

A Smart Fluorescent Light Spectroscope to Identify the Pork Adulteration for Halal Authentication

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Abstract

The complication of adulterated ingredients in processed food items is widely observed in the food industry and remains a continuous disquiet for end users. This problem may affect consumers' spiritual beliefs, likewise with their fitness and diet. Hence commercial foods should be scrutinized for the precision of the avowed ingredients. This study is dedicated to developing a Fluorescent light Spectroscope to identify the pork adulteration. A simple way of DNA extraction process has been introduced to make the system more convenient. The spectral bands linked with pork fat (PF), beef fat (BF) and their combinations in different food formulation were skimmed, and recognized by correlating them to those spectroscopically illustrative to clean Pork or PF and other different items. Every material has the properties to absorb some light of specific wavelength, and our activity is to determine thus wavelength range at which are absorbed or make any change by the target material. The findings have revealed that spectroscopy can be used as one of the procedures to detect and quantify of pork in different foods and beverages formulation for Halal verification purposes. Special laborious procedures and equipment both are essential for the existing testing methods named RT-PCR (Reverse transcription-polymerase chain reaction) and ELISA (enzyme-linked immunosorbent assay). Most of the food processors and dealers are not skillful to conduct sufficient testing for their products with all these sample preparation, extraction, analysis, and obtaining results which can be overcome by our proposed setup.

Keywords

Pork Adulteration, Spectroscope, DNA Extraction, Fluorescence

1. Introduction

The current years have experienced multiple reports about the “Pork Adulteration” at different foods and beverages especially in soft drinks and snack foods. Although some specific brands of the beverage have lost their market in different countries for doubting about mixing pork, most of the people are afraid of pork adulteration because they don’t know which foods are Halal or not for them till now. Moreover, there are a few user-friendly and feasible technologies to detect pork adulteration easily. The findings as well as the evaluation of adulterants are truly essential for the safeguard of the wealth and health of end-users; as a result, several analytical approaches have been advanced for food certification studies. In recent times, there have been some concerns related to the adulteration of pork in different food and beverage products. Overall, the existence of porcine derivatives for instance pork in foodstuffs becomes severe matters in consideration of spiritual disquiets, since religions like Islam, Judaism, and Hinduism prohibit their believers to take food items that contain porcine and its derivatives [1] [2]. A lot of research has been done for the analysis of pork or lard extraction from different food items for example differential scanning calorimetry [3] [4], gas chromatography [5], high-pressure liquid chromatography [6] [7] [8], electronic nose [9], and DNA-based method [10] [11]. As well as different methods have been handed down for the revealing of meat adulteration consists of molecular biology-oriented methods, an enzyme related immune-based techniques, chromatographic techniques, and spectroscopy-based approaches. Molecular biological approaches, such as polymerase chain reaction (PCR), real-time PCR [12], restriction fragment length polymorphism analysis (RFLP) [13], multiplex PCR [14], and species-specific PCR [15] have been characterized for the proof of identity of species and adulteration of meat. Enzyme related immunology-based approaches used in mat-based food items had been gone through by [16]. These approaches are the most precise and delicate for species identification. On the other hand, they need costly laboratory apparatus, highly experienced technical hand, and also agonize from sophisticated false-positive proportions. To get rid of these too laborious and time-consuming methods, an easy, less time consuming and reliable procedure must be developed.

With the improvement of modern technology such as wireless communications [17]-[23], internet of thing [24], remote sensing [25], bio-sensing [26] [27], position tracking, and radar system, the world is entering in the new era, which is known as technology lovers [28] [29] [30] [31]. But also it is a demand of the modern civilization that the improvement will take place in the process of getting our daily food so that it will safe and nutritious which is a basic human right. Now, it has become an appeal for the consumers as well as traders to develop a smart pork detection system to eliminate the doubt. In the earlier researches on adulteration identification, an electronic nose has proved a great role [32] [33] [34]. To give an instance to discriminate adulteration of oil [15] [35] [36] [37], wines [38] and meat. Aimed at meat discernment, studies were

prepared on freshness estimation [39], processing methods valuation [40], meat goods discrepancy, and legitimacy assessment [41]. For the proof of identity and distinction of pork because of halal certification, a study was done on pork from different meat items using E-nose [42]. A fast and non-destructive technique was studied for the detection and quantification of pork in beef meatball using Fourier transform infrared (FTIR) spectroscopy by the authors in [43]. The traditional processes to detect any target materials are very complex. To manipulate the system and devices required a modern laboratory environment and vast knowledge which is not possible for general people. This paper aims to design a reliable, easy-to-perform, and cost-effective process to detect the pork adulteration in any foods and beverages by using some simple steps with a device at a low cost. Moreover no need for vast knowledge about biochemistry or laboratory arrangement.

