorenz-Mie theory. The molecules in principle are considered to be spherical particles and the wavelength of incident light to be less than the scale of the molecules. This relationship according to the range 0.1 $\lambda < P < \lambda$ where P represent particles size and λ: used wavelength of incident light. The scattered light from milk particles provide information about milk composition and can be used to evaluate milk content. it's necessary to note that majority of the scattering light is traveling in the forward direction as shown in shown in Figure 1.

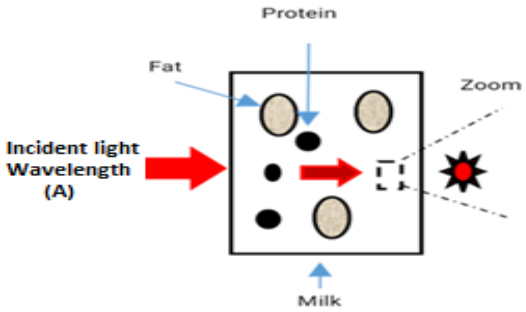

Figure 1: Milk composition (fat, and protein)

The test of the milk sample was carried out at 37 °C. It should be noted that the milk becomes turbid according to concentration and diameter of milk compositions. The scatted light signals of test sample given by [8 and 9]:

$I_s = I_o e^{-\mu_s d}$--------- (1)

Where: I_o intensity of incident light, I_s the scattering coefficient of the mike, d milk container length (interaction path). However the protein and fat scatter should be recognize and taken into account, the scattering transmission ratio (I_{STR}) can then be represent as:

$I_{STR} = \frac{1}{m} e^{-((\mu_s f + \mu_s P) - 1)}$ --------- (2)

This ratio depend on a uniform scatter coefficient (m), μ_s, μ_s represent scatter coefficient of fat and protein, it is important to note that the scatter factor is based on the ratio of incident light cross-section to the output light. The uniform scatter coefficient m is added in order to understand the laser coherent light or no coherent light beam properties and the structure of the studied particles the coefficient govern by cross section area of light source S_0 and particles surface S_p.

In milk sample the components may be known as spherical elements then m=Sp/So. In raw milk it's a struggle to estimates the refractive index (RI) because the fat globule dispersion, temperature and wavelength, and the refractive index of the raw milk at 20 °C was ranged from 1.344 to 1.3485.

Several researches depend on analysis of refractive index to measure the fat and protein concentration.

Cauchy's formula is given below and it is the best model for refractive index

$n(\lambda) = I + \frac{J}{\lambda^2} + \frac{k}{\lambda^4}$ ------- (3)

When the light signal incident on milk sample the absorbed light as clarify previously presented as the following:

$A = log_{10} \frac{I_o}{I} = d * \epsilon * C$ ---------- (4)

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The results of multiply thickness d by molar absorptivity and concentration C give by determine the logarithm response of the ratio between incident intensity I_o and transmitted intensity I. The milk properties can be determined according to absorption spectra where this spectral represent a fingerprint of the materials.

Artificial neural network

ANN refers for artificial neural networks, which are used to model nerve-cell networks in the biological central nervous system. Artificial neurons are created by using ANNs to build machines that act in a similar manner to biological neurons. When given an input pattern, an ANN's job is to generate an output pattern. An artificial neural network was created with the aim of providing a numerical simulation of brain functions. It is now used in a variety of fields, from technical practice to classification problems [as explained by 10]

Materials and Methods

Apparatus

The spectral absorption measurements performed by using Ultraviolet visible radiation UV-Vis UV 1800 SHIMADZU spectrophotometer (japan) with scanning resolution 1 nm compact double source beam instrument with wavelength range (190 to 1100) nm , control through PC with UV probe software using a glass cell with an optical path length of 1 cm and pH meter.

Procedure

Measured a pesticide residues in six samples of cow's milk, The samples obtained from different farms in Hilla city. In this work many samples of raw milk were tested to measure pesticide residue. The presence of pesticide had been evaluated. The research was carried out with the use of spectrum photometer 1800 UV-Vis.

