Pesticides Free Spectrophotometric Filters Creation For Pesticides Quantification in Various Vegetables

Umberto Cerasani
CERAGOS Electronics and Nature
Sophia Antipolis, France
Email: umbertocera@gmail.com

Safa Boudrai
CERAGOS Electronics and Nature
Sophia Antipolis, France
Email: safa.boudrai@gmail.com

Ennio Cerasani
CERAGOS Electronics and Nature
Sophia Antipolis, France
Email: cerasani@ceragos.org

Oumy Diop
Antibes, France,
Email : diop.oumydiop@gmail.com

***Abstract*— A method for estimation of food contaminants, including pesticides, is further described in this document. First, Visible Near InfraRed spectrometric measures were performed on various food samples with different levels of pollution, including pesticides free foods. Methodology and data extraction were meticulously addressed. Based on pesticides free food spectral data, pesticides free filters were created. These filters permitted pesticides isolation without the need of complex chemical procedures from foods natural occurring compounds. The removal of the natural occurring compounds in foods from their artificial counterparts was the initial step, before applying precise pesticides estimation procedures to the remaining spectral data. To accurately quantify foods pesticides contamination, two methods were developed: (1) standard Beer-lambert's concentration law and (2) linear regression. The pesticides contamination evidenced by these two methods were very closed, with less than 3µg/mL of difference. Finally, implementation of these quantification methods in an embedded device, communicating with smartphone application is discussed allowing users to monitor food contaminants.**

Keywords—pesticides; Vis NIR spectroscopy; Beer-Lambert's law; Linear Regression Analysis; Microspectrometer

I. INTRODUCTION

Pesticides are widely used in agriculture to protect crops and seeds and may have contributed to improvement in society health and economy [1]. At the same time, widespread use of pesticides has led to serious harm on the environment and human health [2] [3] [4]. With the increasing demand for a high quality agricultural products, new quality and safety control devices are being investigated. Because pesticides damaging effects are invisible and cannot be directly warned by visible observation or simple testing, pesticides estimation in soil or in foods prior to their consumption, requires complex techniques and remains very challenging [5-7].

A number of analytical methods, including mass spectroscopy gas and liquid chromatography gas chromatography–mass spectrometry (GC-MS), have been reported to detect various pesticides contamination in foods and these methods are very sensitive and reliable [5]. However, these classical analytical approaches are usually confined to a laboratory environment and require costly, long sample preparation time, solvent wasting, and hazardous samples contact [5, 8, 9]. Additional disadvantages of these methods include the restricted database mapping of pesticides analysis and the possibility of false negatives in the results [10].

Biosensors provides a promising alternative for the detection of pesticides [11-15]. Biosensors convert the signal produced by the immobilized biological element that detect the analyte into an electrically detectable signal and can be classified from their signal transduction techniques into electrochemical, optical, piezoelectric and mechanical biosensors [16]. Many biosensors designed for pesticides detection are based on the inhibition reaction or catalytic activity of several enzymes after pesticides contact [17]. Electrochemical transducers are usually simple to design, small and affordable making them candidate of choices for portable pesticides detection [16]. For instance, Enzyme-Linked ImmunoSorbent Assays (ELISA) have grown rapidly as tools for pesticide measurement, although still challenged [17]. Since a number of pesticides have a similar mode of action affecting the activity of the same enzyme, most of ELISA based biosensors suffer poor individual pesticide specificity although improper to detect total pesticide content. On the other hand, immunosensors are biosensors that senses specific pesticides using antibodies (Ab) or antigens (Ag) taking advantage of the newest development of Ab technologies, targeted against pesticide molecules. Immunosensors are therefore able to provide concentration-dependent results in a certain range [17, 18]. For instance in [19], Triazines were assayed using florescent antibodies (conjugated with with fluoresceine isothiocyanate binding to the fibre surface). After contact with the pesticide Triazines the fluorescence signal decreased since less antibodies were binding to the fibre. The detection limit using this immunosensor was very satisfying (around 0.1 ng/ml) [19]. However, there is a time gap between current status in the field and the most recent created immunosensors [17]. In addition, immunosensors usually requires specific testing procedures and have low reusability capacity without loss of sensitivity for most of them [20-22].

Due to the great amount of pesticides currently being used, there is an augmented concern in the investigation and creation of rapid and non-destructive methods for pesticides detection [23]. In the last few years, advanced in optical instruments allowed the residuals of insecticides detection from agricultural samples [10]. Detection of hydrophobic organic pollutants via UV, Raman, and IR spectroscopic methods directly at solid sorbent phases are usually reported to be less sensitive than conventional chromatographic analysis, but permit on-site pollution measurements [9].

Near Infrared spectroscopy (NIR) is a well described method to assess the composition and quality of products in the food industry [24-28], since it has the capability to analyze organic substances rapidly and cost-effectively, although suffering of low spectral resolution for samples in aqueous solutions due to strong water infrared absorption. As an example, a model for the quality control of herbicide Diuron in intact olives with 85.9% of accuracy using reflectance NIR spectroscopy is presented in [29]. Peppers are a frequent object of food safety alerts in various member states of the European Union since they frequently contain unauthorised pesticide residues. Near Infrared Reflectance Spectroscopy (NIRS) for the measurement of pesticide residues in peppers using commercially available spectrophotometers and demonstrating satisfying results is proposed in [30]. Spectral information in the ranges 1644–1772 and 2014–2607 nm without baseline correction and Partial Least Squares (PLS) model interpretation were used to detect Buprofezin, Diuron and Daminozide without any sample pre-treatment and sample destruction in [31].

Diuron determination in pesticide formulations was also analyzed after its extraction with acetonitrile and subsequent transmittance NIR measurements (2021 and 2047 nm). Diuron limit of detection reached 0.013 $mg.g^{-1}$ with this methodology which was 10 times higher than that the results obtained by Liquid Chromatography (LC), making NIR vibrational method appropriate for the quality control of pesticide commercial formulations [32]. Fourier transform near infrared (FT-NIR) spectroscopy is also of value for the determination of pesticides in agrochemicals. Following previous extraction of the active principles and transmission measurements were performed on Chlorsulfuron, Metamitron, Iprodione, Pirimicarb, Procymidone and Tricyclazole, leading to detection values limits ranging from 0.004 to 0.17 $mg.g^{-1}$, 10 times faster than chromatography analysis [33]. Chlopyrifos residue detection in white radish, based on NIR spectroscopy and PLS regression is proposed in [34]. PLS was mainly permitted the determination of the optimum wave number range.

Field portable NIR spectrometer (from 360 to 1690 nm) was able to determine nutrient composition of beef feedlot manure in [35]. On the basis of analysis of dried mannure samples, the field-portable NIR spectrometer allowed fast determination of Carbon, Nitrates, and several other parameters [35]. Field portable pesticides detectors, based on NIR spectrometry could be therefore considered for pesticides detection using a similar procedure.

Infrared (IR) spectroscopy provides a rapid, low cost and highly reproducible diagnostic screening tool. IR spectroscopy is currently employed for soil surveillance systems, crops health and water quality assessment [36]. For instance, soil absorption over the Visible/Near-InfraRed (Vis/NIR) wavelength regions (350–2500 nm) is mostly associated with (1) the vibrational energy transitions of the dominant molecular bonds of Fe-oxides (which have absorptions over the visible (350–780 nm) and short-wave NIR (780–1100 nm) spectral regions), (2) clay minerals (which have absorption over the long-wave NIR (1100–2500 nm) regions), (3) water (which has strong absorption features over Vis/NIR regions, most visibly near 1400 and 1900 nm), and (4) organic matter (which has distinct absorption features over the Vis/NIR, due to the various complex chemical bonds) [37]. Under certain conditions, such as very high concentration in soil, some transition elements (including Ni, Cu, Co) may also exhibit absorption features in the Vis/NIR spectral regions [37], permitting direct soil characterization and estimation of pesticides utilization in fields. A Vis/NIR mobile soil sensor was developed in [38] composed by optical unit to detect soil extractable phosphorous (305 and 1711 nm in reflectance mode).

Vis/NIR spectroscopy also permits pesticides and other food contaminants detection [37, 39]. It may be particularly suited for free space measurement and field studies [40]. Internal and external pesticides damage detection of various fruits in Korea and Japan were detected using non damaging methods, such as Vis/NIR Spectroscopy [41].

Many aromatic pesticides are either naturally fluorescent or photodegrade into fluorescent byproducts and are hence suited for fluorescent spectroscopy detection [42]. For example, Polycyclic Aromatic Hydrocarbons (PAHs), pesticides are naturally fluorescent in aqueous solutions and allows for trace elements detection without previous pesticides concentration procedures [42]. Portable fluorometers are now available on market in a single portative device and with fiber-optic probes that permit remote observations [42].

Progress in Raman spectroscopes and in embedded computation equipment have enabled Raman spectroscopy to be used as an analytical tool for both solid samples and aqueous solutions, offering information permitting to determine the internal content in samples [5]. Because Raman spectrum of compound can furnish narrow and highly resolved bands it contains more complete vibrational information than IR spectrum. It may not require stabilizing materials and needs no chemical or mechanical pretreatment and allows nondestructive extraction of physical information [5, 10].