2. Materials and Methods

2.1. Light Absorption Properties of Meat

If we consider visible and Near-infrared (NIR) spectral regions, it is examined that the light absorption property of most biological soft tissues is comparatively low. This 600 nm to 1300 nm range can be defined as an optical window for biological soft tissues. On the other side, the tissues have reasonably high light absorption property outside the above-mentioned region. Overall, for carrying out non-destructive analysis on biological tissues, fluorescent light can be a good spectral region because the light will be significantly absorbed and scattered within the depth of just a few hundred microns. Light distribution in biological tissues can be determined by optical properties of the tissue. Absorption coefficient for most biological tissues can be calculated with the help of Beer-Lambert law which expressed by the following equations:

$$I = I_0 10^{-\epsilon cl} \quad (1)$$

$$\ln \frac{I_0}{I} = \frac{1}{T} = \epsilon cl \quad (2)$$

where, I_0 = Input light intensity, I = Output light intensity through sample, ϵ = Extinction molar coefficient, c = Molar concentration of substance; l = Sample thickness in cm. The photon diffusion model and the Monte Carlo model are the most popular model to estimate the absorption property of a scattering material from measurements. Volume fractions and distribution of tissue samples have a vital role in the absorption property. As a result, absorption properties may vary sample to sample. Therefore, it is a challenging task to precisely quantify the absorption properties of biological tissues.

2.2. Fatty Acid Mixture and Pure Pork Lard

Mainly, five different types of fatty acids set up in human and pork tissue were blended in the proper ratio shown in **Table 1**.

Table 1. Fatty acid contents of pure animal meat [44].

Fatty acids	Human depot fat	Pork lard	Beef tallow
Oleic (C ₁₈ H ₃₄ O ₂)	46.9%	47.5%	49.6%
Palmitic (C ₁₆ H ₃₂ O ₂)	24.0%	28.3%	27.4%
Linoleic (C ₁₈ H ₃₂ O ₂)	10.2%	6.0%	2.5%
Stearic (C ₁₈ H ₃₆ O ₂)	8.4%	11.9%	14.1%
Palmitoleic (C ₁₆ H ₃₀ O ₂)	5.0%	2.7%	NA

Animal fat holds various types of fatty acids. As an example, at around 96% of pork lard is contained of those five kinds of above mentioned fatty acids. The absorption spectra of these fatty acids have a certain similarity because of their close chemical structures. As a result, to achieve the illustrative absorption spectrum of pork lard, a massive amount of pork lard samples have to be analyzed. We cannot take only tissue as a sample. But, DNA contains all the properties in several biological cells. So, we can use DNA as our desired sample.

2.3. DNA Extraction Process

Three fundamental steps are associated with the DNA extraction process. After the collection of cells, DNA has to expose along with cytoplasm by breaking cell membranes. Lipids from the cell membrane and the nucleus are gone down with detergents and surfactants. Breaking down proteins and RNA by adding a protease and RNase respectively is an optional step [45] [46]. The solution is treated with a concentrated salt solution (saline) to make debris such as broken proteins, lipids, and RNA clump together. DNA purification from detergents, proteins, salts, and reagents used during the cell lysis step. The frequently used ingredient is Ethanol typically well-known as isopropanol or ice-cold ethanol. The flow diagram as shown in **Figure 1** has represented the DNA extraction process for our sample preparation.

Necessary Materials

Pure water, filter paper, pure soap, isopropyl alcohol (70%) and salt.

2.4. Fluorescence Spectroscopy

The incident of emission when there is a changeover happened from singlet excited state to ground state is defined as fluorescence. If in a state all the unpaired electrons have opposite spin then the state can be defined as a singlet state. Fluorescence spectroscopy can be defined as electromagnetic spectroscopy that assesses fluorescence from a sample. It implicates via a beam of light that stimulates the electrons in molecules of certain compounds and causes them to emit light; typically, but not necessarily, visible light. First and foremost fluorescence spectroscopy is involved through electronic and vibrational states of energy levels. In general, the species being inspected has a ground electronic state of lower energy and an agitated electronic state of higher energy. In case of fluores-

cence, absorbing a photon the species is at first excited from its ground electronic state to one of the various vibrational states in the excited electronic state. Because of the collisions with other molecules, the excited molecule loses vibrational energy, and therefore sequentially it reaches up to the lowest vibrational state from the excited electronic state. This procedure is commonly anticipated using a Jablonski diagram which has illustrated in **Figure 2**. Fluorescent light can gain access to relatively extending very far into biological soft tissues. The fluorescent light absorption property of tissue differs with tissue elements specifically water, fat, collagen, and their combined ratio. For that reason, a mixture proportion of tissue constituents can be assessed by analyzing the absorption spectrum to define the light path length in each constituent. This study focuses on the measuring of their absorption spectra from standardized samples.