Extraction Method

The pesticide extracted from milk sample according to [11, 12, and 13] .Take 10 ml of milk and put it into a 50 ml centrifuge tube with 3 replications. And add 15 ml of Acetonitrile, containing 1% acetic acid, homogenized the sample by shaking for a minute, and add 6 grams of anhydrous magnesium sulfate $MgSO_4$ and 1.5 grams of sodium acetate and shake for one minute; Samples are placed in a centrifuge at 5000 / rpm for one minute. We got two phases of the organic phase at the top and the aqueous phase at the bottom. Transfer 2 ml of the organic phase to a 4 ml tube containing 100 mg of C18 and 100 mg of PSA Primary Secondary (Amin) and 300 mg of anhydrous MgSo4. For purification, Shake the vial by hand for 30 seconds, then centrifuge for 1 minute / cycle / minute, transferring the extract to a vial, and it is learned and saved for pending analysis.

Standard solutions

Preparation the standard solution for bifenthrin by diluted it by distiller water at different concentration (0.01, 0.02, 0.05, 0.08, 0.1 mg/l) , and also standard stock solution of glyphosate (1mg./l) was prepared by dissolving 0.1 g of the analytical grade standard of glyphosate in 20mL distilled water and diluted to 100mL. The absorption spectrum of different solutions was obtained (collected) by using UV 1800 SHIMADZU.

Results and Discussion

Bifenthrin Spectrum

The graph of standard absorption spectrum curve was based on standard solutions of bifenthrin shown in

figure 2 for spectral range from 190 – 1100 nm where the x-axis represent the wavelengths of the used light while the y axis indicate to absorbance. Moreover, the formula for bifenthrin is equal to concentrations range ((0.01, 0.02, 0.05, 0.08 and 0.1)) mg/l of curve. As can be observed the absorption peaks is high at 288 nm and the peak decrease from (300 - 370) nm and also at decrease the concentration the absorbance also decrease

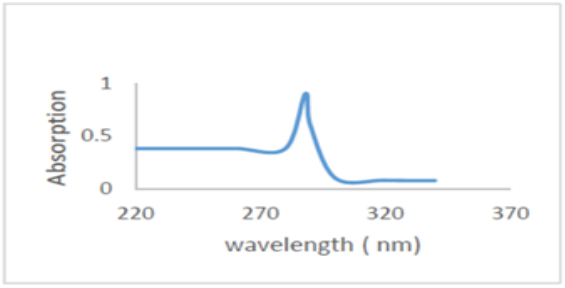

Figure 2: Spectrum of standard solution bifenthrin

Milk Spectrum

Figure 3 Show the absorption spectrum of raw milk sample, in Figure 3 the spectral range from 200 – 800 nm where the x-axis represent the wavelengths of the used light while the y axis indicate to absorbance of milk samples

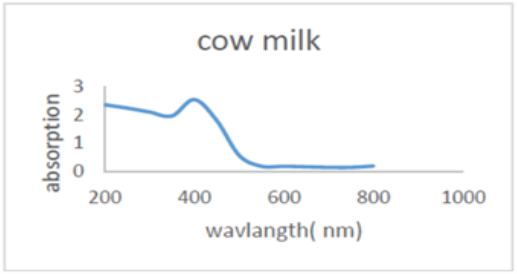

Figure 3: spectrum of cow milk

Bifenthrin cause a change about absorption spectrum of standard milk. The absorption peaks was shift approach (2) nm from 288 nm to 290 nm and the absorption was increased from 1.4 to 3.66 .This because a chemical reaction occur between bifenthrin and milk compositions which cause the absorption peak move to short or long wavelength due to unshared electron pair groups coming from the chemical reaction , the peak absorption of the mixture (milk with pesticide (Bifenthrin)) occurs at 290 nm according equation (3). The rule was applied and the linearity graph is shown in figure 3 within (0.01, 0.02, 0.05, 0.08 and 0.1) mg/l.

Under operating conditions, a linear relationship was discovered between absorbance at ʎ maximum (290) and bifenthrin concentrations in the ranges of 0.01-0.1 mg/l, respectively. The equation describes the calibration graph.

$A = mx + b$: Where A absorption, m, the slop of linear line, b intercept with y axis the absorption. As shown in figures 4, 5 and 6. Obtained using the least squares form

The detection limit of detection (LOD) and the quantification limit (LOQ) calculated as:

$LOD = \frac{3.3\,\sigma}{S}$, $LOQ = \frac{10\,\sigma}{S}$, S , slop of calibration curve , S is a standard deviation .