Raman spectroscopic techniques mostly gathers dispersive Raman spectroscopy, Fourier transform Raman spectroscopy and Surface Enhanced Raman Spectroscopy (SERS) [5]. Because conventional Raman spectroscopy is limited to a small scattering cross section and requires large amount of specimen and strong incident light, the employment of SERS greatly enhances the sensitivity of the conventional Raman spectroscopy and offers more elevated measurement speed and sensitivity [10].

In food industry, spectroscopy has been satisfactorily used to monitor food quality and safety. For instance Raman spectroscopy was applied to discriminate between transgenic and normal crops in various breeding such as tobacco. Different Raman spectrum were obtained between the transgenic tobacco and the wild type, since for transgenic tobacco, the expression of cinnamyl alcohol dehydrogenase was greatly depleted after cinnamaldehyde lignin incorporation [5]. In addition, Raman spectroscopy was successfully used to distinguish the Brassica napus ‘Drakkar’ from the new genetically modified line [5].

Furthermore, the use of Raman spectroscopy permitted carotenoids content quantification in fruits and vegetables [5]. Other antioxidant quantification such as lycopene could be obtained by NIR-FT-Raman spectroscopy method [5]. FT-Raman spectroscopy method in conjunction with Hierarchical Cluster Analysis (HCA) may accurately assess the energetic value and total carbohydrates, protein, and fat of powdered milk infant formulas [5].

Raman spectroscopic techniques are not only applied in quality control but also in safety control of various beverages, most specifically for microorganisms contamination and adulterants adjunction [43-48]. The identification of oil adulteration is of great importance from both market and health perspectives in the olive oil industry [49-55]. Unsaturation of oil Free Fatty Acids (FFA) and total degree of unsaturation could be estimated using spectroscopy measures [5]. Since Raman spectroscopy has been successfully applied to organic compounds detection in food and beverage, its employment for pesticides detection is particularly adequate. Most particularly identification and detection of large family of sulfur-containing pesticide residues at various fruit peels was performed utilizing the shell thickness-dependent Raman enhancement of silver-coated gold nanoparticles [56].

Transmittance spectroscopy is particularly suited for free space measurements without sample preparation and can be applied to pesticides detection. For example, using a transmittance spectroscopy (in the 550 and 980 nm region), insect infested cherries within a tart cherry fruit were detected with accuracy varying from 82% to 87% [57].

Data clustering analysis following spectroscopy data is often required to distinguish food contaminants. Spectroscopy such as SERS coupled with clustering analysis has been shown to enable the trace-level detection of various pesticides [58]. For instance, cluster analysis, following Wavelength Dispersive X Rays Fluorescence Spectroscopy may permit classification of black tea and green tea from tea mineral elements [59]. Clustering algorithms often requires variable fitting or selection methods. Most commonly employed methods are: Stepwise Regression Analysis, Uninformative Variable Elimination, Interval Partial Least Squares (IPLS) regression, Clonal Selection Feature Selection algorithm [23]. As an example improving PLS regression models, used in spectrum data post-processing, may hence result in more specific database inquiries and sample chemical identification.

In this paper, we quantified food pesticides contamination using Vis-NIR reflectance spectroscopy. Since there are several thousand of different active pesticide molecules reported [60, 61], current pesticides detection is limited to few types of pesticides chemical and does not ascertain pesticides free products. We worked from another perspective by comparing the spectral information obtained from pesticides free and pesticides contaminated foods, irrespective of the pesticide types. We searched for particular traces in the spectroscopy spectrum that could be characteristic of pesticides contamination. Mostly two cases could be found: (1) the spectra of foods grown with pesticides contains additional traces which could be related with pesticides own spectral characteristics or modified endogenous food proteins [62-64], (2) oppositely the absence of spectral components in particular wavelength in pesticides contaminated foods compared to organic or totally natural foods (grown without pesticides addition) may also be used as a spectral indicator of food purity since certain food proteins may not be expressed when pesticides are utilized [65-68].

The measured pesticides free foods spectral data were used as filters to isolate the additional non-natural constituents of foods. Pesticides contamination quantification was hence performed on these additional non-natural food components spectral data, avoiding the need of pesticide chemical isolation from the food natural components to increase measures reliability. Transmission spectroscopy was performed for 3 pesticides commonly employed in agriculture: (1) Dicofol, (2) Thiamethoxam and (3) Malathion and their respective absorption spectral data were computed. Since the concentration of the tested pesticides was known, peaks in absorption spectral data permitted to calculate the Dicofol, Thiamethoxam and Malathion absorption coefficients at 791 nm, 774 nm and 475 nm respectively. Pesticides estimation was realized for different foods category from different suppliers, including a market gardener claiming to produce pesticides free foods, an organic food producer and two supermarkets. Pesticides contamination for each food was computed using: (1) the standard concentration law applied at pesticides characteristic wavelength and (2) linear regression model solved with Non Negative Classical Least Squares. Very closed pesticides levels were obtained by both methods, although there were applied to foods of different category and different suppliers.

Finally, algorithm implementation inside a portative device with an embedded spectrometer, as depicted in Figure 1, allows pesticide estimation for non-specialized users. For easier user data reading, a wireless communication device (Bluetooth module) is integrated in the embedded system for user's smartphone communication. A specific smartphone application was created for embedded spectrometer results monitoring. To diminish the costs of the overall device, sunlight is used as spectroscopic light source, limiting device utilization in bright areas.

Our document will be organized into 3 main sections: first, the methodology of our experiment will be introduced with the devices used, then, the Vis-NIR spectroscopy results are reported and analyzed, finally a conclusion is drawn with possible future work direction propositions.

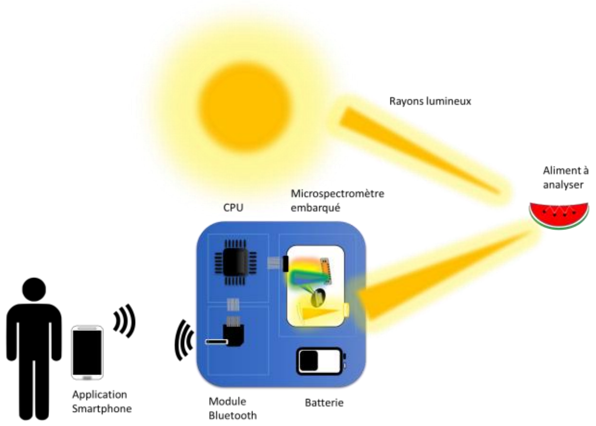

Figure 1. User centric based device for food pesticides monitoring

II. METHODS

Measurements detailed information and equipment used are presented in this Section.

A. *Food samples variety*

We tested 3 different food categories: tomatoes, zucchini and potatoes. The precise variety of vegetable tested is reported in Table I. Food were bought directly from (1) a market gardener that did not used any pesticides or chemical contaminant, (2) an organic store and from 2 famous French supermarket brands, where we assumed that plants were grown using pesticides ((3) and (4)). Although several pesticides are contained in the vegetable peel, we decided to perform the measure on the decorticated vegetable to avoid peel color bias. Each vegetable was tested in 3 different areas.

TABLE I. VEGETABLE TYPES AND VARIETIES USED FOR THE EXERIMENT

Food Variety (botanical name)	**Food suppliers**			
	Market gardener (pesticides free)	**Organic shop**	**Supermarket 1**	**Supermarket 2**
Tomatoes	*Lycopersicon escubentum*	*Lycopersicon escubentum, Solanum lycopersicum*	*Lycopersicon escubentum* *Lycopersicon esculentum*	*Lycopersicon escubentum* *Lycopersicon esculentum*
Zucchini	*Cucurbita moschata, Cucurbita pepo 'De Nice à fruit ronds´*	*Cucurbita pepo ´Verte non coureuse´*	*Cucurbita pepo ´Verte non coureuse´*	*Cucurbita pepo ´Verte non coureuse´*
Poataoes	*Solanum tuberosum ´Charlotte´ (yellow), Solanum tuberosum ´Desiree´ (red),*	*Solanum tuberosum ´Charlotte´ (yellow)*	*'Cherie'*	*'Cherie'*

B. *Test system description:*

Reflectance spectroscopy measures were performed using the test bench described in Figure 2. We used the Ocean Optics kit including the ECOVIS Krypton Lightsource, the USB-650 Red Tide Spectrometer (preconfigured in the 350-1000 nm wavelength range), a 200 µm Bifurcated Fiber (Vis-NIR) and the OceanView software. The results were then extracted and manipulated with Matlab software. The distance between the bifurcated fiber and the sample to measure was fixed to 5mm, and the reflectance robe was positioned at an angle of 90° to the flat surface of the sample to analyze.