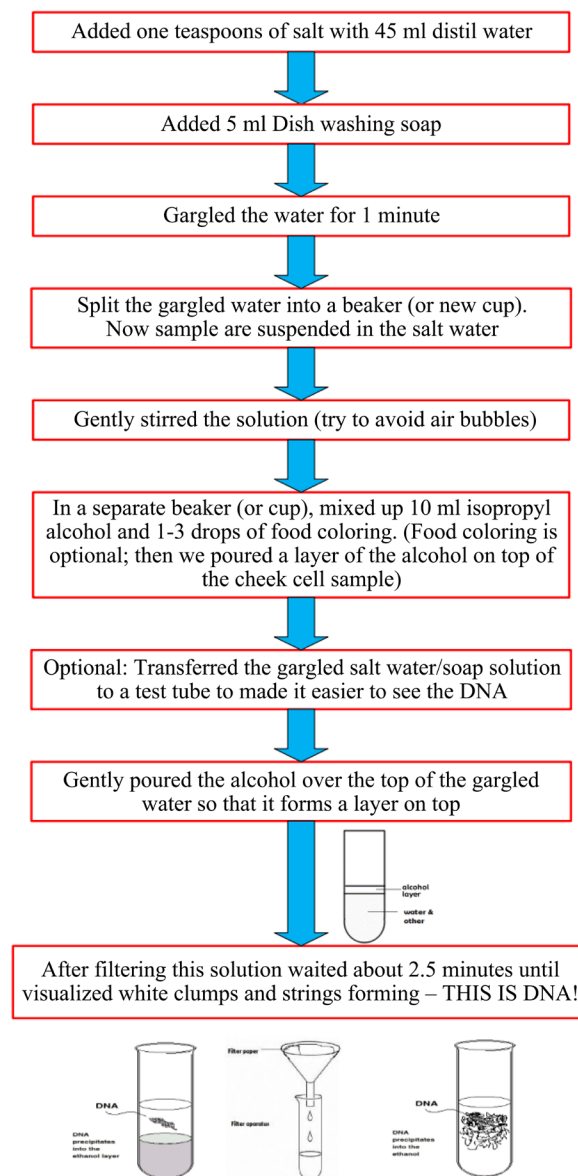


Figure 1. Flow diagram of a DNA extraction process.

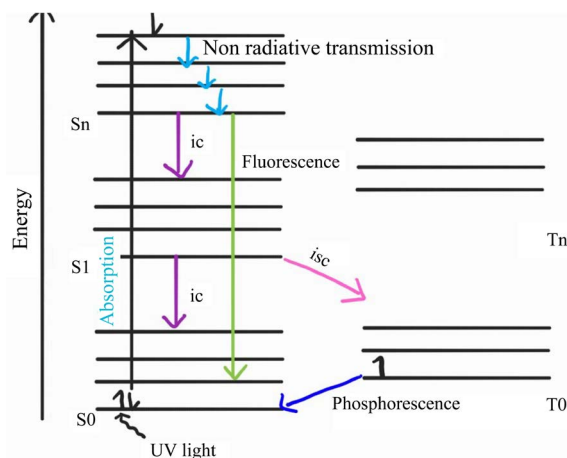


Figure 2. Jablonski Diagram.

3. Hardware Development of Fluorescent Light Spectroscopy

The basic construction of our spectroscope to detect pork DNA is very simple. A specific light source (CFLs lamp) sends a light beam through the DNA sample which is received from opposite side by a light detector through a dispersion material has illustrated in **Figure 3(a)**.

These structures have no moving portions. From the fluorescent light source a light beam comes out which passes through the giving sample. Then the light split into its separate spectral components. After that, the separate wavelengths fall onto the detector where every single absorbance rate is calculated simultaneously. A fluorescent bulb is used as a fluorescent light source that has almost three specific colors spectrum thus are Red, Green, and Blue.