$where : \sigma = 0.0342$

$LOD = \frac{3.3*0.0342}{5.62} = 0.002$ mg/ml

$LOQ = \frac{10*0.0342}{5.62} = 0.06\ mg/ml$

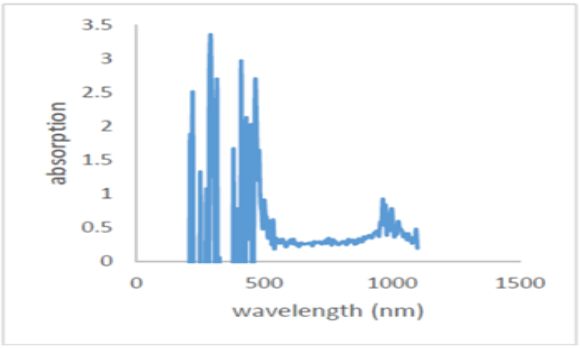

Figure 4: Spectrum of bifenthrin with milk

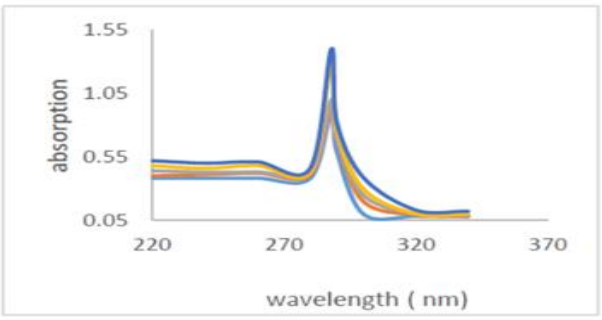

Figure 5: Spectrum of bifenthrin with milk at different concentrations

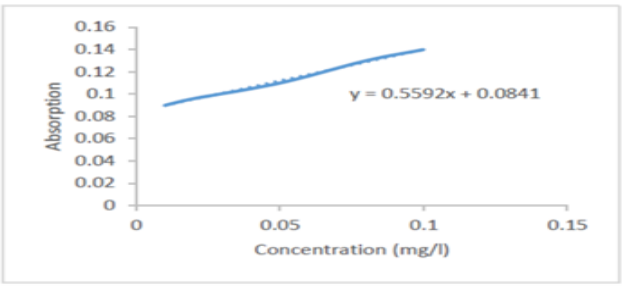

Figure 6: linearity graph of bifenthrin at different concentrations

Glyphosate spectrum

Figure 7 Shows the absorption spectrum of standard glyphosate solution for spectral range from 230 – 290 nm the spectrum clarify the peak absorption of standard glyphosate at 265 nm concentration (0.001) mg/l.

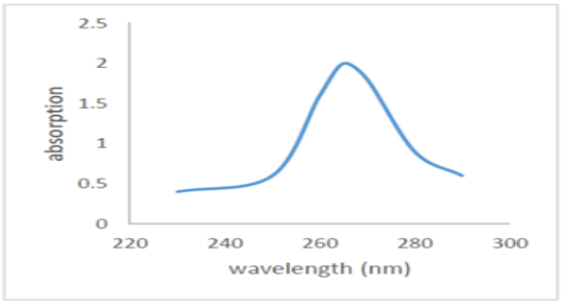

Figure 7: absorption spectrum of standard glyphosate solution

Glyphosate with milk spectrum

Figure 8 shows the absorption spectrum for glyphosate reaction with milk the results of absorption spectrum presented in figure 7 at wavelength range from 190 – 1100 nm with maximum absorption at 275 nm all the measurements at 25 c the 10 nm wavelength shift occur due to glyphosate residue in milk.