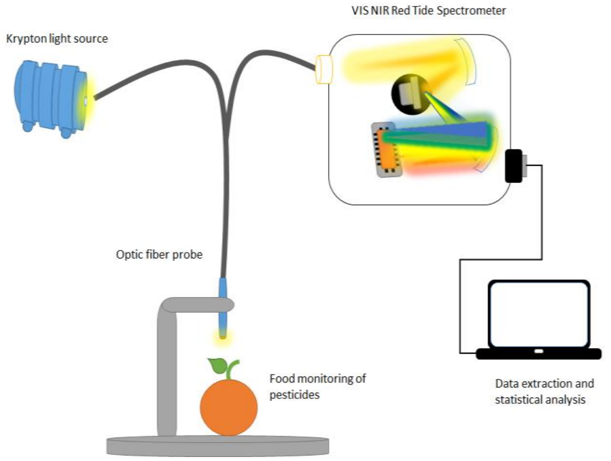

Figure 2. Test bench diagram used for Vis-NIR spectroscopy measurements in various vegetables

C. Absorption spectrum determination

Fresnel's equations are valid in non-near field spectrophotometric analysis and describe the light behavior when moving between media of differing refractive indices. We use reflective Vis-NIR spectroscopy to analyze opaque solutions (foods), which did not necessitate particular sample preparation. On the other hand, transmission spectroscopy was selected to study transparent media of the pesticides Dicofol, Thiamethoxam and Malathion. The three pesticides studied, which are described in Table II, were added to a cuvette of 1 square centimeter area, specifically designed for transmission spectroscopy.

TABLE II. ANALYZED PESTICIDES PHYSICAL CHARACTERISTICS

Pesticides	**Concentration (Mole/L)**	**Density (g/mL)**	**Molar mass (g/Mole)**
Dicofol	*0.75e-3*	*1.23*	*370*
Thiamethoxam	*0.36e-3*	*0.998*	*291*
Malathion	*1.9e-3*	*1.27*	*330*

From the food reflection spectra, the corresponding absorption spectra were immediately determined by noticing that the transmission light was inexistent since the foods were opaque at the considered Vis-NIR wavelengths range (400nm – 850nm).

Secondly the absorption spectra of the pesticides were directly deduced from the pesticides transmission spectra since there was no reflected light (light source was perpendicular to the cuvette containing the analyzed solutions).

To establish the foods absorption spectra and the pesticides absorption spectra, we used the ECOVIS Krypton Light source as the incident reference light and we further removed the light background and electronic device noises. Finally, the absorption spectra were shifted to avoid negative absorption values.

D. Beer Lambert's concentration law

Beer lambert's law (reported in eq. (1)) correlates the solution absorbance to the concentration of the solution and to the thickness of the material sample [69, 70]:

$$-\log_{10}(I_{transmitted}/I_{incident}) = \varepsilon * c * l \qquad (1)$$

where $I_{transmitted}$ is the transmitted light source spectrum, $I_{incident}$ is the incident light source spectrum, c the solution concentration, ε is the absorption coefficient and l is the length of the cuvette. Therefore, the concentration of a solution is directly proportional to the absorbance measurements obtained using a spectrophotometer [71]. Traditional solution concentration determination using Beer Lambert's law is restricted to a single wavelength, corresponding to the absorption spectrum peak. In fact the absorption coefficient (ε) is related to the complex index of refraction (n'') and to the "peak" wavelength (λ), as described in eq. (2) (rewritten from [72]):

$$\varepsilon = 4n''/\lambda \qquad (2)$$

Absorption spectra are additive meaning that absorption spectra of a mixture is the sum of absorption spectra of each mixture components, measured separately.

In many cases absorption calibration curve deviates from this ideal straight behavior. Deviations from linearity are divided into three categories: (1) fundamental which gather the deviations in absorptivity coefficients at high concentrations (>0.01 Moles) due to electrostatic interactions between molecules in close proximity and the changes in refractive index at high analyte concentration, (2) chemical, mainly due to shifts in chemical equilibria as a function of concentration, and (3) instrumental which originate from non-monochromatic radiation and light scattering [70, 73]. In consequence, spectrophotometric study of pesticides concentration using the linear Beer-lambert's law is restricted to a given concentration interval, above the minimum pesticides concentration detection limit.

E. Multiwavelength spectrophotometric analysis

The spectroscopic analysis of mixtures, when the spectra of the components overlap considerably, can be performed using multiple linear regression analysis, which can be solved by Least Squares methods [74]. First, measurement of absorption spectra of each mixture component at a particular concentration permit to determine the absorption coefficient sensitivity (φ) for all the considered Vis-NIR wavelengths interval.

Given the variable of interest c (scalar vector corresponding to the concentration of each component in the mixture), linear regression methods aim to model and estimate the relationship between a scalar dependent variable c and a vector of explanatory variable A (representing the absorption spectrum at the considered wavelength) through the linear relation described in eq. (3):

$$A_\lambda = \varphi_{n,\lambda} .\ c_n \qquad (3)$$

where n represent the number of different components in the mixture and λ the wavelength (also referred as regressors in linear regression models). The classical linear regression model with “standard assumptions”, can be solved using the Least Squares principle, which minimizes the sum-squared error in the reproduction of the values of A, using only the concentration vector c, over all j wavelengths, as expressed in eq. (4):

$$\sum_j (Error)^2 = \sum_j (A_j - \sum_i \varphi_{ij} * c_i)^2 \qquad (4)$$

In the special case of error functions, equating the first order derivative of the squared sum of errors to 0 with respect to the concentrations c_i, results in determination of the concentrations c_i which minimize the differences between the measured absorption spectrum and the reconstituted spectrum. Alternatively, the equations can be converted into matrix expressions, permitting to determine the concentrations c_i of each individual components of the mixture, as described in eq. (5) [74-76]:

$$c_n = (\varphi_{n,\lambda}{}^T * \varphi_{n,\lambda})^{-1} * \varphi_{n,\lambda}{}^T * A_\lambda \qquad (5)$$

For multiple component analysis, we preferred the use of non negative Least Squares, resulting in non negative concentrations c_i estimation of the components forming the analyzed mixture.

The use of multicomponent analysis was however restricted to experimentation purposes and not directly implemented in the created embedded spectrophotometer.

F. *Algorithms implementation*

For embedded device pesticides detection, the choice of the algorithm for pesticides detection was limited by several elements, mainly: (1) spectrophotometer accuracy, (2) the computational resources, (3) simplicity of implementation allowing machine portability, (4) real time computation requirements.

Given these strong restrictions, we decided to select monowavelength Beer-Lambert’s concentration determination, with predetermined absorption coefficients, for samples contamination quantification.

Figure 3 depicts the measurement procedure we followed to extract the absorption coefficient associated with each pesticide, from the measured transmission spectrum. The procedure for pesticides quantification in foods is indicated in Figure 4.

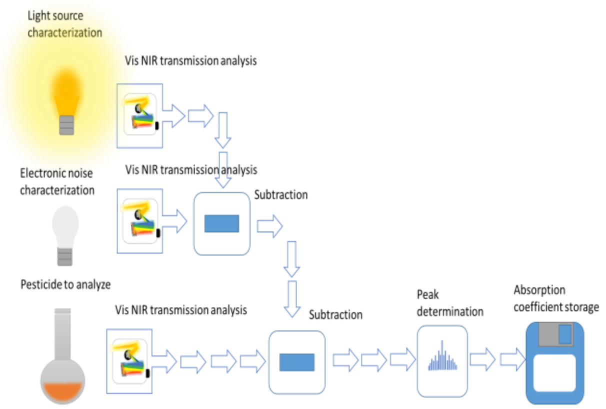

Figure 3. Pesticide absorption coefficient determination procedure

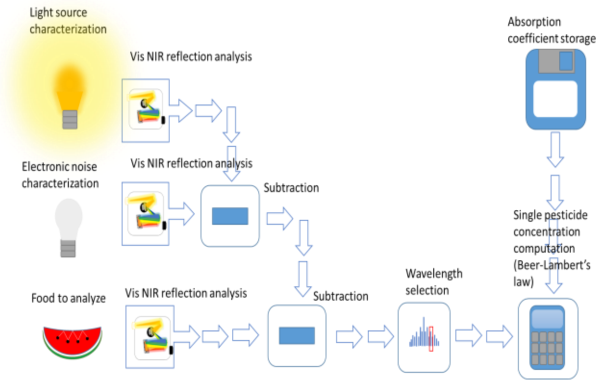

Figure 4. Food analysis procedure using the Vis-NIR reflected spectrum and the Beer-Lambert’s concentration law

III. RESULTS

A. *Additional spectral traces comparison*

Tomatoes spectroscopic measures from different supplier’s origin are presented in Figure 5, after division by their respective Area Under Curve, to remove the light intensity drift differences between samples. Each line represents the spectral measurements realized on tomatoes from a market gardener that did not used any pesticides or chemical contaminant (blue line), an organic store (green line) and from 2 famous French supermarket brands, where we assumed that plants were grown using pesticides (red and pink lines respectively). For each different tomatoes supplier, measures were performed on several vegetable samples to decrease the samples intervariability bias and the mean value was computed and reported on the figures. As an example, the green line in Figure 5 outlines the mean relative light intensity of organic tomatoes sample and the magenta lines in the same figure outlines the mean relative light intensity of the Supermarket 2 tomatoes sample.

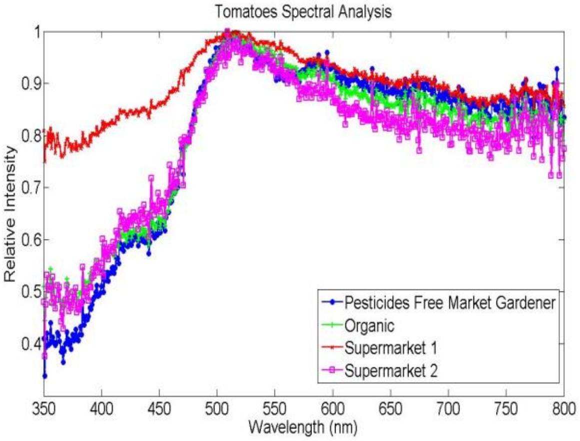

Figure 5. Tomatoes spectral analysis comparison (mean samples values).