3.1. Fluorescent Spectroscopy Arrangement

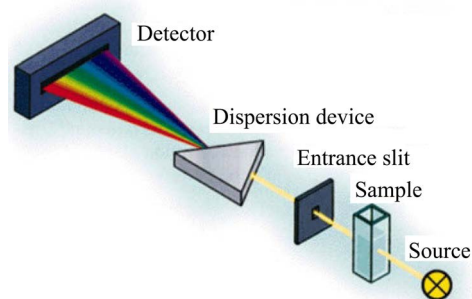
A black colored case apprehended the setup so that no external light can enter. The arrangements of the fluorescent spectroscope to detect Pork Adulteration are shown in **Figure 3(b)**.

A 3D design has done using “Google SketchUp” demonstrated in **Figure 4(a)** for our proposed spectroscope so that the total structure can easily be demonstrated. For the total setup, we needed a white fluorescent light bulb, CCD Camera (Any types of digital camera such as Webcam with use interference system), diffraction Grating (15,000 Lines per inch), cuvette ($\frac{1}{2}'' \times \frac{1}{2}''$ size), and computer (with RSpec Explorer.exe software).

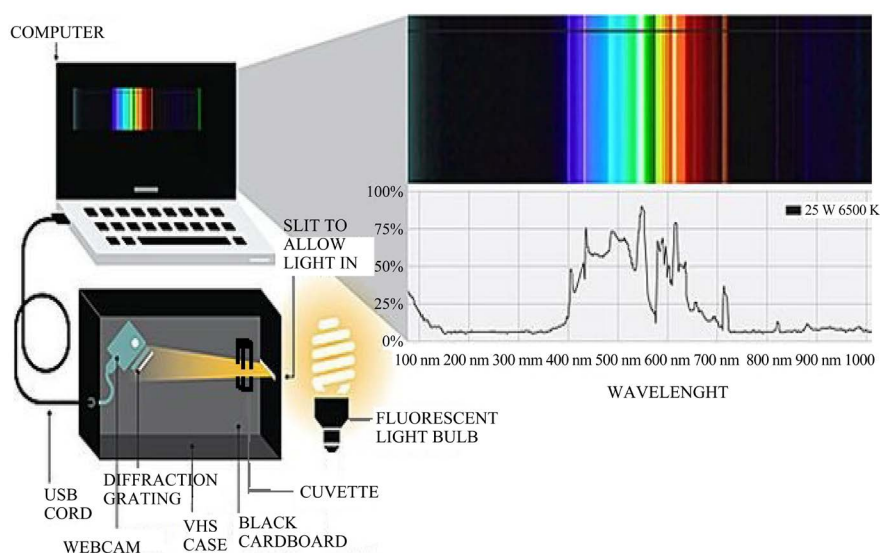
3.2. Construction Procedure

First of all, we fixed the fluorescent light horizontally in the case so that only the bulb is exposed and the rest of the head is covered. Then we cut a hole on both sides of the cuvette holder in such a way that it lines up with horizontally parallel on the case surface. Subsequently, we have fixed the diffraction grating in front

of the camera lens. Later we have mounted the camera with about 60° angles facing the light way. That's how we have turned on the light inside the Styro-foam. At last, we have positioned the camera to take a spectrum image in focus with the main beam on the left side. Real life view of the Fluorescent light Spectroscope setup has exhibited in **Figure 4(b)**.

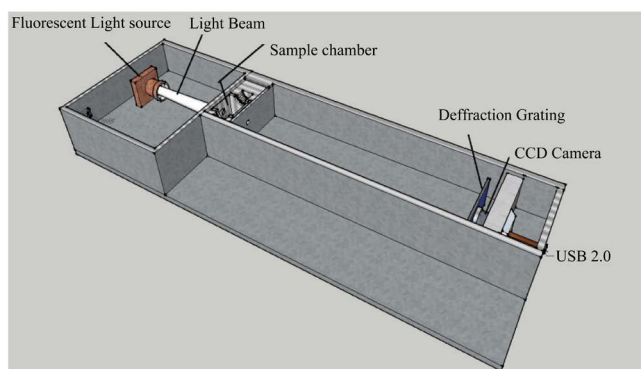


(a) Basic construction of a spectroscopy.



(b) Fluorescent Spectroscopy Arrangement.

Figure 3. Construction and arrangement of a spectroscopy.



(a) Sketch of our fluorescent spectroscopy.