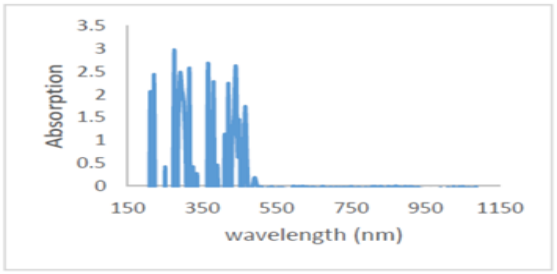

Figure 8: spectrum of glyphosate with milk

Artificial neural network

Pattern recognition will be performed using the output absorbance vectors from all spectrophotometer measurements, which was arranged as input vectors in a matrix form of artificial neural networks. Another vector called target vector, as shown in figures 2 to 8, denotes the classes to which the input vectors are assigned in order to detect pesticide residue in the milk sample. The classification problem strongly affects the number of neurons in the hidden layer. The number of attributes determines the number of neurons in the input layer, while class attributes determine the number of neurons in the output layer. According to the flowchart in figure 9, and table 1, a forward-feed neural network with 20 neurons at the input layer, three hidden layers comprising 8, 10, and 4 neurons, and one neuron at the output layer was simulated. The sigmoid function was used in the hidden layers, where the output neuron's response is linear, and the LM BP algorithm was used to simulate the neuron network to train the network. The training data was taken from spectroscopic data read out using UV-Vis spectroscopy.

The performance of the recognized materials using ANNs trained using LM-BP algorithms was a classification decision as shown in table (1).

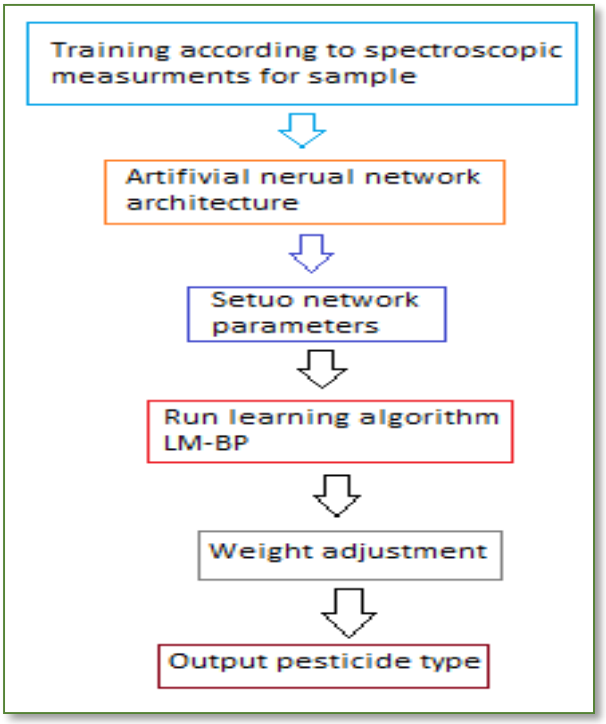

Figure 9: proposal flowchart

Figures 2-8 shows the absorption spectra of milk sample with pesticide at various pesticides concentrations, i.e. 0.01 mg/L, 0.02 mg/L, 0.05 mg/L, 0.1 mg/l. It is clear from the observed absorption spectra that the increment of pesticide concentration leads to the increasing absorbance of the milk sample according to Beer's law where the absorbance and concentration are linearly correlated. Figures 2-8 also shows the measurements of optical spectra for milk sample with pesticides. The peak absorption occurs at wavelength (275-290) nm for bifenthrin glyphosate respectively. The measured absorption spectra of pesticide change are reversible and may be repeated several times. All the reported spectral data was used as a finger print to detect and identify the pesticide residue in the milk by using artificial neural network.

Figure 5 shows the variation of pesticide residue concentration with the absorbance of the milk sample at wavelength 290 nm which revealed that the good linear curve is obtained with an R2 of 0.973 between pesticide (Bifenthrin) concentration and absorbance of milk sample. The fitting equation for the linear curve is $y = 5.6259x + 0.8195$ indicating good fitting of the system response

Table1: Detection and Recognition decision based on absorption spectrum and ANNs

Materials	1	2	3	4	5
Glyphosate	1				
Bifenthrin		1			
Cypermethrin			1		
Malathen [10]				1	
Parathion [10]					1

Conclusions

Low cost easy-to-use method has been developed to detect and measure pesticide residue in raw milk. The method based on spectrophotometric data, the main advantage it's quick, accurate, sensitive, and time-saving and does not require many solvents, the proposed method depend on spectroscopy data and artificial neural network. The method's lower detection limit is about 0.002 mg/l pesticide residues have been determined in milk sample using the proposed method and the results indicate that the residue of bifenthrin in milk within MRL value (200 μg). The developed method can be used in variety of samples such as water, vegetables.

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