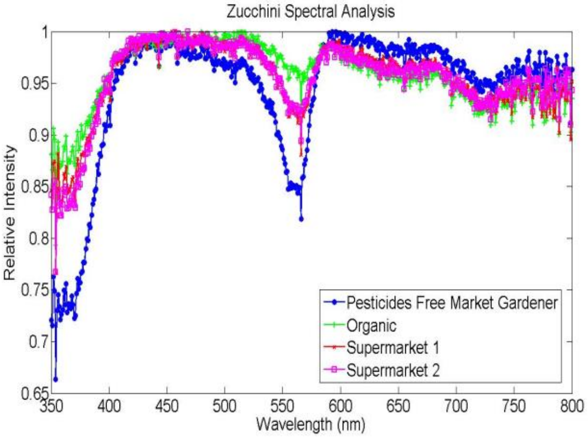

Figure 6. Zucchini spectral data comparison (mean samples values).

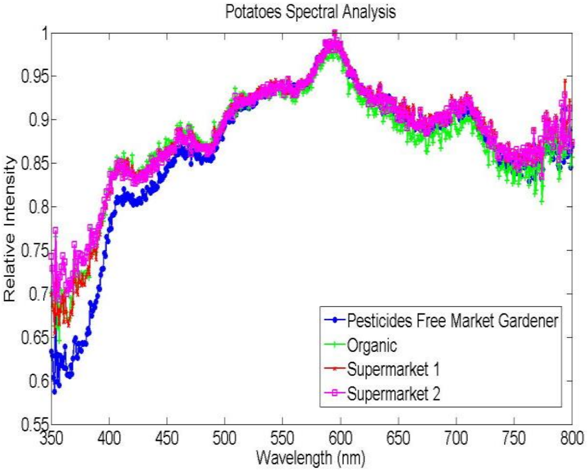

Figure 7. Potatoes spectral data comparison (mean samples values).

Figure 6 and Figure 7 depict the spectral measures associated with zucchini and potatoes from different food supplier's origin respectively. For easier data interpretation, light intensity drift was removed. The mean relative light intensity values of the pesticides free zucchini and potatoes are both reported in blue thick lines in each figure. Similarly, to the previous figure results, each different color line describes the spectral measurements associated with the 4 different origins of the food under study (pesticide free market gardener, organic store, supermarket 1 and supermarket 2).

B. *Spectral filters creation*

Chemical manipulations mostly precede spectrophotometric pesticides analysis to isolate the pesticide from its containing solution. Such manipulations often demand material and expertise. The spectrum of pesticides free foods was extracted for different food category. This spectrum when subtracted to the spectrum of foods grown with pesticides, split each pesticide grown food spectrum into (1) the natural components composing the food spectrum from (2) the extra artificial components of foods spectra (mostly non-naturals adulterants). Supplementary components of foods include airborne pollutants, pesticides, dust, etc. It was assumed that the main supplementary components, following foods mechanical cleansing are mainly the pesticides and conservatives.

Analyzing pesticides content on the spectrum additional non-natural constituents, remove the need of pesticides chemical extraction. The creation of pesticides free Vis-NIR filters is hence of capital importance in food pesticides estimation, provided that the filters created corresponds to the same food category and variety that the food under study.

Reflective spectroscopy spectral data from organic shop (green line), supermarket 1 (red line) and supermarket 2 (pink line) were subtracted to reflective spectral data of pesticides free food of similar variety to obtain the additional non natural components contained in the foods studied. These supplementary artificial compounds for tomatoes, zucchini and potatoes are respectively presented in Figure 8, Figure 9 and in Figure 10.

Figure 8 shows that spectral relative intensity in the 350 nm – 400 nm region is particularly characteristic of the tomatoes added chemicals. Other spectral rays (435 nm, 587 nm, 672 nm, 781nm, 791 nm, etc.) deserve extended analysis and database characterization.

Although characterization of food additives or pesticides contaminants for zucchini seems straightforward with two large peaks at 374 nm and 570 nm compared to pesticides free zucchini (Figure 9), the analysis may be more complex. In fact, as presented in Table I, the zucchini variety of the organic shop, supermarket 1 and supermarket 2 (*Cucurbita pepo 'Verte non coureuse'*) is not exactly the same as the one proposed by the pesticide free market gardener (*Cucurbita moschata* and *Cucurbita pepo 'De Nice à fruit ronds')*. Modification in chromophore molecules between these two varieties of zucchini may explain the large results variation. For more conclusive analysis, data obtained from organic zucchini should be used for spectral filter construction, although we further noted that in these wavelength areas, data obtained from organic stores also differ from the ones associated from the two supermarkets. Of particular interest seems to be the spectral rays of 715 nm, 774 nm, 450 - 456 nm area and need deeper investigation.

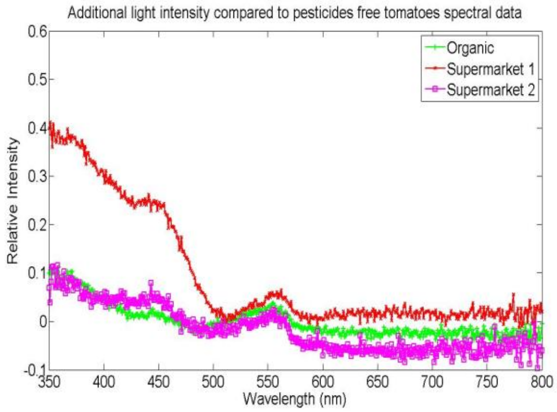

Figure 8. Additional relative light intensity compared to pesticides free tomatoes spectral data

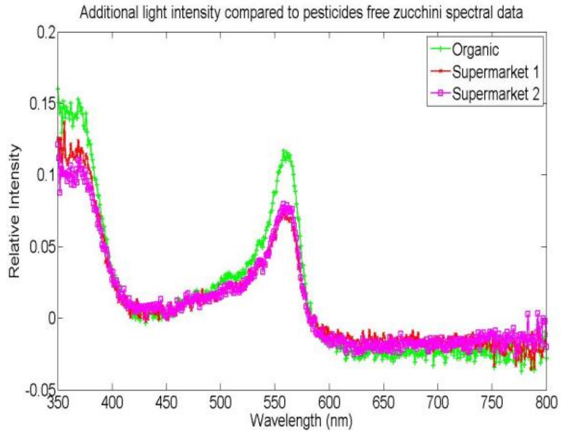

Figure 9. Additional relative light intensity compared to pesticides free zucchini spectral data

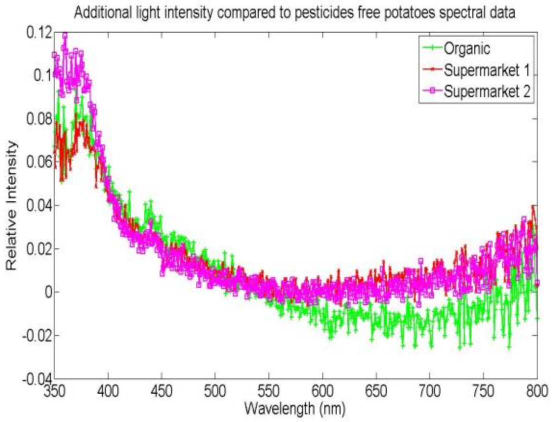

Figure 10. Additional relative light intensity compared to pesticides free potatoes spectral data

Filtration of spectral data from organic shop (green line), supermarket 1 (red line) and supermarket 2 (pink line) with spectral data of potatoes pesticides free mean value is described in Figure 10. Except in the 350 nm - 450 nm and the 600 – 750 nm regions, spectral data obtained from the two supermarkets (red and pink curves) seems to be closer to the pesticide free spectrum. Several explanations may exist. Like for zucchini, not the same potato varieties were compared, explaining the discrepancies between results. A second explanation is the presence of specific food additional substances, possibly pollutants, in organic food compared to supermarket ones (organic foods are not entirely devoid of pesticides, since pesticides from natural sources are allowed in organic gardening [77]).

C. Dicofol, Thiamothexame and Malathion pesticides spectrophotometric characterization

The three pesticides Dicofol, Thiamethoxam and Malathion were studied and since they were almost transparent, transmission measured were performed to determine their corresponding absorption spectrum, reported in

Figure **11**.

In [78], a spectrophotometric method for the determination of Malathion is proposed, involving the decomposition of Malathion in the presence of alcoholic KOH, further reacting with Ammonium meta vanadate in Nitric acid to form a blue color. The absorbance maximum of the Malathion solution was observed at 760 nm and it was shown that the Beer-Lambert's law was obeyed in the 0.5-11 μg/mL interval [78]. Another selective spectrophotometric method developed in [79] for the determination of Malathion required Gention Violet addition. First alkaline hydrolysis of Malathion in presence of sodium ethoxide formed sodium dimethyl dithiophosphate (Na-DMDTP), then the obtained solution was complexed with the cationic dye Gention Violet, before chloroform extraction. The color of the organic layer was measured at 587 nm and the concentration law was respected in the 0.1-10 µg/mL interval. Other authors proposed a cost-effective method for determination of Malathion using spectrophotometric measures with absorbance peak around 520 nm (Amaranth dye titration) [80]. Spectrophotometric organophosphates detection methods require mainly three steps: (1) the reaction of Malathion with an excess of oxidant in acid medium to specifically select Malathion component from the containing solution, followed by (2) the addition of a reacting dye and finally (3) the spectrophotometric measure of the reactive dye variation when added to Malathion, since the reacting dye has notable absorption peaks well characterized [80].