(b) Real life view of the fluorescent light Spectroscopy setup.

Figure 4. Sketch and real life view of our fluorescent light spectroscopy setup.

4. Software Details

RSpec Explorer is normally used for astronomical analysis. This software works on image pixel; it converts pixel at wavelength and plots light intensity according to the pixel intensity. A colorful spectrum can rightly be called a “fingerprint of a star”. In recent times, the cost and complexity of studying spectra have dropped extremely though in the past it was only possible for professionals.

The Procedure We Have Calibrated Our Spectroscopy

To accomplish the calibration our Spectroscopy, we needed a light source with a known wavelength that is available to us. The street sodium lamp shown in **Figure 5(a)** is the one that we choose as a standard light source. Low-pressure sodium (LPS) lamps have borosilicate glass gas discharge tube (arc tube) containing solid sodium, a small amount of neon, and argon gas in a Penning mixture to start the gas discharge. When the lamp has turned on it releases a dim red/pink light to heat up the sodium metal and in a moment, it shifts into the common bright yellow as the sodium metal vaporizes. These lamps produce a virtually monochromatic light averaging a 589.3 nm wavelength illustrated in **Figure 5(b)** and **Figure 6(a)**.

We took few pictures of the sodium lamp for calibration as follows:

These pictures were then used in RSpec Explorer software and accomplished some step to get the spectrum.

After zooming and align the orange color bars for selecting pixel horizontally we completed the two point calibration illustrated in **Figure 6(b)** and **Figure 7**.

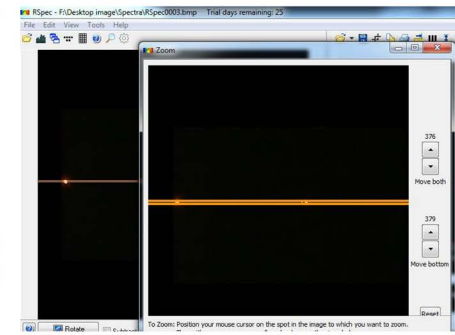
After accomplishing these steps we get this spectrum with the wavelength at nanometer including the color band. Sodium lamp real time spectrum from the software output has presented in **Figure 8**.

Thus we could determine the wavelength of any spectra from this screen by putting the mouse pointer on any desired point. This work measures the spectrum of some other light sources such as incandescent, LED, etc demonstrated in **Figure 9** and **Figure 10(a)**.



(a) Low pressure sodium streetlamp. (b) Low pressure sodium lamp spectrum wavelength.

Figure 5. Low pressure sodium streetlamp and its spectra.



(a) Sodium streetlamp spectra.

(b) Pixel selection by using zoom tool.

Figure 6. Sodium streetlamp spectra and Pixel selection procedure by using zoom tool.

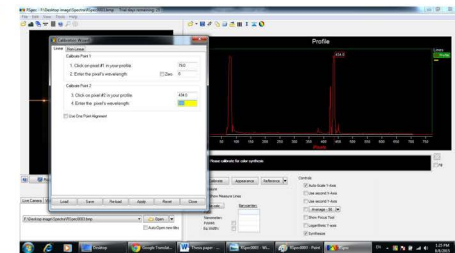
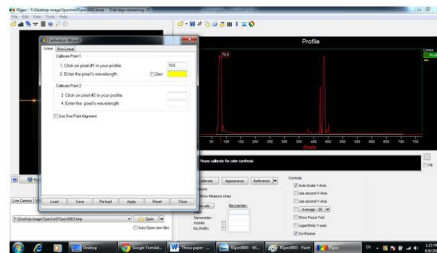


Figure 7. Two point calibration.



Figure 8. Sodium lamp real time spectrum.

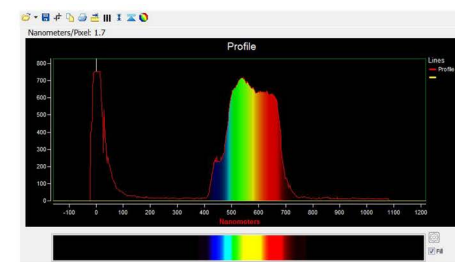


Figure 9. Wavelength and real time spectrum of incandescent light.

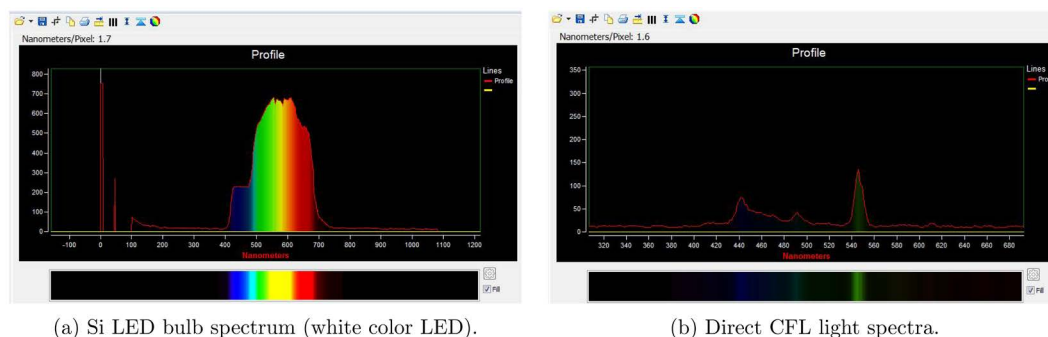


Figure 10. Direct Si LED and CFL light spectrum.

5. Result Analysis

After that, we passed the spectrum through different samples and therefore got the distinguishable difference. Some spectrums through several samples are represented in this section.

5.1. Spectrum Observation through Various Samples

Figure 10(b) represents the spectrum of fluorescent light (CFLs) without any sample through. Water has an absorption area but this area of wavelength is very large near to mid-infrared ray. **Figure 11(a)** has shown that the pure water has an absorption label near to infrared (IR) range and below whose are shown by vertical blue lines by reference library.

Figure 11(b) has shown the Fluorescent light spectra through water and ethanol mixer or solution. There has a noticeable difference that the light intensity through the water with Ethanol is higher than the direct CFLs light intensity because the light is scattered by the water and ethanol molecules also the water cannot absorb in this region.

Figure 11(c) and **Figure 12(a)** has represented the spectra of pure beef and chicken DNA sample respectively.

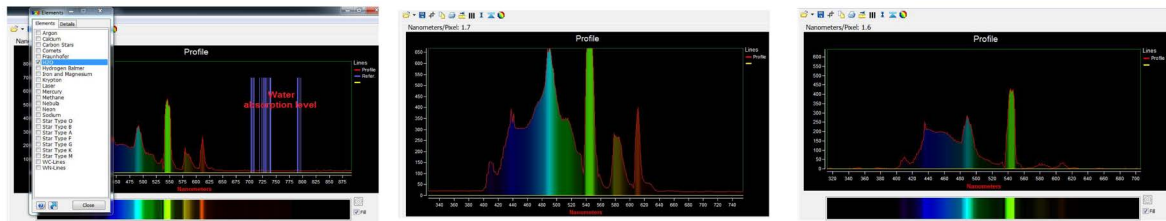
Figure 12(b) has presented the spectra of pure pork where it is clear that only a single beam has found at the range of around 539 nm to 554 nm and others light intensity is very poor because these lights are absorbed.

Figure 12(c) shows the spectra of pork and chicken mixer sample is looking so close to the pure pork spectra.

Figures 13(a)-(c) have presented the spectra of the light passed through the fossil fuel, vegetable oil and edible oil respectively so that different food and beverage characteristics can be observed.

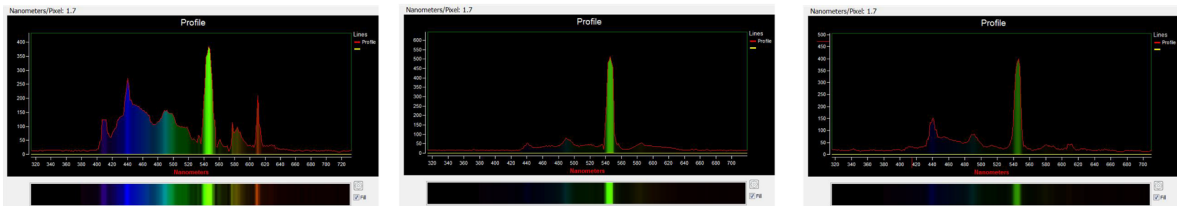
5.2. Result Analysis over Findings

After analysing the above spectrum, it is clearly evident that every sample spectra have the different and specific characteristics. As a result pork sample has a unique spectra output. However, if we find any spectra similar to pork spectra then we can make a decision that this sample content Pork derivatives.



(a) Water spectra with reference level. (b) Water & alcohol solution spectra.