Similar procedures permitted the extraction of concentration's law for Dicofol, with an absorption peak in the UV range (232 nm) [81]. A spectrophotometric method for the determination of Dicofol is described in [82] and based on the Fujiwara reaction (Alkaline hydrolysis of Dicofol, reaction with pyridine to produce red color, addition of glacial acetic acid and final reaction with 4-aminoacetanilide to give an orange-red dye, further extracted in 3-methyl-1-butanol). The extracted dye shows absorption maximum at 525 nm. Beer's law was obeyed in the range of 0.025–0.25 µg mL^{-1} using this method as discussed in [82].

Statistical analysis using UV spectroscopy showed that there was a significant linear relationship between the concentration of Thiamethoxam in tea and the absorbance at 250 nm in the UV spectra of the mixture [83].

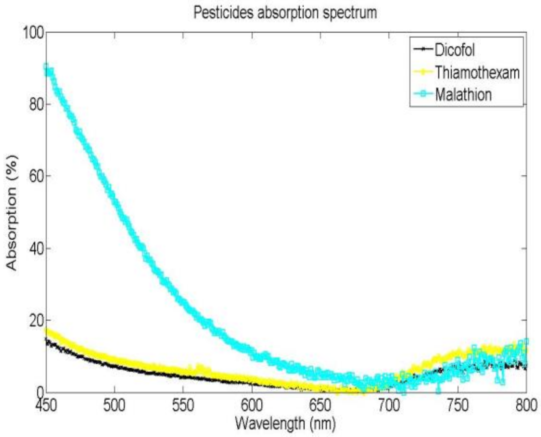

Figure 11. Vis NIR absorption spectra of the Dicofol (concentration: 0.75e-3 Mole/L), Thiamethoxam (concentration: 0.36e-3 Mole/L) and Malathion (concentration: 1.9e-3 Mole/L).

Without the use of the coloring dye and based only on the Vis-NIR absorption spectra measured (reported in Figure **11**), each pesticide we studied most probably has absorption peaks in the UV and IR regions. We retained the 475 nm (Blue region), 774 nm (NIR region) and 791nm (NIR region) as Malathion, Thiamethoxam and Dicofol respective absorption peaks, further used to determine absorption coefficients for pesticides concentration estimation. Because the absorption peaks were possibly non optimal absorption peaks, the concentration range for pesticide detection was reduced compared to other literature studies [70]. Avoiding using the wavelengths associated with the colors green (approximatively 550 nm), and red (approximatively 650 nm) but contrarily using wavelengths in the Blue or NIR area permit to avoid any influence of the food maturation or common food color change due to various natural processes. These wavelengths were used to extract the absorption coefficients according to Beer Lambert's concentration law, since the concentration of the tested pesticides was known.

TABLE III. DICOFOL, THIAMETHOXAM AND MALATHION PESTICIDES CONCENTRATION IN DIFFERENT FOODS CATEGORY OBTAINED FROM DIFFERENT SUPPLIERS

	Dicofol (mg/mL)	**Thiamethoxam (mg/mL)**	**Malathion (mg/mL)**
Peak wavelengths (nm)	*791*	*774*	*475*
Absorption Coefficient (Mole/L)	*9.8e5*	*3.3e6*	*3.8e6*
PESTICIDES CONCENTRATION (MG/ML)			
Organic tomatoes	0,014	0,005	0,003
Supermarket1 tomatoes	0,082	0,017	0,002
Supermarket 2 tomatoes	0,057	0,013	0,008
Organic zucchini	0,167	0,038	0,029
Supermarket 1 zucchini	0,113	0,026	0,019
Supermarket 2 zucchini	0,119	0,026	0,017
Organic potatoes	0,016	0,005	0,003
Supermarket 1 potatoes	0,0099	0,005	0,004
Supermarket 2 potatoes	0,0089	0,002	0,004

To directly estimate the foods pesticide contamination without previous chemical manipulation and dye addition, the main problem to address was to isolate the pesticide contribution from other factors at the selected pesticide wavelength. Although not perfectly accurate the filtering of the food reflection spectra with the corresponding food spectrum obtained from the pesticide free market gardener, permitted to isolate the non-natural added substances. This method is hence an alternative solution to avoid food sample chemical adulteration and dye addition, before spectrophotometric analysis. To further increase the accuracy of the measures, multiple wavelengths can be considered when computing the Beer Lambert's concentration relation and the mean concentration among these multiple wavelengths can be selected instead, for each pesticide. The concentrations of the studied pesticides among each food category studied after pesticides free market gardener food spectra removal is reported in Table III.

Dicofol, Thiamethoxam and Malathion pesticides contamination is markedly lower in tomatoes obtained from organic farming than in tomatoes from supermarkets. Very surprisingly, higher Dicofol, Thiamethoxam and Malathion pesticides traces were found in organic farming zucchini. Difference in variety between market gardener pesticides free zucchini and organic farming zucchini may partially explain their higher absorption coefficients. Another possible hypothesis to interpret these unpredicted results is that the pesticides studied are not the commonly used in foods farming but are rather more adapted for flowers farming and could easily be transported by wind.

Besides, since the foods were bought in a single organic store and in 2 different supermarkets, they are not representative of respectively organic agriculture or conventional agriculture.

D. Non Negative Classical Least Squares analysis

Vis-NIR spectrum of a solution composed of multiple components, knowing the spectrum of each component can be approximated using multiple linear regression models. Multiple linear regression equations can be solved using Least Squares developments. The main idea with multiple linear regression models is to express a function in a different base: from the wavelength base (old base or regressor basis) to a new base (base of concentration). This can be simply performed by multiplying the analyzed function with the inverse transfer matrix. Transfer matrix is used to transform the old base (wavelength or regressor base) into the new base (concentration base). Least Squares is the mathematical method selected to accomplish this operation, permitting to reduce the squared error between the analyzed function expressed in the old base compared to the analyzed function expressed in new base. Because the dimensions in the old base (number of wavelength) is usually much larger than the dimensions in the new base (number of different concentrations of the pesticides studied), direct transfer matrix inversion is most of the time not permitted. It finally results that the foods studied are described with the relative concentrations of the pesticides studied.

Similarly, to the pesticide concentration determination in foods using the previously described method, it is necessary to isolate the pesticide spectrum from the entire foods spectra. Consequently, pesticides free filters determined for each food category were applied before any pesticides concentration estimation, highlighting the importance of the created filters.

The reconstruction of the additional non natural components foods absorption spectra using the Non Negative Least Squares analysis are reported in Figure 12 for organic tomatoes, Figure 13 for organic zucchini and Figure 14 for organic potatoes. These spectra reconstruction was based on the product of (1) the concentration coefficients obtained by the Non Negative Least Squares analysis, (2) by their corresponding pesticides absorption spectra. It should be noted that Non Negative Least Squares algorithm, mainly permitted to obtain the Thiamethoxam pesticides concentration in foods, since only positive concentration coefficients determination were allowed.

In the reconstructed spectrum using the Non Negative Least Squares algorithm for organic tomatoes (Figure 12), only the 470 – 530 nm and the 730 – 800 nm wavelengths regions were correctly modeled. Wavelength regions correctly modeled from the reconstructed spectrum using the Non Negative Least Squares algorithm were further reduced for organic zucchini (Figure 13), with mostly the 520 – 530 nm and the 740 – 800 nm wavelengths regions precisely reproduced. The reproduction of the organic potatoes additional non natural components spectrum from analyzed pesticides spectra using the same algorithm (Figure 14) was very restricted with only few wavelengths correctly replicated (490 – 500 nm and 730 – 740 nm). Obviously the additional non natural components spectra of the various foods did not only contain the three studied pesticides but many other constituents, explaining the reconstruction divergences. Besides the three pesticides studied absorption spectra have a very similar mathematical description (Gaussian distribution with peaks at close wavelengths), impeding correct reconstruction in other wavelengths area. In a general way, stronger are the similarities between the reconstruction from pesticides spectra and the foods spectra, stronger is the validity of the concentration coefficient obtained by the Non Negative Least Squares algorithm.

The algorithm implemented increased the proximities of the solutions at the regressors (wavelengths) maximum and minimum values, corresponding to the wavelength regions of the pesticides spectral apex. However, these wavelength regions are the most corrupted by measurement noise. It is also interesting to mention that to avoid strong boundary noises the Least Squares method can be weighted by the reflectance spectra of the pesticides, leading to better foods spectra reconstruction [74].