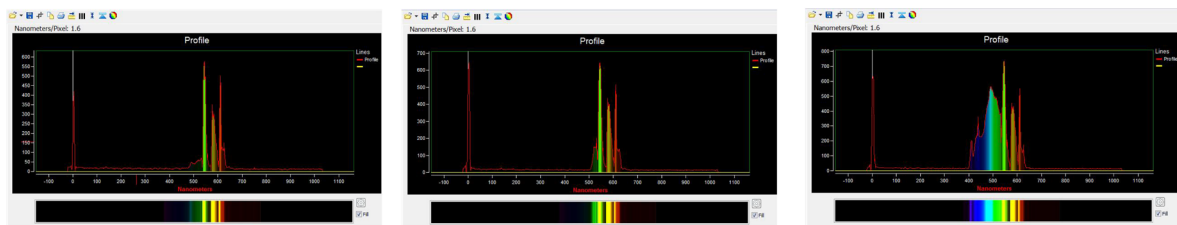
(c) Pure beef spectra.

Figure 11. Spectra of water, water & alcohol, and pure beef.

(a) Pure chicken spectra.

(b) Pure pork spectra.

(c) Pork & chicken mix spectra.

Figure 12. Spectra of pure chicken, pure pork, pork & chicken mix.

(a) Fossil fuels spectra.

(b) Vegetable oil spectra.

(c) Edible oil spectra.

Figure 13. Spectra of fossil fuel, vegetable oil, and edible oil.

5.3. Feasibility Comparison

To take decision between duplicate samples the proposed method shows better outcome than ELISA since it can examine every single wavelength's light intensity. The proposed method takes less time than both RT-PCR and ELISA. About the specificity during reference sample testing it can be 100% accurate after more improvement. Overall, special laborious procedures and equipment both are essential for the existing testing methods named RT-PCR (Reverse transcription polymerase chain reaction) and ELISA (enzyme-linked immunosorbent assay) which can be overcome by our proposed setup.

6. Device Development

6.1. Spectroscope Development

After being observed and reviewed the pattern of the spectrums, we found out a special and unique pattern for pork samples, which showed the way for us to design a instrument. We will replace the camera by a photodetector array and a microcontroller circuit that will read the sample data by the photodetector array. If any reading matches the preset data in the circuit, the display of the device will

show the result “POSITIVE”. Otherwise, it will show “Negative”. Sketch of the Pork detector has been prepared using “Google SketchUp”.

This sketched **Figure 14** has shown that it contains CFLs light, a Diffraction Grating, an LCD display, some buttons, and some photodetectors.

6.2. Electrical Circuit Development

Microcontroller based circuit designed and Simulation in Proteus using PIC16f877 MCU has illustrated in **Figure 15**. The detail pin configuration of PIC16f877 MCU is shown in **Figure 16**.

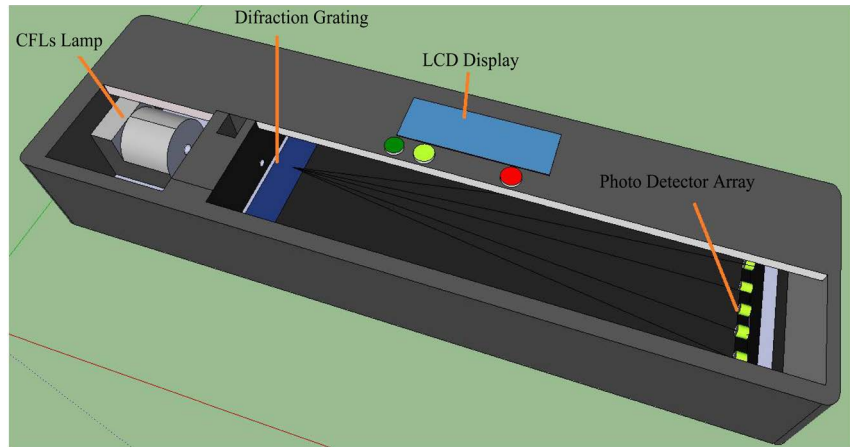


Figure 14. Pork detector sketch up.