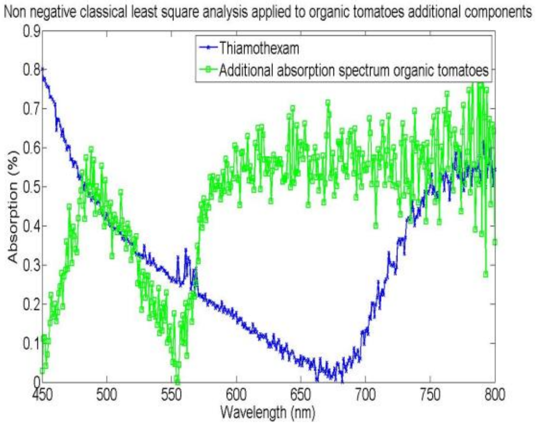

Figure 12. Organic tomatoes additional artificial components spectrum reconstructed from Non Negative Classical Least Squares analysis using the pesticides Dicofol, Thiamethoxam and Malathion spectra.

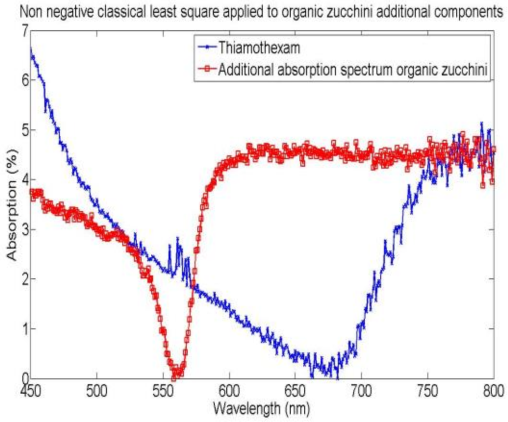

Figure 13. Organic zucchini additional artificial components spectrum reconstructed with Non Negative Classical Least Squares analysis using the pesticides Dicofol, Thiamethoxam and Malathion spectra.

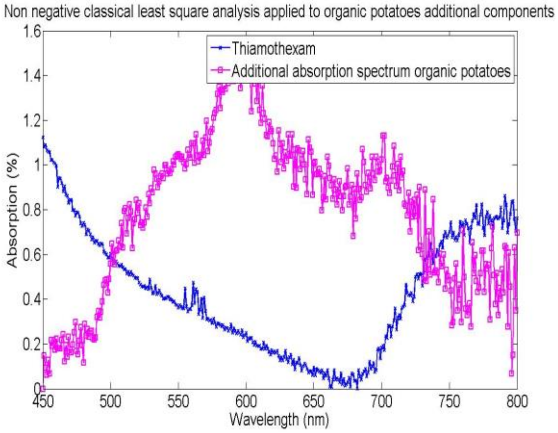

Figure 14. Organic potatoes additional artificial components spectrum reconstructed with Non Negative Classical Least Squares analysis using the pesticides Dicofol, Thiamethoxam and Malathion spectra.

Table IV depicts the comparison between the Thiamethoxam pesticides concentration in various foods categories obtained with the Beer-Lambert procedure or with the Non Negative Least Squares procedure. Both methods permit to obtain very close concentration values with less than 3µg/mL maximum errors.

TABLE IV. THIAMETHOXAM PESTICIDE CONCENTRATION COMPARAISON BETWEEN SINGLE WAVELENGTH CONCENTRATION LAW AND USING NON NEGATIVE LEAST SQUAREs ANALYSIS

	Beer-Lambert's concentration law	**Non Negative Least Squares**
THIAMETHOXAM PESTICIDE CONCENTRATION (MG/ML)		
Organic tomatoes	0.005	0.005
Supermarket1 tomatoes	0.017	0.014
Supermarket 2 tomatoes	0.013	0.016
Organic zucchini	0.038	0.040
Supermarket 1 zucchini	0.026	0.028
Supermarket 2 zucchini	0.026	0.026
Organic potatoes	0.005	0.007
Supermarket 1 potatoes	0.005	0.006
Supermarket 2 potatoes	0.002	0.005

IV. CONCLUSION AND FUTURE WORK

Due to their wide use and toxicity, pesticides quantification in foods are measured by several methods including Liquid Chromatography with Mass Spectrometry (LC-MS), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Voltammetry, Atomic Absorption Spectrophotometry (AAS) and spectrophotometry [80].

Spectrophotometry analysis of pesticides often requires chemical manipulations such as pesticides extraction with solvents and dye addition before dye quantification by spectrophotometric measures. Such manipulations are difficult to reproduce in outside measures and often requires specific material and expertise. In order to directly estimate the pesticides concentration in foods extract using the Beer-Lambert's concentration law, pesticides extraction from foods should be realized first. Avoiding using chemicals for pesticides extraction is therefore a very challenging part, that we partially solved in this document. By performing spectrophotometric measures on food devoid of pesticides contamination and repeating the measures to different foods variety and category, the spectra of pesticides free foods were extracted. These pesticides free foods spectra were used to extract the foods genuine spectra from the added constituents such as pesticides and conservatives. Therefore, we propose an innovative method based on Vis-NIR spectroscopy for detecting food contaminants using spectral filters constructed from pesticides free food samples data.

Our methodology choices were mainly dictated by the currently restricted number of contaminants analyzed using spectrophotometric measures, since pesticides extraction experimental procedures are limited and complex. In contrast, we used the Vis-NIR spectral trace of additional atypical constituents in foods, obtained after application of adequate pesticides free foods filters. The creation of these filters required first the laborious finding of a certified market gardener pesticides free food supplier. Then pesticides free foods spectral data were measured using reflective spectroscopy and used as pesticides free filters. Vis-NIR spectral data of three categories of foods from different food suppliers were compared to the corresponding pesticides free foods spectral data and Vis-NIR spectral traces of contaminants were extracted. We further discussed the most likely wavelengths in the Vis-NIR spectral range associated with pesticides chemicals.

For more rigorous pesticides contamination quantification, we compared two different methods of spectrophotometric pesticides detection: one based on the standard Beer-Lambert's concentration law and the second based on Non Negative Least Squares algorithm. The pesticides of interest were Dicofol, Thiamethoxam and Malathion and their respective absorption spectra were measured. We further extracted the absorption coefficients of these pesticides at appropriate wavelengths.

Measurement of the additional components of the various foods categories in the Vis-NIR spectral trace permitted: (1) the estimation of the pesticides contamination at precise wavelengths (791 nm for Dicofol, 774 nm for Thiamethoxam and 475 nm for Malathion) using the standard Beer-Lambert's law and (2) the estimation of Thiamethoxam food contamination principally, using all the wavelengths (Non Negative Least Squares algorithm). The pesticides concentration results were very similar among the two methods for each category of food with less than 3µg/mL of difference between the two methods.

Globally and from early conclusion, our method is promising, easy to implement and directly permits to interpret pesticides food pollution. Confrontation of the obtained pesticides concentration with another recognized pesticide concentration estimation method such as Liquid Chromatography with Mass Spectrometry is the next step to validate these encouraging results. The use of pesticides free spectral filters and the quantification of pesticides using linear regression models are particularly innovative and should facilitate non-invasive pesticides testing procedures.

The main constraint in the developed method is that meticulous data should be obtained first for numerous pesticide free food variety and category studied, possibly stored in a database.

Astonishingly, when applying the method we developed to foods from different suppliers, it resulted that foods from organic farming were not always the less contaminated with pesticides.

Finally, the implementation of such algorithm in an embedded platform with a microspectrometer module may allow convenient device user estimation of foods chemical contaminants.

REFERENCES

[1] O. Diop and U. Cerasani, "Light Reflection Spectrum Comparison of Pesticides Free Foods, Organic Foods and Conventional Farming Foods for VIS NIR Filter Creation," in *The Ninth International Conference on Advances in Human-oriented and Personalized Mechanisms, Technologies, and Services (CENTRIC 2016)*, Rome, 2016.

[2] D. Pimentel, "Environmental and economic costs of the application of pesticides primarily in the United States," *Environment, development and sustainability*, vol. 7, pp. 229-252, 2005.

[3] N. J. Osborne, R. Cairns, A. H. Dawson, K. M. Chitty, and N. A. Buckley, "Epidemiology of coronial deaths from pesticide ingestion in Australia," *International journal of hygiene and environmental health*, vol. 220, pp. 478-484, 2017.

[4] M. Sarwar, "The dangers of pesticides associated with public health and preventing of the risks," *International Journal of Bioinformatics and Biomedical Engineering*, vol. 1, pp. 130-136, 2015.

[5] D. Yang and Y. Ying, "Applications of Raman spectroscopy in agricultural products and food analysis: A review," *Applied Spectroscopy Reviews*, vol. 46, pp. 539-560, 2011.

[6] B. B. Dzantiev, N. A. Byzova, A. E. Urusov, and A. V. Zherdev, "Immunochromatographic methods in food analysis," *TrAC Trends in Analytical Chemistry*, vol. 55, pp. 81-93, 2014.

[7] G. Marrazza, "Piezoelectric biosensors for organophosphate and carbamate pesticides: a review," *Biosensors*, vol. 4, pp. 301-317, 2014.

[8] D. Lee, S. Lee, G. H. Seong, J. Choo, E. K. Lee, D.-G. Gweon, *et al.*, "Quantitative analysis of methyl parathion pesticides in a polydimethylsiloxane microfluidic channel using confocal surface-enhanced Raman spectroscopy," *Applied spectroscopy*, vol. 60, pp. 373-377, 2006.