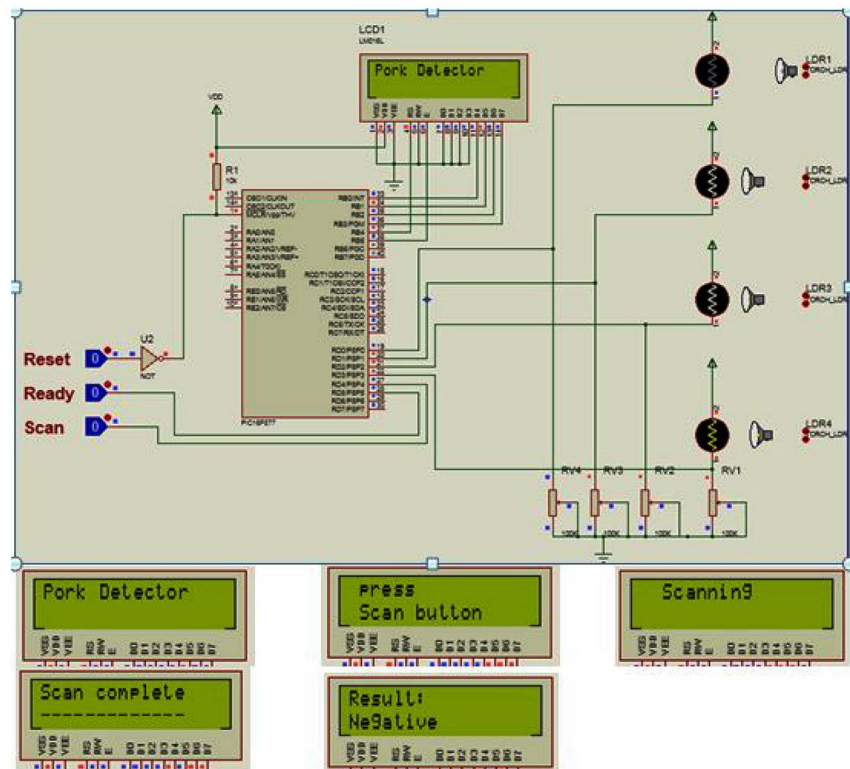


Figure 15. Circuit diagram of CFLs spectroscopy during Simulation.

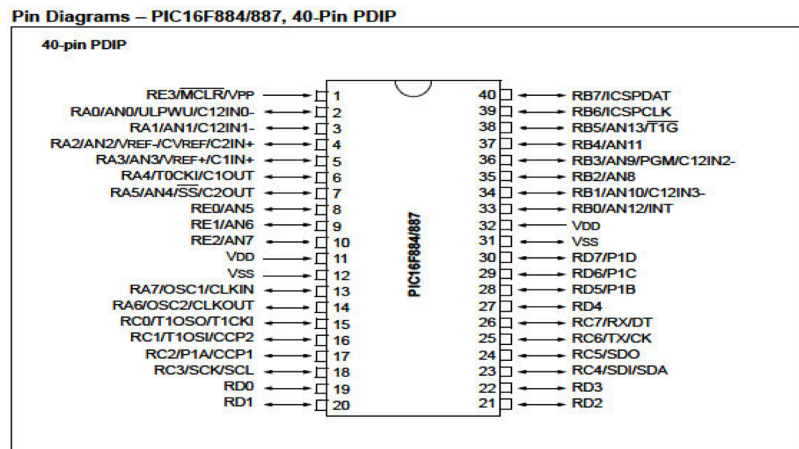


Figure 16. Pin configuration of PIC16f877 MCU.

When MCU's pins 19, 20, 21 & 22 become high for several conditions, MCU displays the results according to the input data from the photodetectors activity. If only pin 21 goes high, the display shows "Positive". Otherwise, shows "Negative". That means if light has been absorbed without the range of defined wavelength then it identifies as pork. Whereas, if multiple beams of light have been identified, the result shows negative which means no pork has been detected. The light intensity and wavelength ranges can be easily changed according to the analysis result. As a result, modification can be easily done by changing the setup.

7. Conclusion

This work addresses the key techniques of developing a smart fluorescence spectroscopy system along with a simple DNA extraction process, which is essential for identifying the pork adulteration in food and beverage. With the help of this spectroscopy, different combinations of material (with and without pork) have been observed and measured the light spectrum through RSpec software. After analyzing the spectrum pattern of different combinations, it is concluded that the proposed system can distinguish pure pork, a mixture of pork, and without any pork. Moreover, an equivalent circuit of the software has been designed to minimize the unnecessary use of RSpec software and computer. The proposed system is reliable, simple, and user friendly, hence can be extensively used in the food industry. It will also restrict illegal profit by ensuring the quality and purity of food items. Finally, the fluorescent light spectroscopy would make it possible to increase the number of sample inspection and testing since every single of the test kits can be promptly executed on-site with no special equipment or trained personnel requirement.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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