[9] M. Karlowatz, M. Kraft, and B. Mizaikoff, "Simultaneous quantitative determination of benzene, toluene, and xylenes in water using mid-infrared evanescent field spectroscopy," *Analytical chemistry*, vol. 76, pp. 2643-2648, 2004.

[10] K. Wong-Ek, M. Horprathum, P. Eiamchai, P. Limnonthakul, V. Patthanasettakul, P. Chindaudom, *et al.*, "Portable surface-enhanced Raman spectroscopy for insecticide detection using silver nanorod film fabricated by magnetron sputtering," in *SPIE BiOS*, 2011, pp. 791108-791108-11.

[11] W. Zhang, A. M. Asiri, D. Liu, D. Du, and Y. Lin, "Nanomaterial-based biosensors for environmental and biological monitoring of organophosphorus pesticides and nerve agents," *TrAC Trends in Analytical Chemistry,* vol. 54, pp. 1-10, 2014.

[12] S. Hassani, S. Momtaz, F. Vakhshiteh, A. S. Maghsoudi, M. R. Ganjali, P. Norouzi, *et al.*, "Biosensors and their applications in detection of organophosphorus pesticides in the environment," *Archives of toxicology,* pp. 1-22, 2017.

[13] M. Stoytcheva, V. Gochev, and Z. Velkova, "Electrochemical biosensors for direct determination of organophosphorus pesticides: a review," *Current Analytical Chemistry,* vol. 12, pp. 37-42, 2016.

[14] L. M. Nollet and H. S. Rathore, *Handbook of pesticides: methods of pesticide residues analysis*: CRC press, 2016.

[15] M. Mutlu, *Biosensors in food processing, safety, and quality control*: CRC Press, 2016.

[16] A. Sassolas, B. Prieto-Simón, and J.-L. Marty, "Biosensors for Pesticide Detection: New Trends," *American Journal of Analytical Chemistry,* pp. 210-232, 2012.

[17] X. Jiang, D. Li, X. Xu, Y. Ying, Y. Li, Z. Ye*, et al.*, "Immunosensors for detection of pesticide residues," *Biosensors and Bioelectronics,* vol. 23, pp. 1577-1587, 2008.

[18] C. R. Suri, M. Raje, and G. C. Varshney, "Immunosensors for pesticide analysis: antibody production and sensor development," *Critical reviews in biotechnology,* vol. 22, pp. 15-32, 2002.

[19] F. Bier, W. Stöcklein, M. Böcher, U. Bilitewski, and R. Schmid, "Use of a fibre optic immunosensor for the detection of pesticides," *Sensors and Actuators B: Chemical,* vol. 7, pp. 509-512, 1992.

[20] M.-I. Baraton, *Sensors for Environment, Health and Security: Advanced Materials and Technologies*: Springer Science & Business Media, 2008.

[21] M. Ferrari, M. Ozkan, and M. Heller, *BioMEMS and Biomedical Nanotechnology: Volume II: Micro/Nano Technologies for Genomics and Proteomics* vol. 2: Springer Science & Business Media, 2007.

[22] O. Tigli, *Novel SAW devices in CMOS for biosensor applications: design, modeling, fabrication and characterization*: ProQuest, 2007.

[23] L. Xue, J. Cai, J. Li, and M. Liu, "Application of particle swarm optimization (PSO) algorithm to determine dichlorvos residue on the surface of navel orange with Vis-NIR spectroscopy," *Procedia Engineering,* vol. 29, pp. 4124-4128, 2012.

[24] J. T. Alander, V. Bochko, B. Martinkauppi, S. Saranwong, and T. Mantere, "A review of optical nondestructive visual and near-infrared methods for food quality and safety," *International Journal of Spectroscopy,* vol. 2013, 2013.

[25] Y.-Z. Feng, G. ElMasry, D.-W. Sun, A. G. Scannell, D. Walsh, and N. Morcy, "Near-infrared hyperspectral imaging and partial least squares regression for rapid and reagentless determination of Enterobacteriaceae on chicken fillets," *Food Chemistry,* vol. 138, pp. 1829-1836, 2013.

[26] E. J. N. Marques, S. T. de Freitas, M. F. Pimentel, and C. Pasquini, "Rapid and non-destructive determination of quality parameters in the 'Tommy Atkins' mango using a novel handheld near infrared spectrometer," *Food chemistry,* vol. 197, pp. 1207-1214, 2016.

[27] M. Manley, "Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of biological materials," *Chemical Society Reviews,* vol. 43, pp. 8200-8214, 2014.

[28] H. Huang, L. Liu, and M. O. Ngadi, "Recent developments in hyperspectral imaging for assessment of food quality and safety," *Sensors,* vol. 14, pp. 7248-7276, 2014.

[29] L. Salguero-Chaparro, A. J. Gaitán-Jurado, V. Ortiz-Somovilla, and F. Peña-Rodríguez, "Feasibility of using NIR spectroscopy to detect herbicide residues in intact olives," *Food control,* vol. 30, pp. 504-509, 2013.

[30] M. T. Sánchez, K. Flores-Rojas, J. E. Guerrero, A. Garrido-Varo, and D. Pérez-Marín, "Measurement of pesticide residues in peppers by near-infrared reflectance spectroscopy," *Pest management science,* vol. 66, pp. 580-586, 2010.

[31] S. Armenta, S. Garrigues, and M. de la Guardia, "Partial least squares-near infrared determination of pesticides in commercial formulations," *Vibrational Spectroscopy,* vol. 44, pp. 273-278, 2007.

[32] J. Moros, S. Armenta, S. Garrigues, and M. de la Guardia, "Near infrared determination of Diuron in pesticide formulations," *Analytica chimica acta,* vol. 543, pp. 124-129, 2005.

[33] J. Moros, S. Armenta, S. Garrigues, and M. de la Guardia, "Univariate near infrared methods for determination of pesticides in agrochemicals," *Analytica chimica acta,* vol. 579, pp. 17-24, 2006.

[34] Y. Zhou, B. Xiang, Z. Wang, and C. Chen, "Determination of chlorpyrifos residue by near-infrared spectroscopy in white radish based on interval partial least square (iPLS) model," *Analytical Letters,* vol. 42, pp. 1518-1526, 2009.

[35] D. Malley, C. McClure, P. Martin, K. Buckley, and W. McCaughey, "Compositional analysis of cattle manure during composting using a field-portable near-infrared spectrometer," *Communications in Soil Science and Plant Analysis,* vol. 36, pp. 455-475, 2005.

[36] K. D. Shepherd and M. G. Walsh, "Infrared spectroscopy—enabling an evidence-based diagnostic surveillance approach to agricultural and environmental management in developing countries," *Journal of Near Infrared Spectroscopy,* vol. 15, pp. 1-19, 2007.

[37] T. Shi, Y. Chen, Y. Liu, and G. Wu, "Visible and near-infrared reflectance spectroscopy—An alternative for monitoring soil contamination by heavy metals," *Journal of hazardous materials,* vol. 265, pp. 166-176, 2014.

[38] M. Maleki, A. Mouazen, H. Ramon, and J. De Baerdemaeker, "Optimisation of soil VIS–NIR sensor-based variable rate application system of soil phosphorus," *Soil and Tillage Research,* vol. 94, pp. 239-250, 2007.

[39] M. Kamruzzaman, Y. Makino, and S. Oshita, "Non-invasive analytical technology for the detection of contamination, adulteration, and authenticity of meat, poultry, and fish: a review," *Analytica chimica acta,* vol. 853, pp. 19-29, 2015.

[40] G. Rateni, P. Dario, and F. Cavallo, "Smartphone-Based Food Diagnostic Technologies: A Review," *Sensors,* vol. 17, p. 1453, 2017.

[41] S.-H. Noh and K.-H. Choi, "Nondestructive quality evaluation technology for fruits and vegetables," in *International Seminar on Enhancing Export Competitiveness of Asian Fruits, Bangkok, Thailand*, 2006.

[42] R. D. JiJi, G. A. Cooper, and K. S. Booksh, "Excitation-emission matrix fluorescence based determination of carbamate pesticides and polycyclic aromatic hydrocarbons," *Analytica Chimica Acta,* vol. 397, pp. 61-72, 1999.

[43] A. Kwiatkowski, M. Czerwicka, J. Smulko, and P. Stepnowski, "Detection of denatonium benzoate (Bitrex) remnants in noncommercial alcoholic beverages by raman spectroscopy," *Journal of forensic sciences,* vol. 59, pp. 1358-1363, 2014.

[44] E. Ali and H. G. Edwards, "The detection of flunitrazepam in beverages using portable Raman spectroscopy," *Drug testing and analysis,* vol. 9, pp. 256-259, 2017.

[45] K. Ilaslan, I. H. Boyaci, and A. Topcu, "Rapid analysis of glucose, fructose and sucrose contents of commercial soft drinks using Raman spectroscopy," *Food Control,* vol. 48, pp. 56-61, 2015.

[46] G. G. Buyukgoz, A. G. Bozkurt, N. B. Akgul, U. Tamer, and I. H. Boyaci, "Spectroscopic detection of aspartame in soft drinks by surface-enhanced Raman spectroscopy," *European Food Research and Technology,* vol. 240, pp. 567-575, 2015.

[47] C. A. F. Penido, M. T. T. Pacheco, I. K. Lednev, and L. Silveira, "Raman spectroscopy in forensic analysis: identification of cocaine and other illegal drugs of abuse," *Journal of Raman Spectroscopy,* vol. 47, pp. 28-38, 2016.

[48] C. Martin, J.-L. Bruneel, F. Guyon, B. Médina, M. Jourdes, P.-L. Teissedre, *et al.*, "Raman spectroscopy of white wines," *Food chemistry,* vol. 181, pp. 235-240, 2015.

[49] T. O. Mendes, R. A. da Rocha, B. L. Porto, M. A. de Oliveira, V. d. C. dos Anjos, and M. J. Bell, "Quantification of extra-virgin olive oil adulteration with soybean oil: a comparative study of NIR, MIR, and Raman spectroscopy associated with chemometric approaches," *Food analytical methods,* vol. 8, pp. 2339-2346, 2015.

[50] V. O. Clavero, A. Weber, W. Schröder, D. Curticapean, N. Javahiraly, and P. Meyrueis, "Monitoring of the molecular structure of lubricant oil using a FT-Raman spectrometer prototype," in *Optical Sensing and Detection III*, 2014, p. 91411W.

[51] K. Czamara, K. Majzner, M. Z. Pacia, K. Kochan, A. Kaczor, and M. Baranska, "Raman spectroscopy of lipids: a review," *Journal of Raman Spectroscopy,* vol. 46, pp. 4-20, 2015.

[52] P. Vandenabeele, H. Edwards, and J. Jehlička, "The role of mobile instrumentation in novel applications of Raman spectroscopy: archaeometry, geosciences, and forensics," *Chemical Society Reviews,* vol. 43, pp. 2628-2649, 2014.

[53] S. Feng, F. Gao, Z. Chen, E. Grant, D. D. Kitts, S. Wang, *et al.*, "Determination of α-Tocopherol in vegetable oils using a molecularly imprinted polymers–surface-enhanced raman spectroscopic biosensor," *Journal of agricultural and food chemistry,* vol. 61, pp. 10467-10475, 2013.

[54] R. S. Uysal, I. H. Boyaci, H. E. Genis, and U. Tamer, "Determination of butter adulteration with margarine using Raman spectroscopy," *Food chemistry,* vol. 141, pp. 4397-4403, 2013.

[55] C. A. Nunes, "Vibrational spectroscopy and chemometrics to assess authenticity, adulteration and intrinsic quality parameters of edible oils and fats," *Food Research International,* vol. 60, pp. 255-261, 2014.

[56] B. Liu, G. Han, Z. Zhang, R. Liu, C. Jiang, S. Wang, *et al.*, "Shell thickness-dependent Raman enhancement for rapid identification and detection of pesticide residues at fruit peels," *Analytical chemistry,* vol. 84, pp. 255-261, 2011.

[57] J. Xing and D. Guyer, "Detecting internal insect infestation in tart cherry using transmittance spectroscopy," *Postharvest biology and technology,* vol. 49, pp. 411-416, 2008.

[58] B. Saute, R. Premasiri, L. Ziegler, and R. Narayanan, "Gold nanorods as surface enhanced Raman spectroscopy substrates for sensitive and selective detection of ultra-low levels of dithiocarbamate pesticides," *Analyst,* vol. 137, pp. 5082-5087, 2012.

[59] G. Chunhui, Z. Guoqiang, G. Liangquan, L. Jun, and W. Ziqiang, "Determination of trace elements in tea by wavelength dispersive X-ray fluorescence spectroscopy," *Nuclear Techniques,* vol. 36, 2013.

[60] L. R. Goldman, "Managing pesticide chronic health risks: US policies," *Journal of agromedicine,* vol. 12, pp. 67-75, 2007.

[61] Centers, for, Disease, Control, and, and Prevention. (2013). *CDC - Pesticide Illness & Injury Surveillance - NIOSH Workplace Safety and Health Topic*. Available: http://www.cdc.gov/

[62] S. Parvez and S. Raisuddin, "Protein carbonyls: novel biomarkers of exposure to oxidative stress-inducing pesticides in freshwater fish Channa punctata (Bloch)," *Environmental Toxicology and Pharmacology,* vol. 20, pp. 112-117, 2005.

[63] J. Dorts, F. Silvestre, H. T. Tu, A.-E. Tyberghein, N. T. Phuong, and P. Kestemont, "Oxidative stress, protein carbonylation and heat shock proteins in the black tiger shrimp, Penaeus monodon, following exposure to endosulfan and deltamethrin," *Environmental toxicology and pharmacology,* vol. 28, pp. 302-310, 2009.

[64] J. J. Fortunato, G. Feier, A. M. Vitali, F. C. Petronilho, F. Dal-Pizzol, and J. Quevedo, "Malathion-induced oxidative stress in rat brain regions," *Neurochemical research,* vol. 31, pp. 671-678, 2006.

[65] S. B. Ceyhun, M. Şentürk, D. Ekinci, O. Erdoğan, A. Çiltaş, and E. M. Kocaman, "Deltamethrin attenuates antioxidant defense system and induces the expression of heat shock protein 70 in rainbow trout," *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,* vol. 152, pp. 215-223, 2010.

[66] R. M. Johnson, J. D. Evans, G. E. Robinson, and M. R. Berenbaum, "Changes in transcript abundance relating to colony collapse disorder in honey bees (Apis mellifera)," *Proceedings of the National Academy of Sciences,* vol. 106, pp. 14790-14795, 2009.

[67] H. T. Hogberg, A. Kinsner-Ovaskainen, T. Hartung, S. Coecke, and A. K. Bal-Price, "Gene expression as a sensitive endpoint to evaluate cell differentiation and maturation of the developing central nervous system in primary cultures of rat cerebellar granule cells (CGCs) exposed to pesticides," *Toxicology and applied pharmacology,* vol. 235, pp. 268-286, 2009.

[68] M. Collotta, P. Bertazzi, and V. Bollati, "Epigenetics and pesticides," *Toxicology*, vol. 307, pp. 35-41, 2013.

[69] J. D. Ingle Jr and S. R. Crouch, "Spectrochemical analysis," 1988.

[70] J. Clack and G. Gunawardena. (Updated in February 2017). *The Beer-Lambert Law (Chemistry LibreTexts ed.)*. Available: https://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Spectroscopy/Electronic_Spectroscopy/Electronic_Spectroscopy_Basics/The_Beer-Lambert_Law

[71] University of California, Los Angeles. (Accesses February 2017). *Colorimetric Analysis (Beer's law or Spectrophotometric Analysis)*. Available: http://www.chem.ucla.edu/~gchemlab/colorimetric_web.htm

[72] Andor, Oxford, Instruments, and Company. (Accessed in February 2017). *Absorption, Transmission and Reflection Spectroscopy* Available: http://www.andor.com/learning-academy/

[73] B. M. Tissue. (Accessed in February 2017). *Beer-Lambert Law*. Available: http://www.tissuegroup.chem.vt.edu

[74] T. C. O'Haver. (Accessed in February 2017). *Curve fitting B: Multicomponent Spectroscopy*. Available: https://terpconnect.umd.edu/~toh/spectrum/CurveFittingB.html

[75] C.-M. Kuan, "Classical Least Squares Theory," in *National Taiwan University, Department of Finance & CRETA*, 2010.

[76] H. Mark and J. Workman, "Classical Least Squares, Part I: Mathematical Theory," 2010.

[77] B. P. Baker, C. M. Benbrook, E. G. III, and K. L. Benbrook, "Pesticide residues in conventional, integrated pest management (IPM)-grown and organic foods: insights from three US data sets," *Food Additives & Contaminants*, vol. 19, pp. 427-446, 2002.

[78] N. Venugopal and B. Sumalatha, "Spectrophotometric determination of malathion in environmental samples," *Journal of Chemistry*, vol. 9, pp. 857-862, 2012.

[79] K. Reddy, K. Suvardhan, K. S. Kumar, D. Rekha, and P. Chiranjeevi, "Extractive spectrophotometric determination of malathion in environmental samples using Gention violet," *Journal of Chemistry*, vol. 2, pp. 187-192, 2005.

[80] A. A. Gouda, A. S. Amin, R. El-Sheikh, and M. A. Akl, "Sensitive spectrophotometric methods for determination of some organophosphorus pesticides in vegetable samples," *Chemical Industry and Chemical Engineering Quarterly*, vol. 16, pp. 11-18, 2010.

[81] A. Bouhaouss, A. Bensaoud, M. El Azzouzi, C. Perrin-Ganier, and M. Schiavon, "Etude par spectroscopie ultraviolet et infrarouge de l'adsorption des pesticides par les apatites," *Phys. Chem. News*, vol. 1, pp. 115-118, 2001.

[82] G. P. Pandey, A. K. Singh, L. Deshmukh, and A. Asthana, "Determination of dicofol in various environmental samples by spectrophotometric method using chromogenic reagent," *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, vol. 45, pp. 1199-1205, 2015.

[83] J. Zhang, Z. Zhao, L. Wang, X. Zhu, L. Shen, and Y. Yu, "Two-Dimensional UV Absorption Correlation Spectroscopy as a Method for the Detection of Thiamethoxam Residue in Tea," *Journal of Applied Spectroscopy*, vol. 82, pp. 311-315, 2015.